ELSEVIER

The Science of the Total Environment 213 (1998) 165–175

the Science of the Total Environment
An International Journal for Scientific Research into the Environment and its Relationship with Man

Hg methylation in sediments and floating meadows of a tropical lake in the Pantanal floodplain, Brazil

Jean R.D. Guimarães[a,*], Markus Meili[b], Olaf Malm[a], Elcia Maria de Souza Brito[a]

[a] *Lab. de Radioisótopos, Inst. de Biofísica da UFRJ, Bloco G CCS, Rio de Janeiro, RJ, CEP 21941-900, Brazil*
[b] *Institute of Earth Sciences, Uppsala University, Norbyvägen 18B, S-752 36 Uppsala, Sweden*

Abstract

Potential net ^{203}Hg methylation was assayed in different substrates and conditions in Fazenda Ipiranga Lake, 30 km downstream from gold mining fields near Poconé, Pantanal, Brazil, during the dry season. Samples and acidified controls of surface sediments and roots of dominant floating macrophytes (*Eichhornia azurea, Salvinia* sp.) were incubated in situ for 3 days with approx. 43 ng Hg.g^{-1} (dry weight), added as $^{203}HgCl_2$. Methylmercury (Me^{203}Hg) was extracted in toluene and measured by beta counting. Net methylation was about 1% in sediments under floating macrophytes, both at an open lake site and at a littoral site. Parallel incubations of sulphate or molybdate amended samples suggest that sulphate reducing bacteria may be important Hg methylators at both sites, and that their activity is sulphate-limited in particular at the littoral site. In laboratory experiments, net methylation in the same sediments was highest at temperatures in the 33–45°C range but was completely inhibited at 55°C. NaCl addition had a strong inhibiting effect on net methylation. In an intact open-lake sediment core, spiked with ^{203}Hg in the overlying water and incubated for 3 days, total ^{203}Hg was detectable down to a depth of 14–16 cm, coinciding with the depth reached by the galleries of chironomid larvae present in the core. Swimming insects caused ^{203}Hg penetration down to 4 cm. Me^{203}Hg was detected only in the upper layers (0–2 cm) of the sediment, with concentrations reaching 0.47–0.75% of total Hg. This suggests an important role for bioturbation in the exchange of Hg and MeHg between sediment and water. Methylation was up to nine times more intense in floating macrophyte roots than in the underlying surface sediments: an average of 10.4% of added Hg was methylated in samples of *Salvinia* sp. roots during the 3-day incubation, and 6.5% in *E. azurea* roots. This adds to previous findings on the role of such macrophyte stands, a distinctive feature of tropical rivers and lakes, as potentially important sites for the production of highly available MeHg.

Keywords: Hg methylation; Pantanal, Brazil; Sulphate reducing bacteria; Bioturbation; Aquatic macrophyte stands

* Corresponding author. Tel.: +55 21 5907147; fax: +55 21 2808193/+55 21 2216844; e-mail: jeanrdg@ibccf.biof.ufrj.br

0048-9697/98/$19.00
PII S0048-9697(98)00089-8

1. Introduction

The Pantanal is one of the largest wetlands in the world and extends over an area of approximately 300 000 km^2 on the Paraguay river basin, along the border area of Brazil, Bolivia, Argentina and Paraguay. Half of this surface is flooded annually. In Brazil, the Pantanal covers an area of 150 000 km^2, mostly in the states of Mato Grosso and Mato Grosso do Sul. It is a region of great wildlife abundance and diversity, exploited by tourism, sport and commercial fishing, and cattle farms. However, since the 18th century, gold has been extracted from quartz veins in some areas of lateritic soil near the Pantanal boundary, using amalgamation as a concentration process and resulting in metallic Hg releases to the atmosphere, soils and sediments. Poconé is the main gold mining area on the Northern Pantanal borders (Fig. 1) and one of the oldest in Brazil. The consequences of Hg emissions to the Pantanal fauna and especially fish are a subject of debate. Some high Hg concentrations in fish have been reported by Vieira (1991) but Lacerda et al. (1991) and Cetem/CNPq (1991) found evidence suggesting a very limited mobility of the metallic Hg present in contaminated tailings, sediments and soils. Hylander et al. (1994) found that among

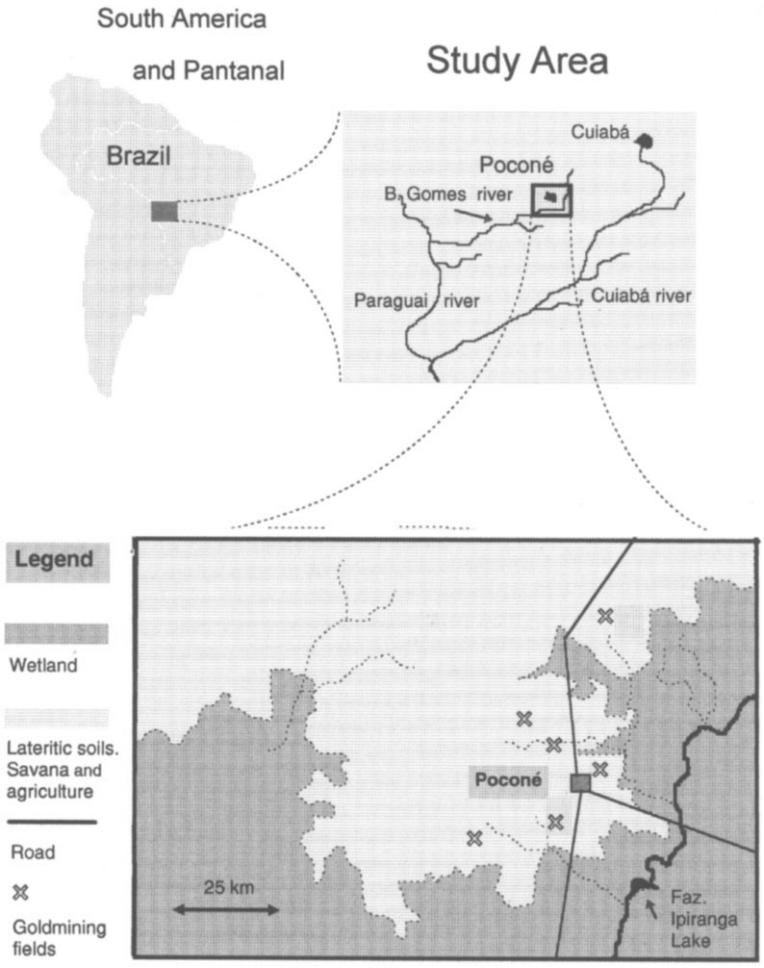

Fig. 1. Location of the study area.

45 samples of carnivorous fish from different areas of the Pantanal, only a few were above the Hg safety limit of 0.5 $\mu g.g^{-1}$ (wet weight). In a more recent survey restricted to the area downstream the Poconé mining fields, we found maximum concentrations of 210 $ng.g^{-1}$ (wet weight) in over 100 fish comprising mainly carnivores. This is in contrast with data from areas in the Amazon basin where a significant fraction of carnivore fish are above this safety limit (Malm et al., 1996b).

The availability to aquatic organisms of Hg^0 released by gold mining activities is limited first by its oxidation rate to Hg^{2+} and then by the environmental conversion equilibrium between methylation of Hg^{2+} to methylmercury (MeHg) and demethylation/volatilization, processes normally mediated by microbial activity. Most studies on Hg methylation have been made in temperate or boreal environments and have demonstrated that Hg methylation occurs in soils, water, periphyton and sediments. The latter have received most attention, as formation of MeHg in the environment was first demonstrated in sediment (Jensen and Jernelöv, 1969) and as methylation appears to be often more intense there than in other sites in aquatic environments, at least on a volumetric basis. However, in tropical rivers and lakes, the abundance and diversity of aquatic macrophytes are a distinctive feature and this very unique biotope deserves attention as a potential Hg methylation site. Some plants such as *Paspalum*, *Eichhornia* and *Salvinia* can form vast floating mats or floating meadows (Junk, 1986a). They represent a high biomass and productivity and are consumed by many herbivores, although most of the plant material passes through the detritus pathway (Junk, 1986a). The dense root layer of the floating mats can reach a thickness of 50 cm and is an essential source of food and shelter for large populations of fish and invertebrates.

The aim of the present study was to compare the potential net ^{203} Hg methylation in samples of different substrates such as surface sediment, roots of floating macrophytes and water, in a lake downstream the Poconé gold mining fields, and to obtain some data on the influence of temperature, salinity and bioturbation on Hg methylation and redistribution in sediments. This study is part of a broader study in the vicinities of Poconé, on total Hg and MeHg in the aquatic fauna, and total Hg in sediments and soils (in preparation).

2. Materials and methods

2.1. Study site

The site chosen for this study was the Fazenda Ipiranga Lake, some 30 km downstream from the gold mining area, and the sampling was made during the dry season (August 1995). Maximum depth at this time of the year is approximately 1.5 m, and the water surface approximately 1 sq.km. The effective area is certainly much higher, but is difficult to estimate due to the vast surface covered by floating meadows. This lake on the Bento Gomes river (Fig. 1) exists as such only during some months of the year and disappears in the river floodplain soon after the beginning of the rainy season.

The water hyacinths *Eichhornia azurea* and *E. crassipes* form extended monospecific floating mats, that are freely floating (*E. crassipes)* or partially rooted in littoral areas. *Salvinia* sp. tends to form smaller mats, mixed with many other floating or rooted plants. The floating mats of these species form a dense root system, the roots of *E. azurea* extending down to 50 cm from the water surface.

2.2. Sampling and methylation assays

The surface (0–1.5 cm) of the sediment at shallow (< 40 cm) sites was sampled with a peristaltic pump. At the center of the lake, sediment was scooped from the surface of samples freshly taken with an Ekman dredge. Sediments were filled to the top of polyethylene vials and capped. Individuals of *E. azurea* and of *Salvinia* sp. were taken by hand in different areas of the floating mats. No attempt was made to exclude the small amounts of periphyton or fine particles attached to the roots, only macrofauna and coarse debris were removed. The roots were transported in sealed plastic bags filled with lake water.

Dissolved oxygen was measured in-situ at vari-

ous depths, in and out of macrophyte stands with a YellowSprings Instruments meter, as well as water temperature, pH, and conductivity. Total Hg and organic matter were determined in the < 74-μm sediment fraction (see Malm et al., 1990 for analytical techniques).

Care was taken to minimize the exposure of the sediment and macrophyte samples to atmospheric oxygen during sampling, transport and preparation of incubations.

Incubations with ^{203}Hg were made in 50-ml Teflon-lined screw-cap borosilicate tubes, previously acid-washed and sterilized. For each assay, 2–3 samples and 1–2 acidified control samples (with 1 ml 4 N HCl) containing roots or surface sediments in amounts equivalent to approx. 1 g dry weight were mixed with 30 ml of lake water and approx. 43 ng of Hg, added as $HgCl_2$, containing approx. 0.7 kBq of ^{203}Hg, supplied by Amersham, UK. Incubations as above but with 40 ml of unfiltered lake water alone were also made, with the same Hg spike level.

Representative root samples were prepared with 3- to 5-cm pieces removed from different plants and positions of the roots. A specific experiment was made to verify if cutting the roots would in itself stimulate Hg methylation. *E. azurea* plants were collected from a small freshwater affluent of Jacarepaguá lagoon, a littoral site southwest of Rio de Janeiro state, and roots were incubated using exactly the same procedures as in the Pantanal, except for a higher Hg spike of 75 ng total Hg per sample (instead of 43 ng) because a different ^{203}Hg solution was used. Fresh root samples equivalent to 1 g dry weight were prepared in four different ways: (a) as described above for the Pantanal experiments, that is, cut into 3- to 5-cm pieces; (b) as in (a) but only with senescent roots; (c) chopped into $<$ 5-mm pieces, and gently mashed in the incubation tubes; (d) terminal sections of roots standing upright in the incubation tubes, with the cut area some millimeters above the water surface, the water being spiked and mixed before the introduction of the roots.

To stimulate or inhibit the activity of sulphate-reducing bacteria (SRB), some sediment samples also received 0.2 ml of sulphate (Na_2SO_4) or molybdate (Na_2MoO_4) solutions, resulting in an added SO_4^{2-} concentration in the samples of 1.3 mM, and 4.8 mM for MoO_4^{2-}. Incubations started within 4–6 h after sampling. The tube racks were totally submerged in the sediment under a macrophyte mat for 3 days (to ensure total darkness and no departure from in-situ temperature). In previous experiments, we found that this is the average time required for reaching or approaching equilibrium in ^{203}MeHg concentration in such small systems (Guimarães et al., 1995b). Methylation in the samples was stopped by addition of 1 ml 4 N HCl and samples were frozen for 5–10 days until MeHg extraction. After addition of NaBr and acid $CuSO_4$ solutions, shaking and centrifuging, Me^{203}Hg was extracted from water or from the supernatant of the sediment and root samples with scintillation cocktail (toluene with scintillation salts) and measured by beta counting on a LKB Wallac Rackbeta 1214 liquid scintillation counter. A more detailed description of procedures and performance of this simplified MeHg extraction can be found in Guimarães et al. (1995a). Net Me^{203}Hg production was expressed as % of added ^{203}Hg as follows:

$$\text{Methylation } \% = (\text{average net dpm in samples} - \text{average net dpm in controls}) \times df \times ef \times 100 / \text{total added dpm}$$

where *df* and *ef* are dilution and extraction efficiency factors, respectively, and dpm refers to decay-corrected radioactive disintegrations per minute. Methylation rates ($\%.g^{-1}.h^{-1}$) for sediments and macrophyte samples, averaged over the incubation period, can be obtained by dividing the % figures by the incubation period (72 h), since sample mass was approx. 1 g dry weight.

An undisturbed open lake sediment core was taken at the center of the lake with a perspex tube (7.0 cm inner diameter) and happened to contain five chironomid larvae and six insects that swam in and out of the flocculent sediment surface. The water column of approximately one liter was spiked with 0.43 μg ^{203}Hg and mixed slowly to avoid sediment resuspension. The spike was calculated to give approximately the same Hg

concentration per unit of sediment area as in the sediment slurries described above. This was as arbitrary as any other choice, because the cores and slurries are not comparable systems and the spike would result in similar Hg concentrations in the sediment only if the spike was homogeneously mixed in the core sediment down to the same depth as the slurries, or approx. 2 cm. After 3 days of incubation at in-situ temperature (25°C) and in very dim light, water was removed and the sediment was sliced. No control cores could be included: the density of insects and other invertebrates in all cores we took in the study lake was high, so the setting up of control cores with little or no bioturbation would require a removal of the organisms, which cannot be achieved without disturbing the natural sediment structure. Total ^{203}Hg in water and sediment was measured by gamma spectrometry in a LKB Wallac Compugamma 1282 and Me^{203}Hg extracted and measured as described above. Me^{203}Hg concentrations in the core sediment layers were calculated as a fraction of total ^{203}Hg.

Surface sediment samples from the littoral site under a macrophyte mat were kept at 4°C in the dark for approx. 3 weeks and allowed 2 days of adaptation time to room temperature before their use in laboratory experiments on the influence of temperature and salinity on Hg methylation. Incubations and extractions were done as described for the in-situ incubations. Samples and acidified controls were incubated for 3 days in the dark at 4, 25, 35, 45 and 55°C, or at 25°C with additions of $NaCl^-$ solutions adjusted to increase the original conductivity by a factor of approx. 2 and 3, just prior to ^{203}Hg addition.

In the present study, a fresh ^{203}Hg solution with a specific activity of 47 μg/MBq was used to perform the incubations with Hg additions of 43 ng Hg.g^{-1} sample dry weight, representing an increase of only 37–60% above natural total Hg concentration in the sediment.

Versions of the present radiochemical approach using high specific activity ^{203}Hg have been applied recently (Gilmour and Riedel, 1995; Stordal and Gill, 1995). They allow study of Hg speciation with tracer level Hg addition and therefore less disturbance of the natural equilibrium among Hg species. The cost and limited availability of the high activity ^{203}Hg have however limited its use.

3. Results and discussion

3.1. Total Hg and other features of the sampling site

The sediments were quite flocculent at the surface and contained 71–116 ng.g^{-1} (dry weight) total Hg and 20% of organic matter in the 0–2 cm layer (see Malm et al., 1990 for analytical methods).

Water temperature was 24.9–25.3°C in the whole water column, and pH varied between 6.7 and 7. The dissolved oxygen at 40 cm below the water surface was lower in a near-shore *E. azurea* mat (54.6% of saturation) than in open water (64.3%), whereas near-bottom concentrations were 16 and 53%, respectively.

Annual ranges of conductivity and pH found by Rodrigues and Maddock (1995) in surface waters of a number of stations along the Bento Gomes river were 48–124 μS.cm^{-1} and 6.1–8.0, respectively. In the interstitial water of Fazenda Ipiranga lake, we found a conductivity of 116 μS.cm^{-1}.

3.2. Hg methylation in water and sediments

Net methylation was 0.4–1.2% in all untreated sediments after incubations at 25°C, both in situ and after 3 weeks of storage at 4°C. This conservative reference range, which includes variations in handling, transportation and storage, was clearly exceeded by several experimental manipulations (see below).

The methylation percentages were slightly lower in surface sediments of an open lake site than in those of a littoral site under the floating macrophytes, composed mainly of *E. azurea*. These figures are comparable with those obtained in similar experiments in different rivers and lakes of the Amazon basin (Guimarães et al., 1995b), although lower Hg additions were used here. Since the fraction of added Hg that is converted to MeHg is smaller at higher Hg concentrations—an intrinsic limitation of any method based on addi-

tion of Hg to the samples—this suggests that in comparable conditions methylation would tend to be smaller in the studied Pantanal lake than in clear or black-water lakes of the Amazon basin. This could, together with the high biomass dilution, possibly explain the low total and MeHg concentrations found in carnivorous fish in this and other studies (Hylander et al., 1994; Malm et al., 1996).

Hg methylation in the water was undetectable ($< 0.05\%$ of total ^{203}Hg, or $< 2 \times 10^{-5}\%.ml^{-1}.h^{-1}$) in unfiltered samples taken in the macrophyte mats, as found in all of our previous measurements in waters of other areas (Guimarães et al., 1995a,b). This is probably due, at least partly, to the small sample size, that increases the achievable detection limits. On the other hand, the total added Hg for water incubations was near 1 $\mu g.l^{-1}$, a level that is probably toxic to microorganisms, except the Hg-resistant ones. However, Gilmour et al. (1996), using tracer-level Hg additions, found no detectable methylation in the water above sediment cores in a series of experiments in the Everglades.

The importance of methylation in water should not be underestimated, as even very low net methylation in water may contribute significantly to Hg bioaccumulation due to the potentially high bioavailability of the MeHg produced. For a reliable estimate, daily and seasonal cycles of Hg methylation in water should also be studied. In addition, strong spatial variations can be found in Hg methylation in the water column of lakes. Regnell (1995) and Watras et al. (1995) found elevated methylation rates just below the oxic–anoxic boundary layer of lakes. Shallow and macrophyte covered large lakes like those found in the Pantanal have forcingly extended oxic/anoxic boundary layers, a feature that probably increases the relative importance of Hg methylation in water in comparison to that in sediments.

3.3. Observations on the role of sulphate-reducers

Table 1a shows the results of methylation experiments with stimulation and inhibition of sulphate-reduction in surface sediment samples. The increase in MeHg production upon addition of sulphate was more pronounced at the littoral site,

Table 1
Net methylation of Hg in the Fazenda Ipiranga Lake, Bento Gomes river, Pantanal, Brazil; (a) Effect of sulphate-reduction stimulation (sulphate addition) or inhibition (molybdate addition) on the net methylation of Hg added to slurries of surface sediments after in-situ incubation (25°C); (b) Net methylation of Hg added to slurries of littoral surface sediments incubated at different salinities (NaCl additions) at 25°C after 3 weeks of storage at 4°C; (c) Comparison of net Hg methylation in samples of floating macrophyte roots and of underlying surface sediments (0–1.5 cm, water depth < 0.5 m) at a littoral site after in-situ incubation (25°C)

Parameter	Hg net methylation (MeHg as % of added inorganic Hg)		
	Untreated	+1.3 mM SO_4^{2-}	+4.8 mM MoO_4^{2-}
(a) Site			
Center of lake	0.92 (0.75–1.1)	1.06 (1.06–1.06)	0.77 (0.72–0.82)
Near shore	1.16 (1.1–1.22)	2.88 (2.87–2.88)	0.66 (0.62–0.7)
(b) Conductivity ($\mu S.cm^{-1}$)			
116	0.91 (0.67–1.1)		
248	0.15 (0.07–0.23)		
314	< 0.05		
(c) Sample type			
Nearshore sediment	1.16 (1.1–1.22)		
Roots of *Eichornia azurea*	6.5 (5.4–7.6)		
Roots of *Salvinia* sp.	10.4 (9–11.7)		

Results are expressed as % of ^{203}Hg converted to $Me^{203}Hg$ (average and range of two to three samples) after 3 days of dark incubation with ca. 43 ng $Hg.g^{-1}$ (dry weight).

as well as the inhibition by molybdate. The fact that sulphate and molybdate had effects on methylation in samples from both sites but more marked for the littoral one suggests that SRB may be important Hg methylators at both sites, but apparently more important and more prone to sulphate limitation at the littoral site. Sulphate reduction rates were unfortunately not measured in this study. The conditions at the more stagnant oxygen-depleted site under the macrophyte mat are in principle more favorable to SRB than those at the open water site, but it has been shown that sulphate reduction occurs even in the oxic layer of marine sediments (Jorgensen and Bak, 1991) and that SRBs are present and can outcompete methanogens even in aerobic sediments (Lovley and Klug, 1983).

3.4. Influence of temperature and salinity

Fig. 2 shows the results of incubations of surface sediments at temperatures ranging over 4–55°C. The highest methylation was found in the 35–45°C range where bacterial activity is expected to be higher. At 55°C, methylation was almost completely suppressed. At such temperatures many enzymes are inactivated, and bacterial activity is probably stopped, suggesting a biological control of methylation. To exclude the hypothesis that MeHg could have been formed at these higher temperatures but degraded before the end of the incubations, shorter experiments (12–36 h) were made and confirmed that at this temperature no MeHg formation is detected in any moment of the incubation. Artifact formation of $Me^{203}Hg$, as described by Bloom et al. (1996), was not observed in controls acidified and heated for 1–3 days at 50 and 100°C or in heated controls with 10% formaldehyde, in accordance with the temperature effects observed here at > 50°C.

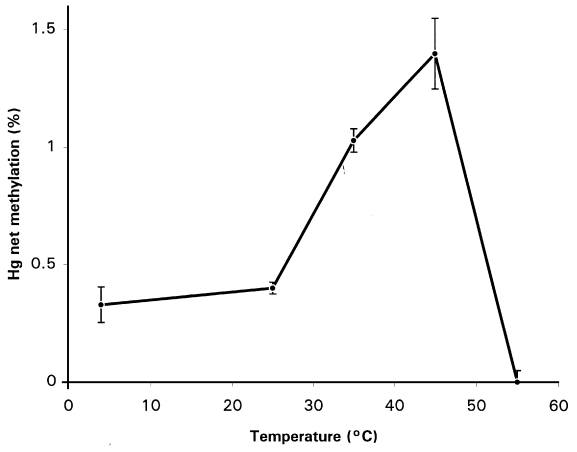

Fig. 2. Net methylation of ^{203}Hg added to slurries of littoral surface sediments from Fazenda Ipiranga lake, Pantanal, Brazil, incubated at different temperatures, after 3 weeks of storage at 4°C. Vertical bars show the range of duplicates.

Water temperatures in the rivers and lakes of the Pantanal or Amazon basin are generally in the 24–31°C range, but temperatures of 40°C can be found in shallow areas and among macrophytes (Sioli, 1990).

3.5. Influence of salinity

Table 1b shows the results of incubations made at in-situ temperature (25°C), at the original conductivity of 116 $\mu S.cm^{-1}$ and at values of 248 and 314 $\mu S.cm^{-1}$, obtained by addition of NaCl solutions. MeHg production was much lower at higher conductivity. Additional experiments using ions other than Cl^- are desirable, to clarify what fraction of this effect could be due to shifts in Hg speciation caused by the formation of Cl^- complexes. Black water rivers have low conductivity and pH, and are rich in humic matter, factors associated with a high MeHg production and/or bioavailability.

The measured conductivity in the Fazenda Ipiranga lake is in the range normally observed in clear to white waters, but this parameter alone can be misleading: conductivities higher than tested here are not uncommon in the Pantanal. Rodrigues and Maddock (1995) measured values of up to 1000 $\mu S.cm^{-1}$ in some tributaries of the Bento Gomes river in the dry season. The *salinas*, high salinity lagoons, are a peculiar feature of the Pantanal and form salt deposits, a few of which are exploited commercially (Magalhães, 1992).

The Hg net methylation observed at in-situ temperature (25°C) in Fig. 2 and conductivity (116 $\mu S.cm^{-1}$) in Table 1b were both obtained from

experiments done under the same conditions as the in-situ incubations in Table 1a, but 3 weeks after sampling and with small differences in starting date and duration. Incubations done some time after sampling tend to yield lower methylation rates than in-situ incubations, irrespective of sample storage at 4°C or at in-situ temperature (Guimarães et al., 1995a).

3.6. Methylation and vertical mixing in a water-spiked intact sediment core

The total ^{203}Hg in unfiltered overlying water at the end of the incubation was close to the detection limit, and Me^{203}Hg was therefore not measurable. Fig. 3 shows the variation of Me^{203}Hg and total ^{203}Hg concentrations along the core layers. Total ^{203}Hg was detectable down to the 14–16 cm layer, corresponding to the depth reached by the U-shaped galleries of five chironomid larvae present in the core, that were actively pumping water during all the incubation period. In the bottom layers of the core between 7 and 19 cm, ^{203}Hg was possibly present only along the walls of the galleries. High concentrations of total ^{203}Hg were found in the top 4 cm of the sediment (Fig. 2), corresponding to the depth reached by the swimming insects present in the core. This suggests that the penetration of ^{203}Hg was the result of the mixing promoted by the activity of these insects, maybe enhanced by the abundant zooplankton on the sediment surface. By the initial gentle mixing and diffusion alone, ^{203}Hg could not penetrate more than some millimeters into the fine-grained sediment ($>90\%$ of sediment dry weight was <74 microns), in a period as short as 3 days. In a similar experiment with intact cores of sediment containing fibers from a pulp mill, Regnell et al. (1996) found that after 78 days, only $9 \pm 4\%$ of ^{203}Hg added to the water reached the 1–5 cm sediment layer.

Me^{203}Hg was detectable in sediment only in the layers 0–0.5, 0.5–1 and 1–2 cm, with concentrations of 0.46%, 0.75% and 0.57% of total ^{203}Hg, respectively (Fig. 3), indicating that Me^{203}Hg and total ^{203}Hg had similar distributions in the top layers. These values are approximately half those obtained in slurries of surface sediments taken at the same site but within the range of results obtained from all slurries (0.4–1.2%). Incubation duration was similar for both types of experiment, but the ^{203}Hg spike level is difficult to compare among them: the slurries were homogeneously spiked with ^{203}Hg at the beginning of the incubation, while the cores were left undisturbed and Me^{203}Hg formation in the core sediment may depend on the progressive penetration of ^{203}Hg.

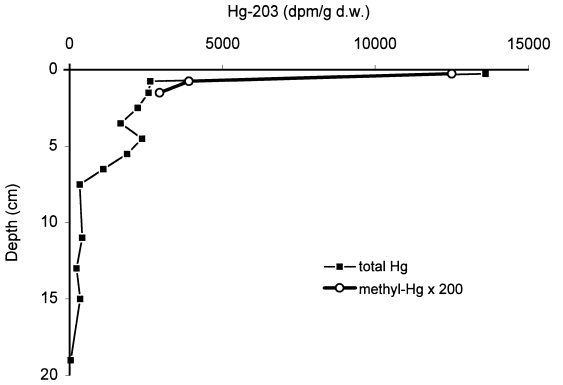

Fig. 3. Penetration of total ^{203}Hg and Me^{203}Hg production in the sediment of a water-spiked intact sediment core from the Fazenda Ipiranga Lake, Pantanal, Brazil, after 3 days of incubation. Unidentified swimming insects penetrated the sediment down to 4 cm, and galleries of chironomid larvae reached 19 cm; d.w.: dry weight.

Though resulting from a single core experiment, these few data illustrate the potential importance of bioturbation in the penetration of Hg in a sediment with a high biological activity, which has implications for the dilution as well as for the retention of Hg added to the ecosystem. In future experiments, we will also include sediment-spiked cores to evaluate the role of bioturbation in the Hg and MeHg sediment–water flux.

3.7. Methylation in floating macrophyte mats

The data in Table 1c represent probably the most striking finding in the present study: the production of MeHg in the roots of two dominant floating macrophytes was higher than in the underlying surface sediments by a factor of 5.6 for *E. azurea*, and 9 for *Salvinia* sp. In similar tests, but during the flood period and in lakes of the Tapajós, Negro and Amazon rivers (Guimarães et

Table 2
Influence of sample preparation on net Hg methylation in *E. azurea* root samples from Canal do Cortado, Rio de Janeiro state, Brazil

Root sample	Hg net methylation (MeHg as % of added inorganic Hg)
3–5 cm pieces	32.2 (31.7–32.8)
Senescent roots only, 3–5 cm pieces	12.2 (8.4–16)
Chopped to < 5 mm and mashed	23.9 (22.6–25.2)
Root tips, with cut area out of water	22.7 (20.5–24.8)

Procedure as in Table 1, but with 75 ng added Hg. Results expressed as % of ^{203}Hg converted to Me^{203}Hg (average and range of two samples).

al., 1995b, 1996), methylation in the root zone of floating *Paspalum* sp. meadows and small periphyton and algal mats was found to be comparable or higher by up to two orders of magnitude than in the sediments of the same sampling sites. In light of the results from a separate experiment (Table 2), it seems unlikely that the higher methylation rate in the presence of cut root fragments is an artifact due to the release of plant compounds that could act as methyl-donors or otherwise stimulate methylation. A lower methylation potential was found in the presence of senescent roots, and potentials for chopped and uncut roots were comparable, suggesting that the observed differences reflect natural variability related to root age, position, amount of associated particles and periphyton, etc. The *E. azurea* samples used in this experiment were taken in a moderately eutrophic freshwater site, 1 mile from the ocean, and formed up to five times more MeHg than those from the Ipiranga lake, additional and unexpected evidence of the importance of macrophyte roots as methylation sites.

Studies on Hg methylation rarely include the measurement of both natural MeHg and ^{203}Hg methylation potentials. In a recent study in a Tapajós river floodplain lake, total Hg and MeHg were analysed in the same type of sediment, macrophyte and soil samples incubated with ^{203}Hg: matching patterns were found for MeHg/total Hg ratios and net ^{203}Hg methylation potential, and both were higher in the floating macrophyte root zone than in the sediments (Guimarães et al., 1996). Gilmour et al. (1996) found that the abundant periphyton in the Everglades, Florida, was a very active methylation site, and also found a good agreement between MeHg concentrations and Hg methylation potentials. Macrophyte roots, and to a lesser extent, periphyton and algal mats seem to be clearly more important Hg methylation sites than sediments, and this appears to be true in different phases of the hydrological cycle and in river basins with different geochemical features.

4. Conclusions

The quantitative importance of SRB in Hg methylation remains unclear. In sediments of the hypoxic littoral zone, about half of the Hg methylation may possibly be attributed to SRB, while their contribution appears to be far less in more exposed sediments, where net methylation was similar. This issue requires more experiments, with better sampling techniques and a wider range of sulphate and molybdate concentrations.

The high potential of Hg methylation in floating macrophyte mats has many implications. This characteristic tropical aquatic vegetation is a potentially important site for the production of highly bioavailable MeHg, because it is in close contact with the water, has a very high relative area, is densely populated by a varied fauna of invertebrates and fish, and represents an essential carbon source for aquatic food chains. A rapid production of MeHg and a MeHg pool of 10% or more of the reactive Hg within this important habitat can constitute a major pathway of MeHg uptake into tropical aquatic food webs. In contrast, the bioavailability of MeHg produced in sediments is probably limited, as well as the sediment–water flux of MeHg. Macrophyte mats may also be the dominant MeHg source to the open water, considering a standing stock of macrophyte mats around 1 kg dw m^{-2} (Junk, 1986b), a mixed sediment layer of 10 kg dw m^{-2} ($\sim$ 10 cm, see above), a tenfold higher net methylation in the macrophyte mats (Table 1c), and a slower exchange of water in the sediment.

Another distinctive feature of tropical aquatic

environments, particularly in the Central Amazon, is the *igapó* or *várzea* forest that is flooded during many months of the year. The organic floc and humus on the surface of the flooded soil of a *várzea* forest on the Tapajós river was shown recently to have a higher methylation potential than the sediments at different sites of an adjoining floodplain lake (Guimarães et al., 1996). These patterns are in agreement with the proportions of MeHg found in boreal wetlands, flooded soils and lake sediments, and support the importance of stagnant hypoxic zones as the main sources of MeHg in surface waters (Meili, 1996).

Macrophyte stands and flooded forest soils are environments of high ecological importance, and very important in the life cycle of commercially important fish species. Due to their important potential role as Hg methylation sites, these unique biotopes should receive the same attention that sediments have received so far in this context.

Acknowledgements

This work was supported by an OPAS/GTZ grant, a CNPq research fellowship for J.R.D. Guimarães, UFRJ and FINEP. We are grateful to the Prefeitura Municipal de Poconé and to the Fazenda Ipiranga, for logistic support during the field work. The help of Jane B.N. Mauro in laboratory experiments is gratefully acknowledged.

References

Bloom NS, Colman JA, Barber L. Artifact formation of methyl mercury during extraction of environmental samples by distillation. In: Fourth International Conference on Hg as a Global Pollutant, Hamburg, Germany, 4–8 Aug. 1996, Abstracts, 1996:51.

Cetem/CNPq. Poconé: um campo de estudo do impacto ambiental do garimpo. Série Tecnologia Ambiental, no. 1, Rio de Janeiro, 1991:113 (in Portuguese).

Gilmour CC, Riedel GS. Measurement of Hg methylation in sediments using high specific activity 203-Hg and ambient incubation. Water Air Soil Pollut 1995;80:747–756.

Gilmour CC, Gill GA, Stordal MC, Spiker E. Mercury methylation and sulfur cycling in the Northern Everglades. In: Fourth International Conference on Hg as a Global Pollutant, Hamburg, Germany, 4–8 Aug. 1996, Abstracts, 1996:166.

Guimarães JRD, Malm O, Pfeiffer WC. A simplified radiochemical technique for measurement of net mercury methylation rates in aquatic systems near goldmining areas, Amazon, Brazil. Sci Total Environ 1995a;172:151–162.

Guimarães JRD, Malm O, Padovani C, Sanches MV, Forsberg B, Pfeiffer WC. A summary of data on net mercury methylation rates in sediment, water, soil and other samples from the Amazon region obtained through radiochemical methods. In: Proceedings of the International Workshop on Environmental Mercury Pollution and its Health Effects in Amazon River Basin, Rio de Janeiro, Nov. 30–Dec. 2, 1994, 1995b:94–99.

Guimarães JRD, Roulet M, Lucotte M. Seasonal and spatial variations of Hg net methylation in a floodplain lake of the Tapajós river, Brazil. In: Fourth International Conference on Mercury as a Global Pollutant, Hamburg, Aug. 1996, Abstracts 1996:418.

Hylander LD, Silva EC, Oliveira LJ, Silva SA, Kuntze EK, Silva DX. Mercury levels in Alto Pantanal: a screening study. Ambio 1994;23:478–484.

Jensen S, Jernelöv A. Biological methylation of mercury in aquatic organisms. Nature 1969;223:753–754.

Jorgensen BB, Bak F. Pathways and microbiology of thiosulphate transformation and sulphate reduction in a marine sediment (Kattegat, Denmark). Appl Environ Microbiol 1991;53:847–856.

Junk WJ. Aquatic plants of the Amazon system. In: Davies EP, Walker RF, editors. The Ecology of River Systems. Dordrecht, The Netherlands: Junk, 1986a.

Junk WJ. Investigations on the ecology and production biology of the 'Floating Meadows' Paspalo-Echinochloetum on the Middle Amazon. I. The floating vegetation and its ecology. Amazoniana 1986b;2(4):449–495.

Lacerda LD, Pfeiffer WC, Marins RV, Rodrigues S, Souza CMM, Bastos WR. Mercury dispersal in water, sediment and aquatic biota of a gold mining tailings drainage in Poconé, Brazil. Water Air Soil Pollut 1991;56:785–796.

Lovley D, Klug MJ. Sulphate-reducers can outcompete methanogens at freshwater sulphate concentrations. Appl Environ Microbiol 1983;45:187–192.

Magalhães NC de. Conheça o Pantanal. Terragraph, S. Paulo, 1992:390 (in Portuguese).

Malm O, Pfeiffer WC, Souza CMM, Reuther R. Mercury pollution due to gold mining in the Madeira river basin, Brazil. Ambio 1990;19:11–15.

Malm O, Akagi H, Guimarães JRD, Bastos WR, Costa LS, Torres JPM. Data on mercury and methyl mercury in environmental and human samples from new study areas in Brazil: Balbina Reservoir and Poconé (Pantanal wetland). JICA/NIMD, Minamata, Japan, Proceedings of the International Workshop on the Fate of Mercury in Gold Mining and Measures to Control the Environmental Pollution in Various Countries, Jakarta, Indonesia, 25–27 November 1996, 1996a.

Malm O, Guimarães JRD, Castro MB, Bastos WR, Viana JP, Branches FJP, Silveira EG, Pfeiffer WC. Follow-up of

mercury levels in fish, human hair and urine in the Madeira and Tapajós basins, Amazon, period 1990–95. In: Proceedings of the Fourth International Conference on Mercury as a Global Pollutant, Hamburg, Aug. 1996, 1996b:10.

Meili M. Mercury in lakes and rivers. In: Sigel H, Sigel A, editors. Mercury and its Effects on Environment and Biology. Metal Ions in Biological Systems. Vol. 34, Chapter 2. New York: Marcel Dekker, 1996:21–51.

Regnell O. Mercury methylation in the water of a stratified meso-eutrophic lake: role of dissolved oxygen, microbial composition and biomass. In: Methyl Mercury in Lakes: Factors Affecting its Production and Partitioning Between Water and Sediment. Ph.D. Thesis, Dept. of Ecology, Chemical Ecology and Ecotoxicology, Lund University, Sweden, 1995.

Regnell O, Tunlid A, Ewald G, Sangfors O. Methyl mercury in freshwater microcosms affected by dissolved oxygen levels: role of cobalamin and microbial community composition. Can J Fish Aquat Sci, 1996:53.

Rodrigues FS, Maddock JEL. Assessment of the heavy metal pollution in a gold garimpo. CETEM/CNPq, Rio de Janeiro, 1995, Série Tecnologia Ambiental, n°. 7, 1995:32.

Sioli H. Amazonia: fundamentos de ecologia da maior região de florestas tropicais. Vozes, Petrópolis, 1990:72 (in Portuguese).

Stordal MC, Gill GA. Determination of mercury methylation rates using a 203-Hg radiotracer technique. Water Air Soil Pollut 1995;80:725–734.

Vieira LM. Avaliação dos níveis de Mercúrio na cadeia trófica como indicador de sua biomagnificação em ambientes aquáticos da região do Pantanal. Ph.D. Thesis, São Carlos Federal University, Brazil, 1991:214 (in Portuguese).

Watras CJ, Bloom NS, Claas SA, Morrisson KA, Gilmour CC, Craig SR. Methylmercury production in the anoxic hypolimnion of a dimictic seepage lake. Water Air Soil Pollut 1995;80:735–745.