Supporting Information

Quantification of Mercury Bioavailability for Methylation Using Diffusive Gradient in Thin-Film Samplers

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Number of pages: 14

Contents:
Supporting Information Materials and Methods
Supporting Figures: 7
Supporting Table: 1
Materials

High purity water (MilliQ Millipore, >18 MΩ-cm) was used to prepare all stock solutions and for all dilution steps. All sample containers and vials were pre-cleaned by acid washing with 10% HCl solution, or entailed trace-metal clean disposable glassware or plasticware.

Dissolved stock solutions of isotopically enriched HgO powders (National Isotope Development Center, Oak Ridge National Laboratory, USA) were made in 1 mM HNO₃ aqueous solution. Sulfide stock solutions were prepared by dissolving sodium sulfide nonahydrate crystals in MilliQ water that had been bubbled with ultra-high purity N₂. A dissolved stock solution of Suwannee River humic acid (2S101H, International Humic Substances Society) was prepared by adding humic acid powders to NaNO₃ solution (100 mM), bringing to pH 7, and filtering with 0.2 µm nylon filter¹. The total organic carbon (TOC) concentration of the filtered humic acid solution as determined by Shimadzu TOC 500 total organic carbon analyzer was 1008 mg L⁻¹. Dissolved glutathione stock solution (100 mM) was prepared in water that had been deoxygenated by bubbling with ultrapure N₂.

Preparation of Isotopically-labelled Hg Endmembers

The dissolved ²⁰⁴Hg²⁺ endmember added to the slurries entailed a dilution of the original ²⁰⁴Hg stock solution. The dissolved ¹⁹⁶Hg-humic acid endmember stock solution was prepared by adding dissolved ¹⁹⁶Hg²⁺ to a solution of Suwannee River humic acid (pH 7, 100 mM NaNO₃) to a final concentration of 0.40 mM Hg and 80 mg-C L⁻¹ of humic acid. The mixtures were held at room temperature for 9 and 18 hours prior to use in the freshwater and mesohaline microcosm slurries respectively.

The nanoparticulate ²⁰⁰HgS stock solutions were prepared according to Pham et al.² In this method, 50 µM of dissolved ²⁰⁰Hg²⁺ and dissolved sulfide were added to a solution containing 0.1 M NaNO₃, 10 mg C L⁻¹ of dissolved Suwannee River humic acid, and 4 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes) buffered at pH 7.5. The mixtures were allowed to age for 15 and 21 hours prior to use in the mesohaline and freshwater slurry experiments respectively. At this aging period, the Z-average hydrodynamic diameter of the nanoparticle suspension was 38.9 ± 9.5 nm, as determined by light intensity-weighted dynamic light scattering (Zetasizer Nano series, Malvern Instruments). This result is similar to our previously published studies, in which the Z-average diameters were 26 – 39 nm for
nanoparticulate HgS stocks aged for 16-24 hr\(^3,4\). These previous papers also showed that the particles comprised predominantly of aggregates of smaller spheroidal particles (3 to 5 nm diameter) and that the particles had metacinnabar-like crystal structure.

The \(^{199}\)Hg-FeS particle stock solution was prepared by synthesizing a suspension of FeS particles and then adsorbing dissolved \(^{199}\)Hg to the particles. FeS was made by adding equimolar concentrations of ferrous ammonium sulfate hexahydrate solution and sodium sulfide nonahydrate to final concentrations (0.2 M). Both solutions consisted of MilliQ water that was deoxygenated by purging with ultrahigh purity N\(_2\) for 30 minutes. Upon mixing of the ferrous iron and sulfide reagents, a black precipitate was formed, and the mixture was allowed to age under static conditions for 7 and 26 days for the mesohaline and freshwater microcosm slurry experiments respectively. The mixture was then centrifuged at 15600 RCF for 30 minutes at 4\(^\circ\)C. The supernatant was removed by decantation. The precipitate was washed with degassed water. 10 mL of degassed 0.3 M NaCl was added to FeS to form a suspension (19.4 g L\(^{-1}\)). FeS particles synthesized in this manner over a range of relevant FeS aging states were shown to be nanocrystalline aggregates of mackinawite by X-ray diffraction (Figure S1). Enriched \(^{199}\)Hg was added to the FeS particle suspension to a final concentration of 18.5 \(\mu\)M (or 0.95 \(\mu\)mol \(^{199}\)Hg per g FeS) and allowed to sorb on the FeS particles for 21 hr. Aliquots of this suspension were subsequently transferred to each sediment slurry jar. In filtered supernatant of this \(^{199}\)Hg-FeS suspension (15600 RCF for 30 min, 0.22 \(\mu\)m PES filter), less than 0.1% of the added \(^{199}\)Hg was measured in the aqueous phase, indicating that most of the \(^{199}\)Hg in the suspension was associated with the solid phase. The aggregate FeS size was not directly quantified. However, the aggregates were likely larger than 0.22 \(\mu\)m because of the removal of Hg from the \(^{199}\)Hg-FeS solution after centrifugation/filtration.
Figure S1. X-ray diffraction spectra of iron sulfide suspensions that were aged for either 7 or 26 days. The spectra indicates that the particles comprised primarily of nanocrystalline mackinawite (7 - 8 nm crystallite diameter by application of the Scherrer formula to the peak at 16.9°). After the aging period, the particles were sorbed with $^{199}\text{Hg}$ and then added to the slurry microcosms. The spectra were collected with a Panalytical X’Pert PRO X-ray diffraction system with a Cu k-alpha X-ray tube.

Table S1. The measured isotopic composition of the stock solution for each Hg(II) isotope endmember added to the slurries. The isotopic composition of the native Hg was based on literature values for natural abundance$^5$.

| Hg endmember                  | Hg isotope abundance (%) |                  |                  |                  |                  |                  |                  |
|-------------------------------|--------------------------|------------------|------------------|------------------|------------------|------------------|------------------|
|                               | $^{196}\text{Hg}$ | $^{198}\text{Hg}$ | $^{199}\text{Hg}$ | $^{200}\text{Hg}$ | $^{201}\text{Hg}$ | $^{202}\text{Hg}$ | $^{204}\text{Hg}$ |
| Native Hg(II) in sediment     | 0.15                     | 9.97             | 16.87            | 23.1             | 13.18            | 29.86            | 6.87             |
| Hg(II)-humic acid             | 13.4                     | 39.78            | 18.30            | 14.0             | 4.98             | 8.31             | 1.24             |
| Hg(II) sorbed to FeS          | 0.009                    | 1.23             | 90.70            | 4.16             | 1.03             | 2.07             | 0.85             |
| Nano-HgS                      | 0.005                    | 0.36             | 1.43             | 94.7             | 1.66             | 1.54             | 0.33             |
| Dissolved Hg(II)              | 0.10                     | 0.23             | 0.40             | 0.58             | 0.43             | 1.44             | 96.8             |
DGT Preparation and Assembly

Preparation and assembly of the DGT samplers were performed as described in Pham et al. and is summarized here. The thiolated silica resin was synthesized by mixing silica resin (5 g), ultrapure water (2 g), ethanol (8 g) and 3-mercaptopropyltrimethoxysilane, and incubating this mixture at 50 °C for 40 hr. This mixture was subsequently washed with ethanol and freeze-dried to remove water/ethanol molecules. The resin was embedded in a polyacrylamide/bis gel by mixing 7.25 mL Milli-Q water, 3.75 mL 40% acrylamide/bis, and 2 g of the thiolated silica. After sonication for 30 seconds, 70 µL of 0.44 M ammonium persulfate and 40 µL tetramethylethylenediamine (Temed) were added to the mixture, and the mixture was poured on glass plates separated with 0.75 mm spacers and laid flat on one side to set for 1 hr. After this period, the thiolated silica resin layer was soaked in 10 mM NaNO₃ and washed twice with this solution in 24 hours before installation in the DGT plastic housing.

The agarose diffusion gel comprised of a 0.75-mm thick layer of agarose (3% w/w). The DGT sampler was assembled by placing the thiolated silica resin on the bottom of a plastic DGT housing (DGT Research Ltd), followed by the diffusive gel, and a 0.45 µm nitrocellulose membrane. The entire assembly was covered with a plastic casing.

Total Hg Analysis, MeHg Analysis, Trace Metal, Major Anion Analysis, and Thiol Extraction Assay

Isotope-specific total Hg content of whole slurry samples was analyzed by first the addition of an internal standard $^{202}$Hg$^{2+}$ (1000 µL of 72 pg µL$^{-1}$) weighed samples (approximately 6.5 g wet weight). These samples were then stored overnight at 4°C. The slurry samples and components of the DGT sampler (thiolated resin layer, agarose gel layer, filter layer) were subsequently extracted by aqua regia digestion at 55 °C on a hot block for 4 hours. After digestion, the samples were diluted with Milli-Q water containing 5% BrCl (v/v). Filtered porewater from the slurry experiments and extracts from the GSH-extraction were also preserved for total Hg analysis by acidification with 5% BrCl (v/v). These acidified samples were stored in the dark at room temperature until analysis.

Total Hg concentration was determined by stannous chloride reduction, cold vapor generation, and amalgamation (MERX-T, Brooks Rand) followed by Hg isotope detection by inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7700). Checks were
conducted to verify that data was within the calibration range of all isotopes measured. In addition to samples from the experiment, a soil certified reference material (San Joaquin soil 2709a) was processed to verify extraction efficiency (hot aqua regia digestion) and measurement accuracy. Our measurements for 3 replicate digestions of the soil reference material resulted an average value of 1.04 ± 0.015 mg kg\(^{-1}\), which is consistent with certified value (0.9 ± 0.2 mg kg\(^{-1}\)). The recovery of the \(^{202}\)Hg internal standard was used to correct all measurements of isotopic endmembers.

MeHg analysis of whole slurry samples was performed with acidified potassium bromide /dichloromethane (DCM) extraction, and followed with ethylation, purge and trap gas chromatography, and detection by ICP-MS\(^6\)\(^8\). Briefly, measured quantities of whole slurry samples (typically 10-15 g) were amended with an isotopically labelled MeHg internal standard (Me\(^{202}\)Hg) and stored overnight in Teflon vials at 4 °C. Then, 5 mL of potassium bromide solution (18%) acidified with sulfuric acid (5%), 1 mL of 1 M copper sulfate solution, and 10 mL DCM were added to the Teflon vials. The mixture was held static at room temperature for 1 hour and then mixed on an end-over-end rotator for an additional 1 hour at 85 rpm. After this mixing period, the sample was centrifuged (15 minutes at 1150 RCF), and 0.5 mL of the DCM supernatant was collected with a syringe and dispensed into 20 mL deionized water in amber glass vials. The vials with DCM were then heated in hot block to 70 °C to remove volatile DCM in a fume hood. The solution remaining in the glass vials was bubbled with ultrahigh purity nitrogen for 10 minutes to ensure that all DCM was removed. MeHg analysis was performed according to EPA Method 1630\(^7\) with a Brooks Rand MERX-M automated methylmercury analyzer interfaced with an ICP-MS (Agilent 7700). Each extraction batch was accompanied by a method blank sample and an extraction of reference materials (Wisconsin lake sediment from Brooks Rand Instruments and CC580-Estuarine Sediment). Our measurements of MeHg in Wisconsin lake sediment and CC580-Estuarine Sediment reference materials were 0.223 ± 0.03 ng g\(^{-1}\) and 78 ± 2 ng g\(^{-1}\) respectively, which were consistent with the certified values of 0.2 ± 0.06 ng g\(^{-1}\) and 75 ± 4 ng g\(^{-1}\) respectively.

Aliquots of filtered pore water were acidified with 15.8 M trace metal grade nitric acid and then analyzed for dissolved Fe by ICP-MS. Other aliquots were analyzed for sulfate and nitrate concentration by ion chromatography (Dionex ICS2100 ion chromatograph with AS18 column). Dissolved organic carbon (DOC) content of pore water was analyzed with a Shimadzu Total
Organic Carbon Analyzer (TOC-L). These samples for anion and DOC analysis were not modified after the filtration step and were stored at 4°C until analysis.

The glutathione (GSH)-extractable Hg content was quantified by adding 1 mM GSH (final concentration) to a mixture of 1 g (wet wt) sample of slurry and 10 ml of deionized water, and mixing on an end-over-end rotator for 1 hour in hungry tubes. This is a selective extraction technique developed in the study of Ticknor et al.¹ The slurries were centrifuged and 5 ml of supernatant was collected, filtered with 0.2 µm polyethersulfone membrane filter, and spiked with bromine chloride and analyzed for total Hg as described above.
Figure S2. Methylmercury content (A, B) and Total Hg content (C, D) from each isotopically labelled endmember of inorganic Hg added to the mesohaline microcosms (A, C) and freshwater microcosms (B, D). Each time point represents the average (± std. error) of 4 or 3 replicate slurries for the mesohaline and freshwater experiments, respectively. The values are based on measurements of whole wet slurry of each replicate jar (sacrificially sampled at that time point) and are reported on dry mass basis.
Figure S3. The percentage of the total Hg from each isotopically-labelled endmember accumulated on top-layer components of the DGT in each slurry microcosm, including (A, B) the 0.45 µm nitrocellulose membrane filter layer and (C, D) the agarose diffusion layer. Each time point represents the average of 4 replicates for the mesohaline microcosms (A, C) and 3 replicates for the freshwater microcosms (B, D).
Figure S4. (A) Dissolved sulfate and (B) dissolved Fe concentrations in filtered porewater (<0.2 µm) of slurry microcosms after the addition of isotopically-labelled Hg spikes. Note that the inset graph for (A) and (B) shows the same data plotted with different scales for the vertical axis. Each data point represents the average (± 1 standard error) of 4 replicate mesohaline microcosms and 3 replicate freshwater microcosms. Error bars not shown are smaller than the symbols.
Figure S5. Principal coordinate analysis (PCoA) of taxonomy using Bray-Curtis distances for mesohaline and freshwater samples from various time points. Mesohaline samples collected in quadruplicate at t = 0.5, 1, 2, 3, 4 days, Freshwater samples collected in triplicate at t = 1, 2, 3, 5, and 7 days. Analysis performed in MEGAN6.9
Figure S6. Taxonomy profile for the top 15 operational taxonomic units (OTUs) at the class level for mesohaline and freshwater samples. Relative abundances based on the total number of bacterial and archaeal reads. Average relative abundances are provided for each time point. Error bars represent one standard deviation.
Figure S7. $hgcA$ abundance for the Deltaproteobacteria (top graph) and Methanogens (bottom graph) in DNA extracts (5 ng) of the mesohaline and freshwater slurry experiments. Lower limits of quantification are $<10^5$ and $<10^3$ $hgcA$ copies per sample (of 5 ng DNA) for the Deltaprobacteria and Methanogens, respectively. Gene abundances for $hgcA^+$ Firmicutes were below detection for both experiments ($< 100$ copies per sample)
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