Simulation of the Chemical Fate and Bioavailability of Liquid Elemental Mercury Drops from Gold Mining in Amazonian Freshwater Systems

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Elemental mercury (Hg⁰) for gold amalgamation is the main process applied by artisanal gold miners in South America, leading to important discharges into freshwater ecosystems. Through a 28-day experimental approach based on indoor microcosms, we simulated the chemical fate and bioavailability of Hg⁰ droplets in the presence or absence of sediment collected from a typical forest creek that is unaffected by gold mining activities. Our results clearly showed significant mercury transfers in the water column in unaffected by gold mining activities. After 4°, elemental mercury (Hg⁰) for gold amalgamation is the main process applied by artisanal gold miners in South America, leading to important discharges into freshwater ecosystems. Through a 28-day experimental approach based on indoor microcosms, we simulated the chemical fate and bioavailability of Hg⁰ droplets in the presence or absence of sediment collected from a typical forest creek that is unaffected by gold mining activities. Our results clearly showed significant mercury transfers in the water column in both the dissolved gaseous Hg⁰ and oxidized (Hg(II)) forms, with a marked effect of the presence of sediment. After 28 days, Hg total (HgT) concentration in the water column was 25 times higher in sediment-free units (108 ± 17 vs 4 ± 0.4 nM). Methylmercury (MeHg) determinations in the dissolved fraction showed a significant increase only in the presence of sediment after 7 and 14 days. Zebrafish (Danio rerio) were used as indicators for mercury bioavailability. The HgT determinations in four organs revealed significant accumulation levels as early as 7 days exposure, with marked differences in favor of fish collected from the sediment-free units. Significant MeHg increases were observed in the four organs only when sediment was present. Genomic tools applied to estimate sulfate-reducing bacteria communities showed mercury impacts on their diversity and distribution in the different compartments (water, sediment, biofilm, fish gut).

Introduction

The Amazon region and the countries of the Cordillera are currently the most popular gold mining exploration targets in South America. In recent years, official gold production in French Guiana, from the activities of artisanal gold miners and companies, has jumped from 3.1 to 9.3 tonnes/a (1). Despite the development of clean technologies (e.g., gravimetric method), gold mining using mercury amalgamation is still today the main extraction process, leading to important losses of elemental mercury (Hg⁰) into the environment, both in liquid form, released directly into the soils and freshwater systems, and as vapor, released into the atmosphere during the amalgam burning process (2). It is normally estimated that 1.4 kg of Hg⁰ are used for 1 kg of gold production (3). In addition, considerable soil erosion due to aggressive gold extraction techniques (water jet, bulldozer, etc.) increases metal releases into aquatic systems through eroded soil particles that are naturally enriched with inorganic mercury (Hg(II)) (4, 5). However Hg(II) bound to these particles is not directly available for organisms (6). Chemical speciation and transformations of mercury inputs from gold mining sites may occur and lead to the formation of methylmercury (MeHg), the most toxic form that biomagnifies along the aquatic food webs (7–9). Methylmercury is produced only from inorganic mercury (Hg(II)); this suggests that inputs from soil erosion are directly available for methylation when anoxic conditions are met, whereas Hg⁰ losses must be oxidized before being methylated. Little is known about Hg⁰ oxidation in an aquatic environment. In the oxic, pH, and Eh conditions usually found in these environments, it is generally admitted that mercury droplets present a low reactivity (3, 10). However, in recent years some laboratory experiments have shown that under certain conditions dissolution/oxidation of Hg⁰ droplets may occur. For instance, in the presence of oxygen, an increase in ligand concentrations such as chloride or dissolved organic acid (humic or fulvic acids) enhances these reactions (11–14). On the other hand, in their experiment Amyot et al. (11) showed that, in well-oxygenated brackish waters, oxidation occurs at the droplet surface, with dissolution of oxidized mercury occurring subsequently. So contact surface between Hg⁰ droplets and water seems to play an important role in the rate at which soluble Hg complexes (11) are formed. In natural aquatic systems, Hg⁰ droplets are only found in sediments or at the water—sediment interface. The physicochemical characteristics of these environments may strongly influence dissolution/oxidation reactions and consequently methylation rates, the superficial sediment layers being considered as the main methylation sites in well-oxygenated waters (8, 9).

Within a multidisciplinary research program on the biogeochemical cycle of mercury in French Guiana and toxic effects on human populations (e.g., refs 6, 15), we set up an experimental approach to simulate the chemical fate and bioavailability of the metal from Hg⁰ droplets added to aquatic systems. We used freshwater indoor microcosms, with or without natural sediment. The different mercury chemical forms—dissolved gaseous mercury (DGM), MeHg, total mercury (HgT)—were followed in the dissolved and particulate fractions of the water column during the 28-day experiment, in the control and contaminated experimental units (EUs). Mercury bioavailability was investigated via small zebrafish (Danio rerio) and HgT/MeHg analysis in four organs (gills, liver, skeletal muscle, brain), after 7, 14, and 28 days. Biofilms were also collected from the tank walls to quantify HgT/MeHg accumulation and potential trophic transfer to fish during the experiment. In parallel, we applied genomic tools to estimate the sulfate-reducing bacteria (SRB) communities in the water column, sediments, biofilms, and fish gut content from control and contaminated microcosms. Sulfate-reducing bacteria (SRB) are the main microbial strains known to methylate Hg(II) in aquatic ecosystems (9, 16).
Materials and Methods

Experiment Design. Four experimental conditions were performed in triplicate: control units (no Hg²⁻ added), with and without sediment in the experimental systems; contaminated units, with Hg²⁻ droplets placed at the bottom of the experimental units (EUs), with and without sediment. The 12 microcosms were set up simultaneously within the HYDRECO laboratory located close to Petit-Saut hydroelectric reservoir dam (French Guiana), using polypropylene tanks (41 × 29 × 22 cm). Sediment and water were collected from Maman-Lézard creek, a typical small tropical creek (4 m wide and 1 m deep). No gold mining or other anthropogenic activities were reported on this river or its watershed. In the laboratory, gravel was eliminated and the sediment was sieved (0.5 cm) to ensure a perfect similarity of this compartment in all EUs of the experimental design. After mechanical homogenization, 5 kg of sediment were placed at the bottom of the six EUs with a mixed biotope, to form a 5 cm layer. Sediment used was typical of French Guiana clear waters: it was sandy sediment containing little organic matter (see Table S1 of the Supporting Information, SI).

Next, 15 L of clear water was carefully added to each of the six EUs to avoid disturbances at the sediment surface, and to the six sediment-free units. The evolution of the physicochemical parameters in the water column of the 12 EUs was followed throughout the 28 days of the experiment, with a multiparameter probe (YSI660 XLM). Organic carbon concentrations in the unfiltered (TOC) and filtered (DOC: <0.45 µm) water samples were analyzed by infrared spectroscopy followed by oxidative or acidic digestion of the samples (see Table S2 of the SI). Water samples (50 mL) were collected and immediately filtered through 0.45 µm filters (Millipore, Bedford, MA) for determination of total suspended solids (TSS) (see Table S2 of the SI).

The EUs were exposed to a natural photoperiod and air was permanently bubbled into each unit (aerator RENA 301). Aerators were placed in one corner of the EUs, close to the surface so as not to disturb the sediment and to maintain well-oxygenated conditions, similar to those found in the reference natural freshwater system. A plastic grid was positioned 2 cm above the sediment surface to avoid direct contact between fish and sediment and mercury droplets. Water was added regularly to compensate for losses associated with evaporation and sampling.

Five days after the EUs were set up, six zebrafish (*Danio rerio*), each weighing approximately 1 g (fresh weight, fw) and measuring 2.5 ± 0.3 cm, were added to each unit. Fish were fed every 2 days with artificial food (Dr. Bassleer’s Biofish (M), Telgte, Netherlands). The quantities added corresponded to 2% of the fish biomass per EU.

Two days after the fish were introduced, four drops of 0.2 mL corresponding to a total of 0.8 mL (11 g/EU) of liquid Hg²⁻ were added using a syringe at the four corners of the six contaminated EUs (three sediment-free and three with sediment). Mercury drops were not cleaned with acid; nevertheless no black layer was observed at their surface. This step corresponded to time zero (T0) of the experiment.

Sample Collection. Water samples were collected from the twelve EUs at 2, 6, 10, and 50 h after the Hg²⁻ drops were added and at days 7, 14, and 28. Water (50 mL) was sampled with a sterile syringe and stored in Teflon vials. During sampling, ultra clean techniques were used. Dissolved gaseous mercury (DGM), HgT and HgDT analyses were performed immediately. Filtrations were performed immediately after sampling using a Teflon syringe filter (pore size, 0.45 µm, Millex-LCR, Millipore, Bedford, MA). Water samples for MeHg determinations were acidified with concentrated HCl (0.5% v/v, Merck Suprapur, Damstadt, Germany) and stored at 4 °C in Teflon bottles.

Fish samples were collected after 7, 14, and 28 days. At each sampling time, two fish per EU were collected, representing a total of six fish per experimental condition. At the end of the experiment, fish were immediately frozen in liquid nitrogen to preserve gut bacteria communities. Samples were stored in dry ice until their transfer to our laboratory in Arcachon (South West France) and then frozen at −80 °C.

At the end of the experiment, biofilms that had developed on the walls of each EU were scraped with a clean Teflon scalpel. Sediment cores were collected from the center of each EU and were cut into 3 layers: 0–1; 1–3; and 3–5 cm. Biofilm and sediment samples were immediately frozen in Teflon vials in liquid nitrogen, to preserve bacteria communities, stored in dry ice until their transfer to Arcachon, and then frozen at −80 °C until analysis.

For each EU, 50 mL of water were collected after 28 days using sterile syringes and filtered through 0.2 µm filters (Millipore, Bedford, MA) to sample pelagic microorganisms. Filters were immediately frozen in liquid nitrogen, stored in dry ice until their transfer to Arcachon, and then frozen at −80 °C until analysis.

Mercury Determination. Mercury species in water samples were detected by cold vapor atomic fluorescence spectrometry (CVAFS). Details on the procedure for HgT, HgTD, DGM, and MeHg were given in the section S6 of the SI.

Total Hg concentrations in fish organs and tissues, sediments, and biofilms were determined by flameless atomic absorption spectrometry; MeHg was determined after KOH–methanol extraction by CVAFS, after ethylation, isothermal gas chromatography and pyrolysis (see details in section S6 of the SI).

Microbiological Analyses. At the last sampling time, sulfate-reducing bacteria communities were studied in control and contaminated microcosms with a mixed biotope “water-column–sediment” by T-RFLP analysis (terminal restriction fragment length polymorphism).

Genomic DNA contained in sediment, biofilms, fish guts, and water column filters was extracted using the UltraClean Soil DNA isolation kit (MoBio Laboratories, Carlsbad, CA) according to the manufacturer’s instructions. All extracted genomic DNA samples were stored at −20 °C until further processing.

T-RFLP analysis was performed with a sulfate-reducing specific primer set, 8f-SRB 385 (17). This primer set targeted all microorganisms able to reduce sulfate (mainly sulfate-reducing bacteria). Restriction enzymes used in T-RFLP analysis were *Hae*III and *Hind*III. For each sample, analyses were performed in triplicate to avoid analytical errors and ensure the reproducibility of the method. The size of each fragment was determined according to molecular weight standard TAMRA 500 (Applied Biosystem, Foster City, CA) with an acceptable error of ±1 bp. The T-RFLP profiles were normalized by calculating relative abundance of each T-RF from eight fluorescence intensities. Normalized T-RFLP mean profiles of all samples were compared by principal component analysis (PCA).

Data Treatment. All the results are expressed as means ± standard errors. Comparative studies of data from the different experimental conditions were performed using variance (ANOVA) and covariance (ANCOVA) analyses after checking assumptions (normality and homoscedasticity of the error term). If the assumption was met, the parametric Student t test was applied. If the assumption was not met, the nonparametric Mann–Whitney U test was used. In each test, *p* < 0.05 was considered significant. All statistical investigations were performed using STATISTICA version 6.1.
software. Computations for PCA were performed using SPAD version 5.5 software (Decisia, France).

Results

Comparative Time Series of Mercury Chemical Species in the Water Compartment. No significant evolution in HgT concentrations was observed throughout the experiment in the water column of control units in the presence or absence of sediment (Figure 1): concentrations remained at 0.04 (± 0.01 nM. These concentrations were in the upper range of background HgT values usually found in lakes or streams lacking substantive, on-site anthropogenic or geological sources (9). However these values were in agreement with those usually found in Guianese freshwaters not impacted by gold mining activity (18).

In contaminated EUs, a very marked increase in HgT concentrations was observed during the first hours after the droplets were introduced, both in the presence and absence of sediment. However, sediment appeared to have a strong influence on the kinetics of HgT transfers to the water column. First, HgT concentrations were significantly lower in EUs with sediment, leading to average values 5 times higher in the water of the sediment-free EUs after 10h, viz. 10 (± 3 and 48 (± 14 nM respectively. On the other hand, the presence of sediment appeared to strongly modify the rate of HgT production and transfer to the water. In sediment-free EUs, a quasi-linear increase was observed after 48 h until the end of the experiment, the final concentration being close to 100 nM; in contrast, in the presence of sediment, HgT concentrations in the water column were drastically reduced after 50 h and reached a plateau after 168 h (4 (± 0.4 nM).

In all EUs, dissolved gaseous mercury (DGM) concentrations were very low compared with HgT concentrations (Figure 1). We must emphasize that permanent aeration of the water column close to the surface contributes to significant transfers of DGM from water to atmosphere. This mechanism was similar to the permanent mixing observed in the reference running water system and which contributes to DGM losses to the atmosphere. In the control units, DGM concentrations were around 1.5 pM and represented only 1–2% of HgT, in agreement with DGM fraction usually observed in natural aquatic systems (9, 19). In contaminated EUs, the contribution of DGM to HgT concentrations increased in the first hours following the addition of Hg° droplets: after 2 h, DGM represented 9 and 6% of HgT in the presence and absence of sediment, respectively. This contribution decreased throughout the experiment, representing only 1–2% of HgT concentrations at the last sampling time. As for HgT concentrations, sediment significantly influenced DGM concentrations: maximum values were measured in sediment-free EUs after only 2 h (1.6 against 0.5 nM). The plateau value observed after 168 h was 10 times higher than those observed in the presence of sediment: 0.5 (± 0.3 and 0.06 (± 0.03 nM, respectively.

Throughout the 28 days’ experiment, the evolution of the dissolved fraction (HgTD) was similar to those observed for the unfiltered water samples. This fraction represented 80 and 85% of HgT during the first hours, in presence or absence of sediment. For the last sampling point, these ratios were 75 and 45%, respectively. In the control units, HgTD represented nearly 50% of HgT concentrations during the entire experiment.

Methylmercury concentrations in the dissolved fraction did not differ significantly in the four experimental conditions at the beginning of the experiment (5.1 ± 0.5 pM); they represented approximately 10% of HgT concentrations (Figure 1). These values were in agreement with ratios usually measured in freshwater systems where MeHg represents 1–10% of HgT concentrations (8, 9). In the control units, no marked changes were observed during the experiment, with
a mean value of 4 ± 1.5 pM. In sediment-free EUs, MeHg concentrations showed no significant differences from those reported for controls. On the other hand, significant MeHg production was observed in the presence of sediment: concentrations increased gradually and reached values 5.7 times higher than those measured in the control units after 336 h, viz. 18.5 ± 2.5 and 3.2 ± 0.3 pM, respectively. Subsequently, a considerable decline in MeHg concentrations was observed, with the mean concentration measured at the end of the experiment being not significantly different from the control level, viz. 3.6 ± 1.3 and 3.2 ± 0.3 pM, respectively.

Mercury in Sediment. Total Hg concentrations in the control and contaminated units were determined from cores collected at the end of the experiment (see Figure S4 of the SI). In the control units, concentrations were less than 0.05 nmol.g⁻¹ (dw). Total Hg concentrations in the three layers of the cores collected in the contaminated EUs were significantly higher. A marked vertical gradient was observed: 0.39 ± 0.02 nmol.g⁻¹ (dw) in the superficial layer (0–1 cm); 0.034 ± 0.005 nmol.g⁻¹ between 1 and 3 cm and 0.013 ± 0.001 nmol.g⁻¹ in the bottom layer.

Mercury in Biofilms. A thin biofilm colonized the walls of all EUs, constituting a potential food source for fish. Total Hg concentrations in biofilms from the control units in the presence or absence of sediment showed no significant differences (see Table S3 of the SI): 0.09 ± 0.03 and 0.13 ± 0.05 nmol.g⁻¹ (dry weight, dw), with an average MeHg fraction around 15%. Total Hg concentrations in biofilms from contaminated EUs were markedly higher. Moreover, in the absence of sediment, accumulation levels were 20 times greater: 990 ± 100 and 44.5 ± 8.5 nmol.g⁻¹ (dw), respectively.

In the absence of sediment, no significant difference was observed between MeHg concentrations in biofilms collected from control and contaminated EUs (0.02 ± 0.005 and 0.025 ± 0.015 pmol.g⁻¹ (dw), respectively), whereas MeHg concentrations in the presence of sediment were 100 times higher in contaminated EUs, viz. 2.25 ± 0.1 pmol.g⁻¹ (dw).

Mercury in Fish. No significant increase in HgT concentrations was observed in tissues or organs of control fish during the experiment (Figure 2). Background HgT levels were 0.9 ± 0.05, 0.5 ± 0.03, 1.5 ± 0.06 and 0.5 ± 0.07 nmol.g⁻¹ (dw) in brain, gills, skeletal muscle and liver, respectively. In the EUs where Hg²⁺ drops were added, significant bioaccumulation was observed in the four organs from the first sampling point, with a marked “sediment” negative effect on metal bioavailability. In sediment-free units, the increase was close to linearity during the 28 days’ exposure. At the end of the experiment, maximum HgT concentrations were observed in brain (132 ± 39 nmol.g⁻¹), followed by gills (94 ± 16 nmol.g⁻¹), liver (89 ± 9 nmol.g⁻¹) and skeletal muscle (27.6 ± 6.5 nmol.g⁻¹). When fish were collected from contaminated EUs in the presence of sediment, maximum HgT concentrations were observed in the four organs after 168 h: 13.6 ± 1.6, 13.2 ± 1.3, 16.1 ± 2.2, and 5 ± 1.5 nmol.g⁻¹, respectively. In the brain and skeletal muscle, these levels were maintained until the end of the experiment; in gills and liver, a decrease was observed at 336 h, after which concentrations remained identical until the end of the experiment (8.3 ± 0.6 and 6 ± 0.6 nmol.g⁻¹, respectively).

Methylmercury concentrations in the four organs of fish from the contaminated units were low (Figure 3). In the absence of sediment, no significant differences were observed between the evolution of MeHg concentrations in the four organs of fish from contaminated and control units. In the presence of sediment, gills, and liver of fish exposed to Hg²⁺ droplets presented significantly higher concentrations after 336 h. Average values were, respectively, 2 and 23 times higher than in the control fish. In the brain and skeletal muscle, significant differences were observed at the end of the experiment (×2.5 and ×4, respectively). The highest MeHg
concentration was determined in the muscle (4.4 ± 1.1 nmol.g⁻¹), corresponding to 88% of HgT.

Characterization of Sulfate-Reducing Bacteria (SRB) Communities. For each microcosm compartment collected (water, sediment, biofilm, fish gut content), characteristic T-RFLP profiles were obtained with approximately 30 T-RFs (HaeIII and HinfI digestion). In control EUs, sediment and fish gut content SRB communities were dominated by SRB corresponding to T-RFs 40 and 41 pb, whereas SRB strains characterized by T-RFs 37, 38, and 42 pb were the main strains in biofilms (see Figure S5 of the SI). In contaminated EUs, T-RFLP profiles obtained for these same compartments were different from those of control EUs. Sulfate reducing bacteria corresponding to T-RFs 37, 38, and 42 became the dominant strains of sediment and fish gut content, whereas T-RFs 39 and 56 dominated SRB communities of biofilms (see Figure S5 of the SI).

Discussion

Our microcosm experiment simulating small French Guiana clear freshwater creeks showed that exchanges occurred between Hg⁰ droplets and water, leading to a production of DGM and the oxidized form of the metal (Hg(II)). Such reactivity was experimentally observed in brackish water and black Amazonian freshwater, with ligand concentrations in these oxic environments being the main factor enhancing dissolution/oxidation of elemental mercury through dissolution-complexation reaction (11, 12, 20, 21). At the beginning of the experiment (T0), total suspended solids (TSS) and dissolved organic carbon (DOC) concentrations measured in the different EUs were close to 5–15 mg.L⁻¹ and 2 mg.L⁻¹, respectively (see Table S2 of the SI). A rapid increase in these concentrations followed the introduction of fish. Dissolved organic carbon values after 168 h were between 5 and 7.8 mg.L⁻¹ which represents more than 50% of total organic carbon. For TSS, the concentrations measured after 168 h presented a net increase compared with those observed at T0. They reached values higher than 15 mg.L⁻¹ in all EUs (see Table S2 of the SI). These increases suggest that fish excrement and food residue were the main component of DOC and TSS. Using the measured HgT, DOC and TSS concentrations, we calculated (WHAM model, (22)) that 55 ± 10 and 45 ± 10% of HgT were bound to DOC and TSS, respectively. These values were representative of the observed HgT partition between dissolved and particulate phases (30–80 and 20–70% of HgT were measured in these two phases, respectively). In our experimental conditions total suspended solids and DOC appeared as efficient ligands involved in metal dissolution—oxidation and then contributed to maintain the high HgT concentrations observed in the water column in the two experimental conditions.

During our experiment we observed higher HgT, DGM, and HgTD concentrations in the water column of EUs without sediment than in EUs with sediment. These results tend to show that the presence of sediment greatly modified transfer of Hg⁰ from droplets toward the water column and the other compartments (biofilms, fish). First, sediment could act as a sink for newly dissolved—oxidized mercury and thus limit transfers of mercury toward the water column. The significantly higher HgT concentrations measured in the superficial layer of exposed EUs vs control EUs tended to support this hypothesis (see Figure S4 of the SI). On the other hand, we estimated HgT burdens in the different compartments of contaminated EUs after 28 days: in the absence of sediment, 1650 nmol were transferred to the water column, 1000 nmol to biofilms and 80 nmol to fish, a total of 2730 nmol. When sediment was present, these values were 75, 50, and 15 nmol, respectively, with 390 nmol accumulated in the superficial layer of exposed EUs vs control EUs. These simulations, which do not take into account transfers to the atmosphere, clearly show that the presence of sediments drastically reduces global metal transfers from Hg⁰ droplets initially introduced (11 g Hg/ EU): 530 nmol vs 2730 nmol in EUs without sediment. On the other hand, sediment acts as an important sink for mercury, with 75% of the total quantity transferred from the Hg⁰ droplets being accumulated in the superficial layer.
The presence of sediment drastically reduces the contact surface between Hg\(^g\) droplets and water from the beginning of the experiment. After this, a progressive burying of droplets was observed when sediment was present, whereas in sediment-free EUs the swimming activity of fish results in the droplets becoming fractioned, which greatly increases contact surface with water, despite the plastic grids put in place to avoid direct contact between fish and Hg\(^g\) droplets. The sediment, therefore, seems to confer a great stability on Hg\(^g\). In the sediment, interactions between Hg\(^g\)/Hg(II) and iron or manganese oxide-hydroxide and/or sulfide could occur, inducing stable and insoluble mercury complexes confined in superficial sediment layers, in agreement with high Hg\(^T\) concentrations measured in the 0–1 cm sediment layer in our experiment.

**Mercury Methylation.** As methylmercury is produced only from Hg(II), oxidation of Hg\(^g\) is the main limiting step in the production of the organic compound from metallic droplets. However, methylation reactions require special conditions and generally occur via two pathways: microbial metabolism and chemical or abiotic processes. Abiotic methylation mainly occurs in organic-rich aquatic systems, where dissolved organic matter can transfer a CH\(_3\) group to Hg(II) (23, 24). Experimental studies have indeed shown that mercury methylation may occur under aerobic conditions via photochemical pathways, in the presence of a CH\(_3\) donor (25). In our experiment, a net MeHg production was observed only in EUs with sediment. Even if the methylation rate in these EUs was low (MeHg:Hg\(^T\) ratio was close to 0.05%), MeHg concentrations measured after 14 days of experiment were close to those reported for surface water affected by mercury mine drainage (5–22 pM (26)). Physicochemical factors measured in the water column of EUs, especially temperature, pH, TOC/DOC, Eh and light exposure, were similar, with or without the presence of sediment. Thus, we can hypothesize that abiotic methylation and especially photochemical Hg(II) methylation, play a minor role or even no role at all, as mercury methylation is supported mainly by biotic processes.

Microorganisms and especially sulfate-reducing bacteria (SRB) are recognized as the main biological agent of mercury methylation (e.g., refs 9, 16). At the end of our experiment, the genomic tools used to investigate the presence of SRB in EUs containing sediment revealed the presence of such organisms in all studied compartments (water column, biofilms, and sediment), suggesting implication of these strains in mercury methylation. In the aquatic environment, mercury methylation by SRB depends on several abiotic parameters such as redox potential (Eh), pH, DOC concentrations. Usually, Eh conditions compatible with sulfate-reducing activity of SRB were met close to the oxic–anoxic freshwater interfaces, which are present in the first superficial layers of sediment or in biofilms, the two freshwater compartments identified as the main sites of mercury methylation (27). Although SRB were found in these two compartments (see Figure S5 of the SI), our results clearly showed that in our conditions, sediment was the main location of mercury methylation. This finding was supported by the fact that MeHg concentrations in controls and sediment-free EUs were identical, whereas MeHg concentrations in biofilms increased significantly only in the presence of sediment. This last result contrasted with other works which emphasize the importance of biofilms as methylation sites for mercury in Amazonian aquatic systems (27). Nevertheless, it should be stressed that biofilms developed on the tank walls during our experiment were thin newly formed biofilms, with different abiotic and biotic conditions from those in the old, thick biofilms usually observed in natural conditions.

It is interesting to point out that the MeHg concentrations measured in the water column during the 28 days’ experiment evolved to those reported in Akagi’s experiment (28). After reintroduction of fish to the experimental system, an increase in methylation rate was observed. This last increase was probably due to indirect reintroduction of organic matter through fish activity.

**Mercury Uptake by Fish.** Our results clearly show that Hg\(^g\) droplets are able to induce significant transfers of the
metal to zebrafish organs during the 28 days’ exposure in the contaminated EUs. Fish were regularly fed with identical amounts of artificial food in the control and contaminated EUs. Total Hg and MeHg concentrations measured in this food were low: 0.25 nmol.g⁻¹ and 0.24 nmol.g⁻¹, for HgT and MeHg, respectively. Moreover, no significant increase in HgT and MeHg concentrations was observed in the organs of control fish during the experiment, showing that this potential Hg trophic source was negligible. Correlation observed between accumulation levels in gills, the biological barrier involved in metal uptake (freshwater fish do not drink), HgT concentrations in the water column, showed that fish contamination was effectively based on direct transfers from the water column in the presence or absence of sediment. Moreover, the organotropism observed after 28 days is typical of direct exposure to inorganic Hg (31, 32), with the highest concentration in the brain, followed by gills and liver and with distinctly lower values in the skeletal muscle (factor close to 5 compared with brain at the end of the experiment).

Methylmercury determinations in the four organs show significant bioaccumulation only for fish collected from the EUs with sediment. These results are in agreement with those obtained for the water column. Nevertheless, MeHg concentrations in the water of the contaminated microcosms were very low, between 5 and 20 pM, compared with HgT (maximal value close to 100 000 pM), and MeHg bioaccumulation levels represent only a small percentage of HgT. The significant increase in MeHg concentrations observed in gills and liver after 336 h exposure (Figure 3) could be linked to the increase in MeHg concentrations in the dissolved fraction of the water column during the first 14 days (Figure 1). In this case, zebrafish would be exposed via the direct route, combined with inorganic Hg transfers at gill level.

However, Eus with sediment, observation of fish gut content showed that they forage on rich MeHg biofilms (2.2 ± 0.1 nmol.g⁻¹ dw), developed on the tank wall. Thus, fish could be exposed to MeHg trough the trophic route of exposition, as suggested by the significant increase in MeHg concentrations observed in the skeletal muscle and brain at the end of experiment. Indeed, these two organs are the most important storage compartment of MeHg after a trophic exposition. Inorganic Hg methylation could also take place in the gut of zebrafish, as SRB were detected in this compartment.

Total Hg and MeHg concentrations measured in skeletal muscle of fish at the end of experiment were in the same range of values that those reported for herbivorous and omnivorous fish collected in Guianese Rivers impacted by gold mining (6). Effectively, as addition of artificial fish food could minimize the impact of trophic route of exposure in our experiment, incorporation of terrestrial items which are characterized by low HgT and MeHg concentrations, minimizes the trophic exposition of herbivorous and omnivorous fish in tropical rivers (33).

This experimental approach simulating the chemical fate of metallic mercury in well-oxygenated waters in French Guiana revealed the existence of transfers between Hg⁹⁹⁹ dropslets and water column, leading to formation of Hg(II) and subsequently of MeHg. This experiment showed the conflicting roles of sediment: on the one hand this compartment trapped and stabilized mercury droplets, limiting dissolution—oxidation processes and also fish exposure. On the other hand, sediment appeared to be the main site of Hg(II) methylation via SRB activity. So goldmining sites, where metallic mercury losses occur in conjunction with organic matter releases and where sediments are subjected to considerable anthropogenic disturbances, appear to be sensitive sites for metallic mercury dissolution—oxidation. Disturbed sediment at those sites appears less sensitive in terms of MeHg production. However, gold mining disturbances of river sediment led to an important remobilization of Hg(II) complexes from the sediment to the particulate phase of the water column (5), allowing their transport to lentic zones, such as hydroelectric reservoirs or floodplains, which are often located downstream from goldmining sites in the Amazon basin and which represent more favorable sites for Hg(II) methylation and its subsequent accumulation by organisms (6).

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Supporting Information Available
Additional analytical details, data tables, and figures. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

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