OT-1 Research on Mercury Pollution in Amazon Basin

(2) Quantification of mercury methylation and Bioconcentration and analysis of influencing environmental factors

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Abstract

Methylmercury (MeHg) is the most neurotoxic mercury compound and it is produced from inorganic mercury, through biological and chemical reactions bottom sediments being the main site of this transformation. The objective of this work is to develop model systems to study the speciation of mercury in the Amazon aquatic systems using a radiochemical approach. For this, sediments from the Minamata river, Japan, were collected 8 km upstream Minamata city, spiked with 0.1, 0.5 and 10ppm of divalent ^{203} Hg and incubated in glass cores with an overlying water column. The water column was periodically sampled and analysed for total ^{203}Hg, inorganic and Me^{203}Hg. At the end of the 3week incubation period, sediment were submitted to the same analysis. Total ^{203}Hg was measured directky gamma spectrometry. Inorganic ^{203}Hg and Me^{203}Hg in water, sediment and fish were separated by thin layer chromatography and quantified by gamma spectrometry or liquid scintillation counting depending on the activity level. For this 1g sediment and fish samples were digested in 8ml of KOH in ethanol, slightly acidified and extracted in 0.1% dithizone in benzene. After centrifugation the organic layer was passed through a Florisil column, the eluate was washed in diluted amonia and evaporated to dryness. The residue was then dissolved acetone and applied to silica-gel coated chromatography paper. The plates were

developed in hexane-benzene and the stripes corresponding to mercuric dethizonate and methylmercury dithizonate were cut and counted for ^{203}Hg. For water samples KOH digestion and Florisil filtration were not necessary.

MeHg production in sediment was proportional to time and Hg^{2+} addition but MeHg % (0.5-5.5) was inversely proportional to sediment Hg^{2+}concentration. The same applied to water, where the MeHg percentage was 10 for 10ppm Hg^{2+} addition and 55 for 0.1ppm. The sediment: water partition coefficient for MeHg was in the 60-330range. It was found, however, that MeHg % in water was overestimated because of Hg^{2+} adsorption by the rubber stoppers and the PVC baskets containing the sediments.

The results show that the radiochemical technique applied here allow a fast and reliable evaluation of MeHg production and distribution among water, sediment and biota. Further experiments are in progress to optimize the MeHg extraction and measurement procedures to allow the use of lower Hg addition levels., The technique will be used in the near future in the fellow's home Institute in studies on Hg methylation in sediments of areas of the Amazon watershed affected by goldmining activities.

1. Introduction

Metallic Hg is used in the Brazilian Amazon for gold prospecting by amalgamation and burning, and since the beginning of the goldrush in the late 70's it is estimated that approximately 130 tons of Hg have been released annually in the local environment, in roughly equal amounts to the atmosphere and soils and sediment (Malm et al, 1995). Metallic Hg can be oxidized to Hg^{2+} by different processes and then converted to the most neurotoxic methylmercury (MeHg) by biological and chemical reactions. Surface river and lake sediments are known to be preferential sites of mercury methylation, are recently, flooded forest soils and the roots of floating macrophytes were shown to be very important Hg methylation sites as well (Guimaraes et al, 0996). MeHg analysis (Akagi et al, 1995). as well as methylation experiments (Guimaraes et al, 1995) show that Hg methylation is actively taking place in the Amazonian environment and as a consequence, High total Hg concentrations are found in fish and in hair of local fisherman, frequently above the recommended safety limits (Malm et al, 1995), total Hg in these compartment being essentially MeHg. It is therefore essential to understand the Hg methylation process and its main sites and controlling factors.

2. Objectives

The aims of the present research are to develop and optimize sediment-freshwater model systems and radiochemical analytical technique, to allow the quantification of Hg methylation in sediment, its distribution among sediment and water and bioconcentration, and evaluate the influence, on the aforementionned processes, of relevant environmental parameters.

3. Materials and Methods

Sampling and incubation of sediments with Hg^{2+}

Surface sediments from the Minamata river, Japan were collected in February 1996, 8km upstream Minamata city, and sieved through a 0.8mm sieve to remove the coarse fraction. Some characteristics of the sediments are listed in Table 1. Sediment Volumes of approximately 200ml were spiked with 0.1, 0.5 and 10ppm (dry weigh basis) of divalent Hg containing ^{203}Hg as tracer, carefully homegenized and poured in baskets made of PVC tube, put in the bottom of Jenkins type glass tubes (I.D. 8cm) containing 800ml of Minamata river unfiltered water and stopped at both ends with butyl rubber stoppers. The stoppers were perforated by thin glass tubes for bubbling air in the water column and allowing water sampling without removing the stoppers. The water-sediment systems were incubated at in-situ temperature (11-13℃) and in dim light conditions for 3weeks. The water column was periodically sampled and analysed for total 203 Hg, inorganic ^{203}Hg and $Me^{203}Hg$. At the end of the 3week incubation period, sediments were homegenized and submitted to the same analysis. Total ^{203}Hg was measured directly by gamma spectrometry. Inorganic ^{203}Hg and $Me^{203}Hg$ in water, sediment and fish were separated by thin layer chromatography and quantified by gamma spectrometry or liquid scintillation counting depending on their radioactivity.

Table 1: Characteristics of Minamata river sandy (<0.8mm) sediment

Water content (%)	Organic matter (% of dry weight)	Original total Hg (ng/g of dry weight)
10.7	0.44	9

MeHg and Hg^{2+} extraction and measurement in sediment samples

Sediment samples of 1-2g wet weight were measured for total ^{203}Hg by gamma spectrometry in glass scintillation vials and digested in the same vials by shaking 20-30minutes with 8ml of 1N KOH in etanol. After addition of 5 ml HCL 2.4N, the samples

were shaken for 5minutes with 5ml of 0.1% dithizone in benzene (Dz: Bz), centrifuged at 1000rpm for 5minutes and 1-2ml of the Dz: Bz layer were transferred to another vial and washed with 10ml of 2.5% amonia and centrifuged at 1000rpm for 5minutes. The Dz: Bz layer was dries and cleaned by percolation trough a column made in a disposable Pasteur pipette, containing 0.5g of Florisil topped by 0.5g of anydrous Na_2So_4. A precise volume of the Dz: Bz (usually 0.5-1ml) 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 2-3drops of acetone, and applied with glass capillaries on a thin-layer chromatography plate together with similarly prepared standards of non-radioactive Hg^{2+} and MeHg. The paper was developed for 1-2hours in 1:1 benzene: hexane solvent and the stripes corresponding to Hg^{2+}-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 or liquid scintillation counting.

MeHg extraction and measurement in water samples

Total water samples of 50-75ml were collected in 100ml erlenmeyers, mixed with 5drops of 0.5% $KMnO_4$ and 1ml of 2.4N HCl and extracted in 5ml of 0.1% Dz: Bz by shaking for 5minutes. The aqueous layer was aspirated off, the benzene layer washed with 10ml of 2.5% amonia and 1ml of the benzene was evaporated to dryness, dissolved in a few drops of acetone and submitted to TLC as described above.

4. Results and Discussion

The evolution of MeHg production and partition among sediment and water in all systems is described in Figure 1. MeHg production in both sediment and water increased with time and sediment Hg^{2+} addition (0.1-10ppm range) but MeHg% in sediment and water was inversely proportional to sediment Hg^{2+} concentration and, for the 10ppm spiking level, the increase with time was very small. In water, the MeHg percentage was 10 for 10ppm Hg^{2+} addition and 55 for 0.1ppm. The sediment: water partition coefficient for MeHg was in the 60-330range. It was found by parallel experiments that MeHg as a % of total Hg in water was overestimated because of preferential Hg^{2+} adsorption by the stoppers and the PVC baskets containing the sediments. As a result of the process, sediment: water partition coefficients were also overestimated. In new experiments presently in course, the sediments are put directly on the bottom of the glass tubes and the rubber stoppers are lined with Teflon sheets, that absorb or absorb practically no Hg^{2+} or MeHg.

In the first incubation week, the MeHg production was small in all systems, possibly reflecting acclimation of the natural sediment bacterial community to the added Hg levels. Maximum production occurred in the second week, and after 3weeks of incubation, MeHg production in the sediments seems close to reach an apparent equilibrium. An exemption to this pattern was the 10ppm system, where the MeHg% was low and relatively constant, which may reflect, on one hand, the finite Hg methylating capacity of the sandy sediment and on the other, a possible onset of bacterial Hg poisoning.

The Table 2 displays the same date as Fig. 1, but with equilibrium concentrations of total Hg, Hg^{2+} and MeHg and MeHg%. As mentioned earlier, the date in the 7th and 8th columns are probably overestimated, the others being considered reliable.

Table 2: Mercury distribution in water-sediment system, after 3weeks incubation at in-situ temperature(11-13℃).

Total Hg	Hg in sediment(ppb)			Hg in water(ppt)			Hg sed/Hg water	
	Total	MeHg	%MeHg	Total	MeHg	%MeHg	Total	MeHg
109	106	6.1	5.7	37	21	55	2900	300
509	430	12	2.8	380	210	54	1100	60
10.010	8800	51	0.6	1500	150	10	6000	330

Preliminary results of the experiments presently underway with sediments of the Minamata river containing a higher proportion of fine particles and organic matter, spiked with Hg^{2+} to a concentration of 0.7ppm (dry weight basis) indicated, after 3weeks of incubation, a proportion of MeHg in sediment of 13% and 3% in the water.

5. Conclusions

A simple model system was developed that allows observing Hg behavior in water-sediment-biota systems. An optimizes thin-layer chromatographic technique allowed fast an reliable separation and quantification of organic Hg and MeHg, which permits to detect low Hg methylation rates, and sediment-water MeHg fluxes, even under relatively unfavorable conditions (sandy sediment, low incubation temperatures). In Amazon river and lake sediments, Hg methylation is expected to be more intense, due to higher water temperatures (24-30℃) and sediment organic content. With such