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## Mercury methylation along a lake–forest transect in the Tapajós river floodplain, Brazilian Amazon: seasonal and vertical variations

Jean Remy Davée Guimarães<sup>a,\*</sup>, Marc Roulet<sup>b</sup>, Marc Lucotte<sup>b</sup>,  
Donna Mergler<sup>c</sup>

<sup>a</sup>Lab. de Radioisótopos, Inst. de Biofísica, Universidade Federal do Rio de Janeiro (UFRJ), Bloco G CCS, Rio de Janeiro,  
CEP 21949-900 Brazil

<sup>b</sup>Chaire de Recherche en Environnement, Université du Québec à Montréal (UQaM), CP 8888, Montréal, H3C 3P8, Canada  
<sup>c</sup>CINBIOSE, UQaM, CP 8888, succ. Centre-ville, Montréal, H3C 3P8, Canada

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### Abstract

The seasonal and spatial variations of net methylmercury production in sediments, soils and other sites were evaluated by assays with  $^{203}\text{Hg}$  at different depths and locations along a lake–forest transect at lake Enseada Grande, Tapajós river. Soil and sediment samples were taken at the surface and at different depths up to 9 cm. Fresh samples and acidified controls (1–3 g dry wt.) were slurried with local water and incubated in the dark at 25–28°C for 3 days with 0.5–1.6  $\mu\text{g Hg g}^{-1}$  (dry wt.) added as  $^{203}\text{HgCl}_2$ .  $\text{CH}_3^{203}\text{Hg}$  was extracted and measured in scintillation cocktail after acid leaching. Methylmercury production varied by orders of magnitude among sites and among sediment or soil layers. Seasonal variations were smaller than those with sample depth and location. In both seasons, MeHg formation in sediment and soil or flooded soil decreased with depth and was, in the top layers, one order of magnitude higher in the C-rich littoral macrophyte zone (2.3–8.9%) and flooded forest (3.2–4.5%) than in the center of the lake (0.2–0.56%). Similar MeHg production was found in slurried dry soils (dry season) and in soils already flooded for months. In the macrophyte zone soil (dry season), methylation was mainly associated with the thin *Paspalum* sp. rootlet layer. In the forest site, vertical variation in methylation was less pronounced in flooded than in dry soils and during the inundation the higher methylation rate was found in the flocculent sediment settled over the litter layer. The roots of floating *Paspalum* sp. were an important Hg methylation site, particularly those heavily colonized with periphyton (3.4–5.4%). Methylation in surface or near-bottom water was undetectable ( $< 3 \times 10^{-2}\%$ )

\* Corresponding author. Tel.: +55-21-5615339; fax: +55-21-2808193.  
E-mail address: jeanrdg@biof.ufrj.br (J.R. Guimarães).

at all sites. Flooded forests and macrophyte mats are specific features of the Amazon and are important links between Hg inputs from natural and manmade sources and MeHg exposure of local populations through fish intake. © 2000 Elsevier Science B.V. All rights reserved.

*Keywords:* Methylmercury; Sediment; Soil; Macrophytes; Floodplain

## 1. Introduction

Though many recent advances have been made in the understanding of the origins and environmental pathways of mercury in the Amazon most aspects of the cycle of methylmercury (MeHg) in this complex system remain unknown. High total Hg and/or MeHg concentrations have been found in fish and human hair from areas affected by Hg<sup>0</sup> released by gold mining areas (Malm et al., 1990, 1997; Akagi et al., 1995; Lacerda, 1995; Kehrig et al., 1998) but also from pristine areas such as the Rio Negro (Forsberg et al., 1994) and lakes in the Amapá coastal plain (Guimarães et al., 1999a). The actual impact of gold mining activity on environmental Hg levels is a subject of debate, principally after Roulet et al. (1999a,b) demonstrated the importance of soil erosion as an intense natural Hg source to aquatic systems in the Tapajós river basin. Irrespective of the relative importance of the different Hg sources, many riverine populations in the Amazon basin are exposed to MeHg concentrations in their diet that have subtle but quantifiable neurotoxic effects (Lebel et al., 1996). Despite this, the factors controlling MeHg production, flux and bioaccumulation in the Amazon aquatic systems remain essentially unknown.

Mercury methylation in different types of samples has been studied in a variety of rivers and lakes in the Brazilian Amazon, Pantanal and other areas in Brazil (Guimarães et al., 1995, 1998; Lemos et al., 1997; Mauro, 1997) and the root layer of the floating aquatic plants — ‘floating meadows’, Junk (1986) — appeared to be a preferential methylation site. The first assessment of MeHg concentrations in water, epiphyton and plankton in the Tapajós river was made by Roulet et al. (1999b) in the same study area as described herein. Similarly to the floating meadows, flooded

forests or *igapó* are peculiar to the Amazon and are essential habitats for a varied fauna, including most fish of commercial importance. Herbaceous macrophytes and forest trees constitute 52 and 32%, respectively, of total primary production in the Amazon floodplain (Forsberg et al., 1993). Although there is some debate about the contribution of these and other carbon sources to the fish food web, most fish feed in these flooded aquatic habitats. Despite their potential importance in the Hg cycle, Hg biogeochemistry in these environments has not yet been studied.

In the frame of a collaboration project between UFPA, UQaM and UFRJ, total Hg, MeHg, <sup>203</sup>Hg net methylation and a set of biogeochemical parameters were simultaneously determined, along a lake–forest transect in a floodplain lake of the lower Tapajós river. This manuscript describes the study of the vertical and seasonal variations of net MeHg production in sediments, soils and other sites such as floating macrophytes along this transect.

## 2. Materials and methods

### 2.1. Study site

The lake Enseada Grande is part of the flood-plain of the lower Tapajós river and is connected to the river during all year (Fig. 1). Maximum depth is 6 m at the peak of flooding and 1.5 m in the dry season. The lake is separated from the river and from a river channel by *igapó* forest fringes of approximately 200 m width that are flooded during 4–6 months of the year. During the wet season the lake contour is densely colonized by floating mats of *Paspalum* sp. and to a much lesser degree, *Eichhornia azurea*, while in the dry season the exposed shallow areas are

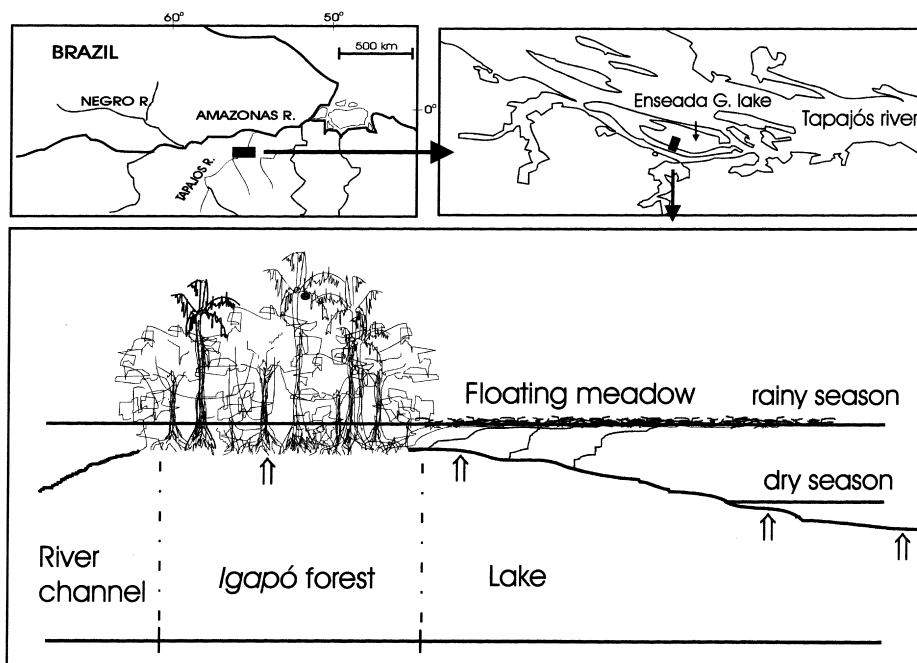


Fig. 1. Geographical location of study area and schematic representation of the lake forest transect in Lake Enseada Grande, Tapajós river. Arrows represent the location of sediment or soil cores.

converted to pasture, exploited by cattle and aquatic birds.

## 2.2. Sampling and incubations with $^{203}\text{Hg}$

Sediment or soil cores were taken by hand or SCUBA diving, by means of PVC tubes of 15 cm diameter. Samples were taken during the wet (March 1995) and dry season (November 1995), at the center of the lake, on the littoral of the lake, at the lake–forest interface and in the *igapó* forest (Fig. 1). On board, Eh profiles were recorded with thin platinum electrodes introduced in lateral perforations at each centimeter of the PVC cores and cores were sliced each 1–1.5 cm within 6 h after sampling. Sub-samples were reserved and frozen for analysis of total Hg, MeHg, carbon and nitrogen, Fe and Al oxyhydroxides. Hg net methylation was determined on sediment and soil samples from the surface layer (0–1 cm) and from the 4 to 5 and 8 to 9 cm layers. In forest soils, the surface sample corresponded to a mixture of fine litter, fine roots and

humic soil. Coarse litter was not incubated in any of the sampling dates, but in the wet season, the organic floc settled over the flooded litter in shallow areas was sampled by aspiration. The sub-surface forest soil layers were alluvial clayey mineral soils. The sediments at the center of the lake were clayey and showed no profile development, while the surface of the sediments in the macrophyte region were rich in *Paspalum* sp. detritus, with deeper layers similar to the sediment at the center of the lake.

Samples of roots of the floating macrophyte *Paspalum* sp., that forms dense mats on the lake shore, were taken at 0.5 m or more from the lake–macrophyte interface, and care was taken to minimize the loss, during sampling, of the fine detritus, periphyton and sediment attached to the roots. Though the samples could not be manipulated in anaerobic conditions, care was taken to minimize their exposure to atmospheric oxygen.

All samples were incubated at the in situ water temperature (25–28°C) and in the dark with 30 ml of lake water, in acid-washed Teflon<sup>®</sup>-lined

screw-capped 50-ml borosilicate tubes. All incubations were started in the field within a few hours after sampling. Duplicates and an acidified control, with 1 ml of 4 N HCl, received  $^{203}\text{Hg}$ , added as  $^{203}\text{HgCl}_2$  obtained from Amersham International, UK. Although different  $^{203}\text{Hg}$  lots were used at each sampling season, care was taken to calculate the added activity to obtain the same total added Hg (0.5–1.6 ng/g dry wt.) concentration in each type of sample. Fresh samples were equivalent to 1–3 g dry wt., and for each sample type, care was taken to incubate samples of same mass in the two sampling periods. After 72 h of incubation, Hg methylation was stopped by addition of 1 ml of 4 N HCl and samples were frozen until MeHg extraction, performed within 10 days after incubations. Previous tests showed that in these incubation conditions MeHg formation in sediment and macrophyte root samples reaches equilibrium within 2–5 days (Guimarães, 1992; Mauro, 1997).

MeHg was leached with 6 ml of 3 M NaBr in 11%  $\text{H}_2\text{SO}_4$  and 1.5 ml of 0.5 M  $\text{CuSO}_4$ : after 1 min of shaking, the samples were centrifuged and the supernatant (~25 ml) transferred to 60-ml glass separatory funnels and shaken for 15 min with 10 ml of scintillation cocktail (toluene + POP + POPOP). The aqueous phase was discarded and the overlying toluene layer transferred to glass tubes for centrifugation at 3000  $\text{rev. min}^{-1}$  for 1–2 min. The clean toluene was shaken in a vial with 0.5 g of anhydrous  $\text{Na}_2\text{SO}_4$  to remove traces of water containing inorganic  $^{203}\text{Hg}$  and transferred to clean scintillation vials for measurement on a Wallac-LKB 1482 Rack-beta liquid scintillation detector. Dpm data, corrected for decay, extraction efficiency and quenching were used to calculate MeHg as a percentage of total added Hg.

The  $^{203}\text{HgCl}_2$  solution used to spike the samples in field was not stabilized with acids or oxidants — to avoid changing the pH or Eh of the samples — and could undergo Hg losses by volatilization. For posterior control of added activity, every day in which incubations were performed, aliquots of the  $^{203}\text{Hg}$  spike, of same volume as added to the samples, were transferred

to test tubes containing 1 ml of 0.1 M  $\text{K}_2\text{Cr}_2\text{O}_7$  in 2 M  $\text{HNO}_3$ .

### 3. Results and discussion

Net methylmercury production from added  $\text{Hg}^{2+}$  in cores taken along the lake–forest transect in the dry and wet season is shown in Figs. 2 and 3. Hg methylation in surface soils and sediments varied by one order of magnitude along the transect, and pronounced vertical variations were also found, seasonal variations appearing less important than those due to site or depth. In the wet season, methylation was highest at the surface of sediments taken under the floating macrophyte mats of the littoral zone (2.3–8.9%) and in the floc settled at the surface of the litter in flooded soils (3.2–3.6%). In contrast, Hg methylation in sediments taken in the center of the lake was low (0.2–0.35%).

The vertical variation in methylation with depth was less pronounced in the flooded forest core than at the other two sites. It is noteworthy that as much MeHg was produced in the 4–5-cm layer as in the organic floc over the litter surface. However, MeHg produced in situ at this soil depth would be less available than when production takes place at the sediment–water interface.

During the wet season, the higher methylation in the flooded forest site was found in the flocculent organic sediment settled over the recent litter layer. This organic floc is formed by the most labile components of litter-fall, such as fruits and flowers, that decompose much faster than the leaves and branches, and by decaying plankton and other settled particles. Flux from this layer is direct to the overlying water column and readily bioavailable to insects and zooplankton. Tremblay and Lucotte (1997) demonstrated that insect emergence from flooded soils was the main pathway of MeHg from reservoir sediments to fish. Detritivorous fish also feed directly on this flocculent layer (Forsberg et al., 1993). It should also be pointed out that, unless specific sampling techniques are used, this surface layer is most often lost during soil and sediment coring or when slicing the cores.

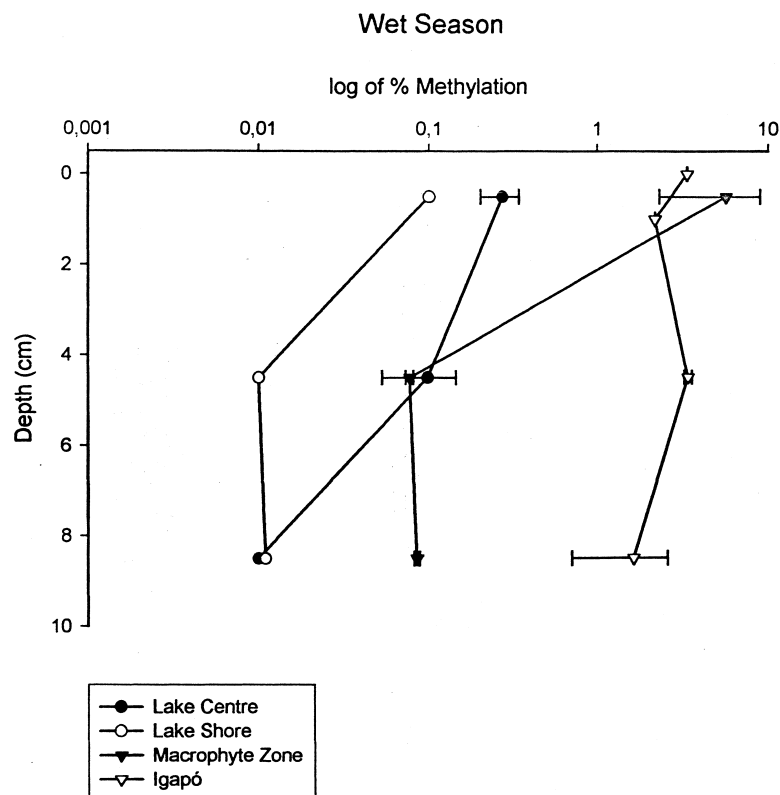


Fig. 2. Net MeHg production along the lake–forest transect (Lake Enseada Grande, Tapajós river) during the wet season. Methylation is expressed as percent of added  $\text{Hg}^{2+}$ . Horizontal bars represent the ranges for duplicate or triplicate samples, when bigger than the symbols.

In the dry season, methylation in sediments from a shallow (< 10 cm) littoral site was 0.2–0.42%, comparable to the center of the lake, regardless of season. In this core, methylation did not decrease with depth, in contrast with the other cores taken in this study, possibly due to the bioturbation promoted on this soft sediment by birds, cattle and other factors.

In the dry season, the soils from the lake shore — taken at the same spot where water was covered with macrophyte mats in the wet season — and from the *igapó* were slurried with lake water to make the methylation assays. The MeHg production was similar to that in soils already flooded for at least 2 months, suggesting that these periodically flooded soils conserve their methylation capacity during the dry season and

express it as soon as reached by seasonal flooding or simply heavy rain.

In the lake shore sediment (dry season), methylation was mainly associated to the top 1-cm sediment layer, rich in *Paspalum* sp. rootlets. Like most plants in the seasonally flooded areas of the Amazon basin, *Paspalum* sp. has developed strategies to deal with the rapid variations in the river and lake water level. Its rhizome and shoots cover the dry soil and when flooding begins, the rhizome shoots grow rapidly and *Paspalum* sp. forms dense floating mats, partially rooted to the bottom. It is interesting to note that similar high Hg methylation was found in the floating mats, the surface sediments under the mats — rich in *Paspalum* sp. detritus — and in the soils in which these sediments are converted to in the dry sea-

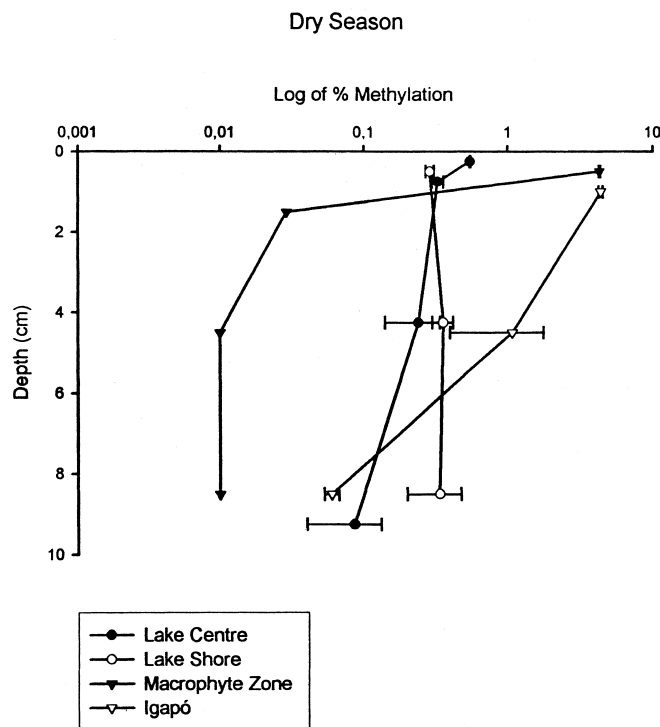


Fig. 3. Net MeHg production along the lake–forest transect (Lake Enseada Grande, Tapajós river) during the dry season. Methylation is expressed as percent of added  $Hg^{2+}$ . Horizontal bars represent the ranges for duplicate or triplicate samples, when bigger than the symbols.

son. Sulfate-reducing bacteria seem to play an important role in Hg methylation in floating macrophyte mats as well as in bottom sediments (Mauro, 1997; Guimarães et al., 1998) but the type of microorganisms involved in Hg methylation in the studied soils was not investigated. Roulet et al. (1999b) found that MeHg in roots of floating *Paspalum* sp. was associated with detritus, periphyton and trapped suspended sediments. Accordingly, in methylation experiments in the Paraguay river floodplain, Guimarães et al. (1999b) found that the associated solids are the main methylation site in roots of floating *Eichhornia azurea*.

Floating *Paspalum* sp. roots were an important Hg methylation site, particularly in roots heavily colonized with periphyton (3.4–5.4%). In similar experiments with other macrophytes at different study sites in Brazil, Lemos et al. (1997), Mauro

(1997) and Guimarães et al. (1998) found higher MeHg production in floating macrophyte roots than in the underlying sediments, and MeHg production in the submersed parts of macrophytes such as *Eichhornia* and *Salvinia*, evaluated in comparable conditions, was in the 25–40% range, much higher than found in the present study. Heller and Weber (1998) observed that along a seasonal cycle, MeHg was 6–48% of total Hg in *Spartina alterniflora* from an estuarine marsh in USA.

Few studies have simultaneously addressed Hg methylation and natural MeHg concentrations. Roulet et al. (1999a,b) determined total Hg and MeHg on replicates of the samples used herein and also in water, epiphyton and plankton. They found that the *igapó* forest and macrophyte floating mats were the only sites where MeHg was significantly detected in water, representing

3–22% of total Hg in filtered water, while in other lentic or lotic sites in the same area < 4% of MeHg was found. The proportion of MeHg to total Hg in the epiphytic material collected from the roots of *Paspalum* sp. floating mats ranged 1.5–8.3% and correlated with the organic C and total N content.

The finding of higher MeHg concentrations in the water column at the same sites of high Hg methylation suggests that MeHg accumulates in these sites and is also an evidence of the bioavailability of MeHg formed in these environments.

Methylation in surface or near-bottom total water was undetectable ( $< 3 \times 10^{-2}\%$ ) at all sites, but further studies on MeHg production in water, with larger samples and a more sensitive method, would be justified by the importance of this compartment to the biota. Concentrations can represent > 60% of total Hg in filtered water (Kelly et al., 1997).

When data on  $^{203}\text{Hg}$  methylation from all cores and depths are plotted against MeHg, total C and N, Fe and Al hydroxides, pH or Eh, no significant correlations appear. Matilainen et al. (1992) studied  $^{203}\text{Hg}$  methylation and demethylation in surface sediments of different lakes in Finland and found significant positive correlations between methylation/demethylation ratios and water pH, sediment Fe and Mn, while negative correlation were found with sediment organic matter and total Hg.

Despite the lack of significant correlations between  $^{203}\text{Hg}$  methylation and the data on MeHg and other sediment and soil parameters in the present study, the vertical variations of MeHg concentrations as well as of  $^{203}\text{Hg}$  methylation in the different cores were similar and, more importantly, both approaches identified the flooded forest soils, the semi-aquatic soils under *Paspalum* sp. mats and the macrophyte mats themselves as main methylation sites. The agreement between trends of net potential Hg methylation and of MeHg suggests that the former approach, despite its inherent limitations (see discussion on that matter by Gilmour and Henry, 1991) is a useful tool in the prediction of environments with higher MeHg.

#### 4. Concluding remarks

In the lower Tapajós valley it was demonstrated that Hg of natural origin accumulates in soils and is released by the weathering caused by erosion, following the colonization of the Tapajós basin (Roulet et al., 1998). Increased erosion, promoted by different anthropogenic activities including agriculture and gold mining activities, leads to the higher transport and deposition of particulate total Hg as measured in the Tapajós river water column (Roulet et al., 1998).

Though the relative contribution of human perturbations in the watershed to an increased production and flux of MeHg to humans through the food chain remains to be evaluated, flooded forests and macrophyte mats are clearly important in the production and bioaccumulation of MeHg, and are also efficient traps for the suspended particles responsible for most Hg transport. Methylation and other aspects of the Hg cycle in these environments should therefore receive more attention, since they appear as essential links between local ecosystems and human health.

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