STUDY OF METHYLATION SITES AND FACTORS IN CONTAMINATED AQUATIC SYSTEMS IN THE AMAZON USING AN OPTIMIZED RADIOCHEMICAL TECHNIQUE - BRAZIL

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1. BACKGROUND AND SCOPE

In the last few years, some new data have brought light on the Hg cycle in the Amazon. Roulet et al (1998 and 1999) found high natural Hg contents in soils and showed that soil erosion, due to agriculture and other human activities, had increased the Hg burdens in aquatic systems. They also showed that, surprisingly, the activity of goldminers on many uupstream affluents of the Tapajós river did not result in downstream gradients in dissolved or particulate Hg. Our own data (Malm et al, 1995, 1997) from long term surveys show little or no reduction in fish or human hair Hg levels in different water basins, despite a 3 to 10-fold decrease in goldmining activities since 1990.

Regardless of the on-going debate on the relative magnitude of natural and man-made Hg sources in the Amazon, Hg is being transported and increasingly accumulated in productive lakes and floodplains in all the Amazon basin, leading riverine populations to unsafe Hg exposure levels. This Hg transport is done mainly in the particulate form, and the floating vegetation is a very efficient particle trap, besides providing support to an abundant periphyton, features that favor MeHg formation and bio-availability.

If the relative importance of the different Hg sources in the Amazon is uncertain, available data clearly show that Hg deposition in sediments and floodplain soils has increased in the recent past (Roulet et al, 1999). Hg concentrations in hair among many riverine communities are near or above the safety limits due to the high fish ingestion rate and cause subtle neurotoxic effects (Lebel et al, 1996). Very little is known about the biogeochemistry of Hg and MeHg in floodplain lakes of the Amazon, despite their importance for most aquatic biota, including most fish species of commercial importance. Few studies have addressed the issue of MeHg in the Amazon aquatic systems. Akagi et al. (1995) and Kherig et al. (1998) measured MeHg in fish and human hair, but data on MeHg in different compartments of flooded areas in the Amazon were obtained only recently (Roulet et al, 1999). In Brazil, studies on Hg methylation using ^{203}Hg focused initially on bottom river and lake sediments (Guimarães et al, 1994, 1995) but the highest net methylation potentials were later found below the water surface, in the submerged roots of the dense floating macrophyte mats, common in tropical rivers and lakes (Guimarães et al, 1998, Mauro et al, 1999, Lemos et al, 1999). Floating meadows are peculiar to the Amazon and are essencial habitats for a varied

fauna, including most fish of commercial importance. Herbaceaous macrophytes respond for 52 % of total primary production in the Amazon floodplain (Forsberg et al, 1993).
We present here data on net potential Hg methylation potentials in surface sediments and in roots or other underwater parts of aquatic macrophytes, lakes and floodplain lakes from different regions of Brazil, mainly in the Pantanal (Cuiaba and Paraguay rivers) and in the Amazon (Negro, Solimoes and Tapajós rivers) but also in coastal and inland lakes in the states of Rio de Janeiro and São Paulo. Our objective is to present characteristics of Hg methylation which might be specific for the tropics or subtropics.

2. METHODS

All samples were incubated in the dark, in Teflon- lined screw-capped 50 ml borosilicate tubes with 30 ml of lake water at in-situ water temperature (22-28 °C). Duplicate or triplicate samples and an acidified control (sample with 1 ml of 4 N HCl) received ^{203}Hg as $^{203}HgCl_2$, obtained from Amersham International, UK. Though different ^{203}Hg lots were used in each study or sampling season, the mass of incubated samples and the Hg additions were calculated to obtain, in each study, similar total added Hg concentrations for the different sample types. These concentrations ranged 40-1600 ng Hg $.g^{-1}$ dry weight and fresh samples were equivalent to 0.5-2 g dry weight. All incubations were started in-situ and within a few hours after sampling. Previous tests showed that under these conditions the MeHg formation in sediment and macrophyte root samples reaches equilibrium within 2-5 days (Guimarães et al, 1995). After 2 to 4 day-long incubations, Hg methylation was stopped by addition of 1 ml of 4 N HCl and the samples were frozen until MeHg extraction, performed within 10 to 45 days after the end of methylation essays. MeHg was leached with 6 ml of 3 M NaBr in 11% H_2SO_4 and 1.5 ml of 0,5 M $CuSO_4$. After one minute shaking, the samples were centrifuged and the supernatant (~25 ml) transferred to 60 ml glass separatory funnels and shaken for 15 minutes with 10 ml of a scintillation cocktail prepared with toluene and the scintillation salts POP (2,5-diphenyloxazole) and POPOP (1,4-bis[5-phenyl-2-oxazolil]-benzene). The aqueous phase was discarded and the overlying toluene layer transferred to glass tubes for centrifugation at 3000 rpm for 1-2 minutes. The clean toluene was shaken in a vial with 0.5 g of anhydrous Na_2SO_4 to remove traces of water containing inorganic ^{203}Hg and then transferred to clean scintillation vials for measurement on a Wallac-LKB 1482 Rackbeta liquid scintillation detector. Counting data were used to calculate $Me^{203}Hg$ as a percentage of total added ^{203}Hg, after correction for decay, extraction efficiency and quenching. The MeHg extraction procedure is a simplified version of the technique proposed by Furutani and Rudd (1980), and is described in more detail in Guimarães et al. (1995). It allows performing methylation tests with total Hg additions as low as 40 $ng.g^{-1}$ sample dry weight, if the ^{203}Hg solution has a specific activity of 1mg Hg. mCi^{-1} and is used within one half-life (47 days) after production date. The assumption that all activity in the extracts is $Me^{203}Hg$ was confirmed by thin-layer chromatography (Guimarães et al, 1995). Brito and Guimarães (1999) showed that similar efficiencies are obtained for MeHg extraction from sediment and macrophyte root samples when using this technique or other alternatives such as alkaline digestion, extraction in dithizone/benzene and separation by thin-layer chromatography (Akagi and Nishimura, 1991) or acid distillation (Horvat, 1993).

3. RESULTS AND DISCUSSION

Table 1 presents the data on net Hg methylation potentials in surface sediments and aquatic macrophytes, obtained in experiments made from 1994 to date in 14 different sites in Brazil. The average methylation in macrophytes from all the different study areas was 13.7 %, while in surface sediments it was only 0.6 %. This difference alone suggest that the former are an important methylation site but their importance is probably better measured by the ratio between net methylation potentials in macrophytes and sediments at each site, also shown in Table 1. Part of the range in the methylation data for sediment and macrophytes is due to variations in the amount of added total Hg from one study to another, caused mainly by the use of ^{203}Hg solutions with different ages and belonging to different ^{203}Hg lots. This limits the comparisons between data of different studies because, though the amount of formed MeHg increases when more total Hg is added, the proportion of formed MeHg actually decreases (Jensen and Jernelöv, 1969; Guimarães et al, 1994). However, in each study reported here, the Hg addition to sediment or macrophyte root samples was the same or very similar, and samples were incubated under identical conditions. Therefore the ratios between methylation potentials in these two type of samples are a better parameter for comparisons between different sites or studies. Table 1 shows that, on average, methylation potentials in the submerged parts of the studied macrophytes are 27.5 times higher (range 0.85-113) than in the underlying lake sediments or flooded soils at the same sites. In the Pantanal lakes studied in 1998, methylation in E. azurea roots was on average 58.8 times higher than in sediments (range 20-113).

The percentages of $Me^{203}Hg$ at equilibrium in sediments were in the range found for MeHg as percentage of total Hg in different water systems (see compilation in Roulet et al, 1999). Hg methylation potentials in sediments can be higher than reported here. Brito and Guimarães (1999) found methylation potentials of 8.7 % (N=10) in sediments of a forest stream in Floresta da Tijuca, Rio de Janeiro, and Guimarães et al. (1995) found up to 5.0 % methylation in sediments of the Jamari river, Rondonia, downstream the Samuel reservoir, while methylation in sediments of other rivers in the region was typically lower, in the 0.01-3 % range.

In comparison to bottom or river sediment, few data are available on methylation in flooded soils. The data presented here refer to two contrasting types of soils, which were flooded pasture soils in Tupé lake (Negro river) and C-rich flooded forest soil and semi-aquatic sediment (covered by mats of Paspalum sp., during the wet season) in a Tapajós floodplain lake. The flooded pasture soils showed the lowest methylation (0.022%, Table 1), while in the Tapajós floodplain lake, the flooded forest soil and semi-aquatic sediment, presented respectively up to 4.5 and 7.9 % methylation, (Guimarães et al, 1999). Roulet et al. (1999) and Guimarães et al. (1999) made detailed studies in Enseada Grande lake, Tapajós floodplain, and showed that both Hg methylation potentials and in-situ MeHg concentrations were much higher in flooded forest soil than in lake sediment.

A high methylation (avg. 34 %) was observed in E. azurea from the black water lake Baía Siá Mariana, Pantanal, in 1998. In the other lakes from Pantanal, methylation in this species ranged 1.8-25%. Higher methylation was found in Salvinia rotundifolia than in E. azurea in Fazenda Ipiranga lake, Pantanal, and also in Salvinia sp. from Lagoa do Diogo, São Paulo. In all study areas, E. azurea was much more abundant than Salvinia sp. The roots of E. crassipes are also an efficient methylation site, demonstrated by that up to 33 % MeHg formation was found in incubated root samples from Lagoinha, Rio de Janeiro. This may be related to the

eutrophication of this lagoon, but no data from the other areas are available for comparison, as E. crassipes is not common in these areas.
Less methylation was observed in samples of Paspalum sp. in Catalão lake, Solimões river, than in two Tapajós river floodplain lakes, possibly due to the fact that in the former case the submerged parts were mainly decomposing leaves, while in the latter only submerged roots were incubated.
Different techniques are now available for speciating Hg in different environmental samples, but data on MeHg in aquatic macrophytes are still scarce. Heller and Weber (1998) succeeded to speciate Hg in Spartina alterniflora from the Great Bay estuary, NH, USA, and found that MeHg ranged 6.23-48.1 % of total Hg along the growing season.
Methylmercury formation of such intensity is not expected in the environment unless in similar total Hg concentrations and availability as in the methylation experiments described here. Nevertheless, in the only studies in Brazil were methylation potentials and MeHg concentrations were measured simultaneously (Guimarães et al., 1999; Roulet et al., 1999), higher MeHg concentrations in the water column were found at the sites of higher Hg methylation. MeHg made up 3 to 22 % of total Hg in filtered water sampled in floating Paspalum sp mats. In other lentic or lotic sites in the same area were MeHg levels in water were<4% of total Hg. The proportion of MeHg to total Hg in the epiphytic material collected from the roots of Paspalum sp. ranged 1.5-8.3 % and correlated with the organic C and total N content (Roulet et al., 1999).
This suggests that MeHg accumulates in these sites and is also an evidence of the bioavailability of MeHg formed in these environments. Accordingly, Mauro et al. (1999) observed that upon incubation of Eichhornia crassipes root samples, 20 % of total $Me^{203}Hg$ formed was found in the filtered incubation water, while in parallel incubations of lake sediment, methylation was much less and $Me^{203}Hg$ remained bound to the solid phase.
The submerged roots of floating macrophyte mats are efficient traps for suspended particles and have a high surface area for the fixation of periphyton and bacteria that are directly grazed or enter the detritus pathway. The mildly anoxic conditions in the dense macrophyte mats favor Hg methylation and the root exudates are a carbon source that stimulates bacterial activity. Hg methylation in E. crassipes roots is strongly influenced by temperature, with a maxima at 35 °C and strong inhibition above 50 °C; it is higher at pH 6-7 than above or below this range and also stimulated by low conductivity (Mauro et al, 1999). Experiments with stimulation and inhibition of sulfate-reduction activity suggested that sulfidogen bacteria are the main methylating bacteria in macrophyte roots as well as in sediments (Guimarães et al, 1998; Mauro et al, 1999).
Measurements of Hg methylation potentials in the solids associated to E. azurea roots from four floodplain lakes of the Cuiabá and Paraguay rivers indicated that methylation in root associated solids was lower and more variable (avg. 4.7 %, range <0.3 to 13%, N=8) than in untreated roots (avg. 14%, range 8.3 to 20, N=8). In Baía do Burro, untreated samples of E. azurea roots were incubated in parallel with roots that had been stripped from associated solids by shaking and centrifugation. Methylation was 4 times lower in the latter. The treatment used here to separate the solids from the roots does not remove all root-associated solids and these cannot be quantitatively removed without some damage to the root tissue. The few evidence presented above, though limited to one macrophyte specie in four lakes in the High Pantanal, suggest that Hg methylation in macrophyte roots is carried out by the microorganisms attached to the roots and their diverse associated solids. It is probable, however, that the roots are more than a simple physical support for periphyton and a trap for suspended particles and fine detritus. The production of exudates by the roots and the amount

of decomposing root tissue are affected by the metabolism of the macrophyte and in turn affect microbiological activity and Hg methylation.

4. CONCLUSIONS

A high Hg methylation potential in macrophytes is relevant for many reasons.. This characteristic tropical aquatic vegetation produces highly bioavailable MeHg, because of its high standing stock (1 kg dw. m^{-2}, Sioli, 1986) in direct contact with the water column and very high relative area. Because the root zone of these floating aquatic plants is densely populated by a varied fauna of invertebrates and fish and represents an essential carbon source for aquatic food chains, it may constitute a major pathway of MeHg uptake into tropical aquatic food webs. In contrast, the production of MeHg in surface sediments is ~30 times lower than in macrophyte roots, its bioavailability is probably limited, as well as the sediment-water flux of MeHg.

Moreover, the role of floating meadows as important links in the formation and food-web transfer of MeHg is likely to increase due to the growing exploitation of floodplains in the Amazon and Pantanal, where the burning of forests to create more pasture at the borders of floodplain lakes during the dry season forms new environments to be colonized by macrophytes in the next wet season.

Table 1: Net Hg methylation potentials ($Me^{203}Hg$ as % of total added ^{203}Hg) in surface sediments (0-1 cm) and underwater roots of floating macrophytes in different study areas in Brazil. N = number of replicates. M/S is the ratio between the % methylation in macrophyte samples and in sediment samples.

		% Methylation				
Study area	**Lake (River)**	**Sediment**	**Macrophytes**	**N**	**M/S**	**Reference**
Amazon	Catalão (Solimões)	0.33	0.28 Paspalum sp.	3	0.85	Guimarães et al, 1994
	Tupé (Negro)	0.022	0.047 green algae mat	3	2.1	"
	Unnamed (Tapajós)	0.25	9.9 Paspalum sp.	3	40	"
	E. Grande (Tapajós)	0.41	3.4 Paspalum sp.	6	8.2	Guimarães et al, 1999
Pantanal	B. Paraiso (Paraguay)	0.3	18 E. azurea	2	58	This study
	B. Amolar (Paraguay/Cuiabá)	0.3	5.9 E. azurea	4	20	"
	B. Burro (Cuiabá)	0.3	9.0 E. azurea	6	30	"
	B. Cachorrada (Paraguay)	0.3	19 E. azurea	2	64	"
	B. Chacorore (Cuiabá).	0.3	20 E. azurea	11	68	"

Table 1 (cont.)

Study area	Lake (River)	Sediment	Macrophytes	N	M/S	Reference
	B. Sia Mariana (Cuiabá)	0.3	34 E. azurea	3	113	"
	Fazenda Ipiranga (B. Gomes)	1.16	10 Salvinia rotundifolia[a]	3	9	Guimarães et al, 1998
	Fazenda Ipiranga (B. Gomes).	1.16	6.5 E. azurea	3	5.6	"
Rio de Janeiro	Lagoinha	1	24 E. crassipes	6	24	Mauro et al, 1999
	Lagoinha	-	27 E. crassipes	10	-	Brito & Guimarães, 1999
	Canal do Cortado	-	23 E. crassipes	8	-	Guimarães et al, 1998
	Brejo do Canal de Itaipuaçú	1.4	14 E. sellowiana[b]	6	10	Lemos et al, 1997
São Paulo	L. do Diogo (Mogi-Guaçú)	2.5	25 Salvinia sp.	3	10	Lemos et al, 1999
	"	"	7 E. azurea	12	2.8	"
	"	"	6 Scirpus cubensis	3	2.4	"
Average		**0.6**	**13.8**		**27.5**	

a; Salvinia "roots" are root-like modified submerged fronds b: submerged decomposing leaves.

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