Mercury Speciation and Microbial Transformations in Mine Wastes, Stream Sediments, and Surface Waters at the Almadén Mining District, Spain

JOHN E. GRAY,^{*,†} MARK E. HINES,[‡]
PABLO L. HIGUERAS,[§]
ISAAC ADATTO,[‡] AND
BRENDA K. LASORSA^{||}

U.S. Geological Survey, P.O. Box 25046, MS 973,
Denver, Colorado 80225, University of Massachusetts Lowell,
Department of Biological Sciences,
Lowell, Massachusetts 01854, Universidad de Castilla-La Mancha, Plaza M. Meca 1, 13400 Almadén, Spain, and
Battelle Marine Sciences Laboratory,
1529 West Sequim Bay Road, Sequim, Washington 98382

Speciation of Hg and conversion to methyl-Hg were evaluated in mine wastes, sediments, and water collected from the Almadén District, Spain, the world's largest Hg producing region. Our data for methyl-Hg, a neurotoxin hazardous to humans, are the first reported for sediment and water from the Almadén area. Concentrations of Hg and methyl-Hg in mine waste, sediment, and water from Almadén are among the highest found at Hg mines worldwide. Mine wastes from Almadén contain highly elevated Hg concentrations, ranging from 160 to 34 000 $\mu\text{g/g}$, and methyl-Hg varies from <0.20 to 3100 ng/g. Isotopic tracer methods indicate that mine wastes at one site (Almadenejos) exhibit unusually high rates of Hg-methylation, which correspond with mine wastes containing the highest methyl-Hg concentrations. Streamwater collected near the Almadén mine is also contaminated, containing Hg as high as 13 000 ng/L and methyl-Hg as high as 30 ng/L; corresponding stream sediments contain Hg concentrations as high as 2300 $\mu\text{g/g}$ and methyl-Hg concentrations as high as 82 ng/g. Several streamwaters contain Hg concentrations in excess of the 1000 ng/L World Health Organization (WHO) drinking water standard. Methyl-Hg formation and degradation was rapid in mines wastes and stream sediments demonstrating the dynamic nature of Hg cycling. These data indicate substantial downstream transport of Hg from the Almadén mine and significant conversion to methyl-Hg in the surface environment.

Introduction

Mining of Hg worldwide has steadily declined because of low prices and low demand and as a result of environmental concerns over the highly toxic nature of Hg. Almadén, Spain,

is the world's largest Hg mining district and has produced over 285 000 t of Hg (about 8 300 000 flasks) during more than 2000 yr of mining. Production from Almadén represents more than 30% of the total known Hg produced throughout the world (1, 2). Mining activity at the Almadén mine ceased in May 2002, but significant quantities of ore were stockpiled prior to that time. Retorting of ore was discontinued at Almadén in February 2004 following a breakdown of the retorts. It is presently unclear if mining or retorting of ore at Almadén will resume, but any future mining or retorting will depend on the price and demand of Hg.

Similar to most Hg mines, Hg ore at Almadén is dominantly cinnabar (HgS), but elemental Hg (Hg^0) is also present and in some deposits it is locally abundant (Figure 1). As a result of the Hg ore retorting process, most Hg compounds are converted to Hg^0 . A significant part of this Hg^0 is lost to the surrounding environment or remains in mine wastes that are commonly discarded on site. As much as 25% of the total Hg recovered from some Hg mines may be lost, contaminating surrounding environments (3). Even after many years of inactivity, areas of Hg mining contain highly elevated Hg concentrations primarily as a result of inefficient mining procedures and incomplete extraction of Hg, and Hg contamination can be significant over 100 km from the source (3–7). In addition, highly reactive, water-soluble ionic Hg salts such as calomel (Hg_2Cl_2) and mercury oxychlorides (i.e., Hg_2ClO or $\text{Hg}_4\text{Cl}_2\text{O}$), which are probably formed during Hg extraction, have been observed in Hg mine wastes (8). Due to the large size of the Almadén District, Hg emissions and downstream dispersion has potentially contaminated a significant part of the surrounding environment. Numerous Hg mine sites worldwide have been studied for effects to surrounding environments (e.g., refs 3–5, 7, and 8), but relatively little environmental research has been carried out at Almadén (6, 9, 10).

There is continuing concern, and abundant ongoing research, related to natural and anthropogenic Hg contamination of atmospheric, terrestrial, aquatic, and biological ecosystems. The U.S. Environmental Protection Agency (U.S. EPA) has indicated that, of all pollutants included in the Clean Air Act, Hg has the greatest potential to impact human health (11). The greatest potential threat of Hg to human health is conversion of inorganic Hg (such as Hg^0 and Hg^{2+} in contaminated mine sites) to highly toxic organic Hg compounds such as methyl-Hg (CH_3Hg^+). Mercury methylation is primarily the result of microbial activity that is more common in anaerobic, organic-rich environments (11). Methyl-Hg is water soluble, is toxic to all organisms, and is a neurotoxin that damages the central nervous system in humans (12, 13).

Sites of past and active Hg mining worldwide are of environmental concern because of toxic Hg compounds in discarded mine wastes at these sites. The most significant environmental concerns of Hg mines include downstream transport of Hg in sediment and water as well as chemical and microbial transformations of inorganic Hg to highly toxic bioavailable compounds such as methyl-Hg, which are readily transferred from the sediment column, to water, and then to biota. The exceptionally long history of mining and abundant Hg production has produced significant deposits of mine-waste calcine (retorted ore) in the Almadén area that potentially leach Hg into the surrounding environment. Previous studies at Almadén have not measured Hg species such as methyl-Hg in mine-waste calcines, sediment, or water in the mine area, and thus, ecosystem effects related to the mine are not well constrained. The purpose of this study was

* Corresponding author phone: (303)236-2446; fax: (303)236-3200; e-mail: jgray@usgs.gov.

[†] U.S. Geological Survey.

[‡] University of Massachusetts Lowell.

[§] Universidad de Castilla-La Mancha.

^{||} Battelle Marine Sciences Laboratory.

FIGURE 1. Cinnabar (HgS , red mineral) is the dominant ore mineral at the Almadén mine, whereas elemental Hg (Hg^0 , gray beads) is abundant locally.

FIGURE 2. Location of samples for sediment, water, and mine-waste calcine collected in this study. Sample site localities (e.g., V1, V2, etc.) are shown for reference.

to evaluate Hg contamination and transformation to methyl-Hg through the measurement of (i) total Hg, Hg^{2+} , and methyl-Hg concentrations in mine-waste calcines and stream sediments collected in the Almadén area; (ii) Hg and methyl-Hg in surface waters, some of which are drinking water supplies for residents in the region; and (iii) the potential rate of Hg methylation and methyl-Hg demethylation in mine-waste calcines and stream-sediment samples. We also conducted several laboratory water leaching experiments to evaluate the capacity of the mine wastes to release (or leach) Hg during weathering.

Experimental Section

Sample Collection. In June 2003, we collected samples of mine-waste calcine from the Almadén and the Almadenejos retort sites as well as stream sediment and water from several downstream watershed sites (Figure 2). Stream and reservoir sediment samples were collected from surface-layer bed-load alluvium. Mine-waste calcines were collected as grab samples about 25–50 cm below the surface to avoid the highly oxidized near-surface environment. Weather conditions were hot, sunny, and dry (temperatures generally 20–26 °C) during collection of all samples, and there was no rainfall during our sampling or in the month before. All solid samples were stored in glass vessels until analysis, and samples for Hg speciation analysis were immediately frozen until analysis.

Samples for Hg methylation and demethylation rate measurements were refrigerated but not frozen. Prior to geochemical analysis, the stream-sediment and calcine samples were air-dried. Sediment and mine-waste samples were sieved to –80 mesh (0.18 mm) and pulverized.

Water samples were not filtered because comparative reference standards for Hg such as international drinking water and aquatic life standards are established for unfiltered water (14, 15) and there are no clearly established standards for Hg in filtered water. Thus, filtered water samples were not collected in this study. Unfiltered water samples for total Hg and methyl-Hg analysis were collected in precleaned, Teflon bottles, which were acidified on-site with ultrapure HCl using a final acid concentration of 0.5% v/v. Unfiltered water samples for cation determinations were collected in precleaned polypropylene bottles and acidified on-site with ultrapure HNO_3 . Water parameters such as pH, conductivity, total dissolved solids, alkalinity, turbidity, temperature, Fe^{2+} , oxidation–reduction potential, and dissolved oxygen (data not shown) were measured at each sample site using portable meters, field test kits, or a Hydrolab instrument.

Laboratory water leaching experiments were also carried out on samples of mine-waste calcine using the U.S. EPA 1312 Synthetic Precipitation Leaching Procedure (SPLP) (16). For these tests, we collected 19 grab samples from mine-waste piles near the Almadén mine ($n = 15$) and the Almadenejos retort site ($n = 4$). These samples were air-dried and sieved to –9.5 mm prior to leaching. Following the 1312 SPLP method, 100 g of sample was leached with 2 L of deionized water acidified to pH 4.2, and then the samples were rotated at 28 rpm for 18 h. Our only modification of the 1312 method was that the leachate was extracted and filtered to 0.45- μm , rather than with a 0.7- μm filter used in the 1312 method. Both unfiltered and filtered leachate samples were analyzed for total Hg and other trace-elements.

Sample Analysis. Major and trace elements were measured in sediment, water, and leachate samples by inductively coupled plasma-mass spectrometry. Total Hg and methyl-Hg were determined in the sediment, water, and leachate samples using cold-vapor atomic fluorescence (17, 18). Measurement of total Hg followed U.S. EPA Method 1631, and analysis of methyl-Hg followed U.S. EPA draft Method 1630 (18). Total Hg, Hg^{2+} , and methyl-Hg determinations in sediment and water were performed by Battelle Marine Science Laboratory in Sequim, WA. Total Hg concentrations in leachate samples during leaching studies were determined at the U.S. Geological Survey, Denver, CO (USGS). During methyl-Hg analysis, the sediment samples were extracted into methylene chloride during digestion to avoid possible methylation artifact effects (19). Measurement of Hg^{2+} (ionic Hg) was also made using cold-vapor atomic fluorescence following cold-leaching of the sediment samples for 24 h with 1 N HCl (17). This operationally defined technique liberates most Hg not bound in cinnabar or elemental Hg. Quality control for geochemical analyses, including Hg and methyl-Hg determinations, was addressed with method blanks, blank spikes, matrix spikes, certified reference materials, and blind duplicates. Recoveries on blank and matrix spikes were 92–121% for total Hg. The relative percent difference was $\leq 11\%$ from certified values on standard reference materials for total Hg in sediment and water (sediment standards NIST-2704 and PACS-2; water standard 1641d). Water samples were corrected for distillation recovery efficiency, which averaged 88%. Recovery efficiency was 100% for sediments. Field and method blanks were below the limits of determination for total Hg. Limits of determination were 0.002 $\mu\text{g/g}$ for total Hg, 0.005 $\mu\text{g/g}$ for Hg^{2+} , and 0.2 ng/g for methyl-Hg in sediment samples and 0.3 ng/L for total Hg and 0.02 ng/L for methyl-Hg in water samples.

TABLE 1. Mercury Speciation Data for Hg Mines Worldwide, Comparative Baselines, and Regulatory Standards

location	unfiltered water		sediments, calcine, or soil		Hg ²⁺ (μg/g)
	Hg (ng/L)	methyl-Hg (ng/L)	Hg (μg/g)	methyl-Hg (ng/g)	
Almadén Hg District, Spain ^a (6, 9)					
downstream from mines	7.6–13 000	0.41–30	3.0–2300	0.32–82	<0.005–17
mine-waste calcines			160–34 000	<0.2–3,100	0.97–11,000
soils			6–8889		
mine-pit lake	2200–2800	0.040–0.065	935	3.0	17
Idrija Hg Mine, Slovenia (3, 29, 30)					
downstream from mines	6.0–322	0.01–0.6	0.77–1347	0.01–11	
mine-waste calcine			10–727	6.5–14	
Hg Mines, California (21, 31)					
downstream from mines	1400–19 000	0.96–4.5	0.4–220	1.1–150	
mine-waste calcines	2–450 000	<0.003–47	10–1500		
Hg Mines, Southwest Alaska (4, 22)					
downstream from mines	1.0–2500	0.01–1.2	0.90–5500	0.05–31	0.01–34
mine-waste calcine and soil			3.5–46 000	0.2–41	0.01–483
Hg Mines, Nevada (32)					
downstream from mines	6.0–2000	0.039–0.92	0.17–170	0.12–0.95	
mine-waste calcine			14–14 000	<0.05–96	
Palawan Hg Mine, Philippines (33)					
downstream from mines	170–330	<0.02–0.33	3.7–15	0.28–3.9	
mine-waste calcine	18 000–31 000	<0.02–1.4	28–660	0.13–3.2	
mine-pit lake	120–940	1.7–3.1	6.9–400	2.0–21	
Comparative Baselines Distal from Mines					
baselines, Almadén, Spain ^a (6)	9.1–43	0.048–0.34	0.36–0.60	0.23–4.3	0.025–0.029
Lake Baikal, Russia (34)	0.14–2.02	0.002–0.161	0.005–0.072		
Lake San Antonio, CA (35)	0.6–1.8				
baselines streams, SW Alaska (4)	0.1–1.4	0.04–0.2	0.02–0.78	0.1–0.3	
uncontaminated streams, Canada (36)			0.01–0.7		
streams and lakes, Antarctica (37)	0.27–1.9	0.02–0.33			
Regulatory Standards					
international drinking water standard (14)	1000				
U.S. EPA drinking water standard (38)	2000				
U.S. EPA, acute aquatic life water standard (15)	2400				
U.S. EPA, chronic aquatic life water standard (15)	12				

^a This study.

The potential rates of methyl-Hg production and consumption were determined using radioisotopic methods (3). For these assays, sample slurries consisting of 3.0 mL of sediment or 3.0 g of calcines or soils diluted with 3.0 mL of water were injected (2.0 μL) with the radiotracers ²⁰³HgCl₂ (0.33 μCi equivalent to 3.6 μg of Hg) and ¹⁴C-methyl-Hg chloride (0.36 μCi equivalent to 1.26 μg of Hg) for methylation and demethylation, respectively, and incubated at room temperature in darkness for 1 d. The amount of Hg added as ²⁰³Hg was equal to or less than ambient total Hg in samples. The ¹⁴C-methyl-Hg added was approximately equal to ambient methyl-Hg (in some calcines) to as much as 1000-fold higher than ambient methyl-Hg (sediments and soils). For most samples, HCl (6 N) was added following incubation to stop the reaction. Samples were tested prior to incubation for the presence of carbonate material by adding HCl and observing CO₂ formation. Demethylation reactions in samples that exhibited significant CO₂ formation were terminated using NaOH (3 N) instead of HCl. For methylation assays, radiolabeled methyl-Hg was extracted twice into toluene following treatment with CuSO₄ and KCl in H₂SO₄. Pooled toluene extracts were dehydrated using anhydrous NaSO₄, and radioactivity was determined by scintillation counting. Demethylation was determined by measuring ¹⁴C in CO₂ and CH₄ using a gas-proportional counter after separation of gases on a chromatographic column. ¹⁴CH₄ was also determined by gas-proportional counting in samples in which the reaction was terminated by NaOH (those containing significant quantities of carbonate minerals). However, following ¹⁴CH₄ measurements, the samples were slowly acidified with HCl, and ¹⁴CO₂ was stripped via a stream of N₂ and trapped in a

mixture of phenethylamine, methanol, and scintillation fluid (3), which was counted.

Results and Discussion

Mine-Waste Calcines. Samples of mine-waste calcines collected from Almadén (*n* = 8) and the Almadenejos retort site (*n* = 2) contain highly elevated concentrations of total Hg ranging from 160 to 34 000 μg/g, which are several orders of magnitude higher than sediments collected from uncontaminated watershed baseline areas in the Almadén region and those worldwide (Table 1). Retorting of Hg ore at temperatures of about 500–700 °C easily converts cinnabar to elemental Hg, the final product of Hg processing; this ease of conversion combined with the unique properties of metallic Hg is the reason Hg has been mined for such a long time. However, retorting of Hg-bearing ore is known to be an inefficient and incomplete process, and as a result, mine-waste calcines found at most Hg mines contain unconverted cinnabar and other Hg compounds formed during processing (5, 8, 20, 21). Calcines in the Almadén area are similar in character to those found at other Hg mines worldwide: they are red-brown in color due to the presence of abundant iron-oxides and contain fine-grained cinnabar and elemental Hg sorbed to particulates and contain several byproduct Hg compounds (8, 9). Highly elevated total Hg concentrations in Almadén calcines (Table 1) are related to the inefficient retorting process and the presence of numerous Hg compounds in these wastes.

Calcines containing the highest total Hg contents were collected from the Almadenejos retort site (11 000 and 34 000 μg/g). The Almadenejos site has been inactive since the 1850s,

TABLE 2. Mercury Transformation Rate Constants (k) and Potential Rates of Hg Transformations in Various Sample Types from the Almadén Mining District, Spain

location	n	Hg transformation constant (% d ⁻¹)		% CH ₄ ^a	methyl-Hg formation ^b (ng g ⁻¹ d ⁻¹)	methyl-Hg degradation ^c (ng g ⁻¹ d ⁻¹)	net formation ^d (ng g ⁻¹ d ⁻¹)
		methylation, k_{meth}	demethylation, k_{deg}				
Almadenejos calcines	2	0.32 (0.12–0.52) ^e	7.8 (0–16)	100	11 300 (9900–13 000)	210 (0–420)	11 100 (9400–13 000)
Almadén calcines	5	0.021 (0–0.10)	5.7 (0–21)	100	2.3 (0–11.3)	0.3 (0–0.7)	2.0 (–0.69 to 11) ^f
Azogado Creek sediments	2	0.88 (0.55–1.2)	31 (9.0–53)	7.5 (0–15)	6.4 (4.3–8.6)	24 (4.8–43)	–18 (–35 to –0.59)
Valdeazogues River sediments	7	3.4 (0.38–13)	4.4 (0.04–17)	22 (0–100)	1.8 (0.04–9.1)	0.11 (0.001–0.68)	1.7 (–0.35 to 9.3)
El Entredicho mine pit	1	0.01	0	na ^g	1.9	0	1.9
Gargantiel Reservoir sediments	1	9.6	3.0	100	2.4	0.13	2.2
soil	1	0.09	9.7	100	14	0.4	13

^a Percent of ¹⁴C recovered as CH₄ during the demethylation of ¹⁴C-methyl-Hg. ^b Product of methylation rate constant times Hg²⁺ concentration.

^c Product of demethylation rate constant times methyl-Hg concentration. ^d Methyl-Hg formation rate minus methyl-Hg degradation rate. ^e Range.

^f Negative numbers indicate net degradation of methyl-Hg. ^g Not applicable.

and the highly elevated Hg results for these calcines suggest that significant Hg was lost during processing at this site, probably as a result of inefficient retorting. In addition, ore that was rich in elemental Hg was also known to have been processed at Almadenejos. Highly elevated Hg concentrations in mine wastes from other Hg mines worldwide are common, but total Hg concentrations in mine-waste calcines from the Almadén District are among the highest previously reported (Table 1). Only soils collected from Hg mines in southwestern Alaska (22) have been reported to contain higher Hg concentrations (46 000 µg/g).

Concentrations of methyl-Hg in the mine wastes are more important than total Hg concentrations simply because methyl-Hg is highly toxic to all organisms. Our methyl-Hg concentrations in mine waste and sediment are the first reported for the Almadén area. Similar to the results for total Hg, methyl-Hg concentrations found in Almadén mine-waste calcines are highly elevated, ranging from <0.2 to 3100 ng/g. The highest methyl-Hg concentrations were found at the Almadenejos retort site (2700 and 3100 ng/g) and are more than an order of magnitude higher than previously reported from other Hg mines (Table 1). Concentrations of Hg²⁺ are also highly elevated in calcines collected from the Almadén area, but especially in samples from the Almadenejos site (1900 and 11 000 µg/g). These data suggest that high concentrations of reactive Hg²⁺ compounds could be bioavailable for microbial transformation of inorganic Hg to methyl-Hg.

Mercury transformation studies confirm the finding that Almadenejos calcines are “hot spots” for Hg methylation. Methylation rate constants (k_{meth}) in Almadenejos samples, determined from the conversion of ²⁰³Hg to methyl-²⁰³Hg, are about an order of magnitude higher than those noted for Almadén calcine samples (Table 2). In fact, methylation rate constants in most of the calcine samples collected at Almadén are below the detection limit of the method (~0.01% d⁻¹), which is not surprising because this material was dry and oxic, conditions generally not conducive to methylation, which is primarily an anaerobic process (23, 24).

Rate constants are used to estimate the rate at which Hg is methylated and demethylated by multiplying by the independently determined Hg²⁺ and methyl-Hg concentrations, respectively. Demethylation destroys methyl-Hg, so demethylation rates are subtracted from methylation data to obtain a potential net methylation rate (Table 2). Because Almadenejos calcines contain about 100-fold more Hg²⁺ than those from Almadén, the potential net methylation rate in Almadenejos calcines is about 5000 times higher than that found at Almadén. Ambient methyl-Hg in the Almadenejos samples is about 300-fold higher than at Almadén. Net

methylation calculations must be viewed with caution because introduced radioisotopes tend to be more bioavailable than ambient species (25, 26). However, high Hg transformation rates are found at sites rich in methyl-Hg (i.e., Almadenejos), indicating that the rates we measured are good indicators of the ability of sites to create and accumulate methyl-Hg.

Although rate constants for demethylation (k_{deg}) are generally higher than methylation (k_{meth}), the quantity of ambient methyl-Hg is low as compared to Hg²⁺, so the estimated rate of demethylation is small and less than 2.0% of the methylation rate. These data suggest that methyl-Hg should be accumulating rapidly and should double in 0.3–5.0 d. This is obviously much faster than what is occurring, and the discrepancy is most likely due to (i) the relative lability of the ²⁰³Hg tracer and (ii) the high concentrations of natural Hg²⁺ that are not as bioavailable as the added tracer. However, the wide differences between sites in rate constants and net rates indicate that material at the Almadenejos mine is easily methylated and that this methyl-Hg accumulates to high concentrations. For example, despite the several orders of magnitude difference in Hg concentrations and in activity rates at the two calcine sites, the ratio of the methylation to methyl-Hg concentration at each was within a factor of 3–6, suggesting that the rate measurements are useful for comparing sites exhibiting extreme differences in Hg speciation and activity. It is also interesting that a natural soil sample collected in the Almadén area displayed low k_{meth} and high k_{deg} like the calcine samples, and it also exhibited a potential rate of methyl-Hg formation that is high relative to the degradation rate. The ratio of methyl-Hg formation/methyl-Hg degradation in the soil sample (Table 1) is similar to that noted in Almadenejos calcines despite the fact that both sites are significantly different in terms of Hg species.

¹⁴C-methyl-Hg was degraded solely to ¹⁴CH₄ in calcine samples from both Almadén and Almadenejos (Table 2), which is indicative of reductive demethylation processes, presumably catalyzed by the *mer* detoxification process (27). The *mer* system is comprised of genetic loci harboring genes conferring resistance to inorganic Hg and, sometimes, organomercurial compounds. The latter occurs via an organomercurial lyase that catalyzes the reductive cleavage of the methyl group of methyl-Hg to CH₄, as opposed to the oxidative demethylation of methyl-Hg that liberates CO₂ (3, 28). Studies suggest that the reductive *mer* mediated demethylation dominates at high Hg concentrations in more aerobic settings, whereas the oxidative demethylation pathway dominates at lower Hg concentrations in more anaerobic settings (25). The calcines we studied are rich in Hg, but it has been shown that oxidizing conditions support the

FIGURE 3. Total Hg concentration in sediments and mine wastes versus distance from the Almadén mine. Sample site localities (e.g., V1, V2, etc.) are the same as shown in Figure 2. Pit-lake sediment is from the El Entredicho mine. Negative distances are those downstream from the Almadén mine; positive distances are those upstream from Almadén.

formation of CH_4 from methyl-Hg even when Hg levels are quite low (3). Indeed, a soil sample containing low Hg collected approximately 100 km from Almadén also yielded only CH_4 from methyl-Hg (data not shown).

On the basis of the data collected, we conclude that the Almadenejos retort site has much higher Hg-methylation than calcines from the Almadén mine site. Our results indicate that significant elemental Hg was lost at this site either through retort stack emissions, spillage, or in discarded mine wastes. Through time, the majority of this elemental Hg was converted to Hg^{2+} compounds, mostly by oxidation, and there are also reactive Hg^{2+} salts present in the mine wastes, which were formed during the retorting process. Previous work at Almadenejos has shown the presence of Hg^0 and Hg^{2+} compounds sorbed to particulates in mine waste (9). At Almadenejos, extremely high concentrations of bioavailable Hg^{2+} have resulted in high microbial Hg-methylation, which is surprising considering that the surface environment where the samples were collected is oxic and generally considered nonconductive to Hg-methylation. We suggest that methylating bacteria were probably dormant when dry but were reactivated when saturated with water during the laboratory methylation experiments.

Stream Sediments. Stream and reservoir sediment samples were collected from sites along the Valdezogues River and its tributaries (Figure 2) to evaluate Hg contamination and Hg methylation in sediments derived from the Almadén mine-waste calcines, which erode into this watershed. Stream sediments collected from Azogado Creek, within about 7 km downstream from waste piles at the Almadén site, contained the highest total Hg concentrations, ranging from 770 to 2,300 $\mu\text{g/g}$ (Figure 3). Such total Hg concentrations are similar to those found in sediments collected downstream from other Hg mines worldwide (Table 1). Few structures are found preventing downstream erosion of mine wastes at the Almadén retort site, and as a result, detrital fragments of calcine and fine-grained cinnabar are found in stream bed material at sample sites on Azogado Creek, indicating that mine wastes are eroding into streams downgradient from Almadén mine-waste piles. However, stream sediments collected more distant from mined areas along the Valdezogues River show significant geochemical dispersion, where total Hg concentrations vary from 2.6 to 36 $\mu\text{g/g}$ (Figure 3). Sediment samples collected from two baseline sites distant from Hg mines in the region contain the lowest total Hg concentrations 0.36 $\mu\text{g/g}$ (upstream on the Valdezogues River, site V9, Figure 3) and 0.60 $\mu\text{g/g}$ (Gargantiel Reservoir, site G1, Figure 3). Total Hg concentrations in these samples are similar to those found in sediments collected from

FIGURE 4. Concentration of total Hg vs methyl-Hg in sediments and calcines collected in the Almadén area. The range of data for Hg mines in Alaska and Nevada are shown for reference.

uncontaminated sites worldwide (Table 1). However, it is probable that the landscape in a significant area around Almadén is affected by Hg because of the wide distribution of Hg mines and the duration of Hg mining in this region.

Stream-sediment samples collected most proximal to mines or mine wastes also contain highly elevated concentrations of methyl-Hg and Hg^{2+} . For example, the three sediments collected from Azogado Creek contain methyl-Hg ranging from 11 to 82 ng/g and Hg^{2+} concentrations ranging from 0.19 to 1.6 $\mu\text{g/g}$. A pit-lake sediment sample collected from El Entredicho mine contained a methyl-Hg concentration of 3.0 ng/g and a Hg^{2+} concentration of 17 $\mu\text{g/g}$. These results indicate significant Hg methylation in areas proximal to the mines. Concentrations of methyl-Hg in stream sediments in the Almadén area are similar to, or higher than, those found in sediment samples collected downstream from other Hg mines worldwide (Table 1, Figure 4). For example, only sediments collected downstream from Hg mines in the California Coast Ranges (31) have been reported to contain higher methyl-Hg concentrations (as much as 150 ng/g; Table 1). Stream sediments collected more distally from mines in the Almadén area along the Valdezogues River contain methyl-Hg concentrations ranging from 0.32 to 4.0 ng/g and Hg^{2+} concentrations ranging from <0.005 to 0.23 $\mu\text{g/g}$. Methyl-Hg and Hg^{2+} concentrations in the Valdezogues River sediments are generally higher than those in the baseline sites, although sediment collected from Gargantiel Reservoir contained a methyl-Hg concentration of 4.3 ng/g, suggesting elevated Hg methylation at this site as well.

The k_{meth} values for sediment samples are up to 1–2 orders of magnitude higher than those noted in most calcines (Table 2). The k_{deg} values in Valdezogues River sediments are similar to those in calcines, whereas k_{deg} values are highest in the Azogado Creek sediments. Sediments sampled were clearly anoxic, which would enhance methylation, whereas demethylation is active under both anaerobic and aerobic conditions (27). Concentrations of Hg^{2+} in Azogado Creek sediments are about 10-fold higher than in Valdezogues River sediments, which accounts for the higher potential methylation rates in Azogado Creek sediments. However, the high k_{deg} values up to 53%/d and the high methyl-Hg concentrations up to 82 ng/g in Azogado Creek result in high methyl-Hg degradation rates leading to an average net degradation of methyl-Hg. Only two of three sediment samples from Azogado Creek yield measurable Hg transformation data, and one of those exhibit high values of k_{deg} (>50%/d) and a high concentration of methyl-Hg (82 ng/g).

The other site yields gross rates of formation and degradation that are nearly equal. Hence, average data reported in Table 2 are somewhat misleading. However, rapid rates of activity in Azogado Creek suggest that methyl-Hg in those sediments is recycled rapidly through methylation and demethylation (39), but the net result is a significant accumulation of methyl-Hg.

Even though Valdezogues River sediments do not transform Hg as rapidly as Azogado Creek sediments, Hg is actively transformed. Like the calcine samples, Valdezogues River sediments yield an average net Hg transformation rate that is similar in magnitude to the pool size of methyl-Hg. In other words, the net quantity of methyl-Hg produced per day is similar to the amount of methyl-Hg measured. In fact, when data from all the sites are examined, the net quantity of methyl-Hg produced per day is within a factor of 4 of the quantity of ambient methyl-Hg. It follows that rate measurements provide data that agree with Hg speciation data and that the formation of methyl-Hg is tightly coupled with its demethylation. The degradation of ^{14}C -methyl-Hg in sediment samples occurs primarily via the oxidative pathway as indicated by the production of $^{14}\text{CO}_2$ (Table 2). Only one sediment sample produced 100% $^{14}\text{CH}_4$ from ^{14}C -methyl-Hg, and this sediment was collected from a reservoir in the Valdezogues River system (Figure 2, site V5). Six of the nine sediment samples analyzed yield <3% CH_4 . These data agree with the assumption that the anaerobic degradation of methyl-Hg usually occurs by an oxidative demethylation pathway (3, 27), as opposed to the reductive path that is evident in oxic soil and calcine samples.

Our data indicate that sediments collected from Azogado Creek and Valdezogues River downstream from mined areas have high potential to transform Hg to methyl-Hg and are host to active Hg-methylators and methyl-Hg demethylators. The stream environments are obviously wet, anoxic, and contain more organic matter than the mine-waste calcines; these conditions are conducive to microbial methylation. Stream-sediment data suggest significant Hg contamination and Hg-methylation along at least 60 km of the Valdezogues River from El Entredicho Reservoir to the La Serena Reservoir (Figures 2 and 3).

Water. Total Hg concentrations are highly elevated in water samples collected from the Azogado Creek ($n = 2$; 11 000 and 13 000 ng/L) and El Entredicho mine-pit lake ($n = 2$; 2200 and 2800 ng/L) and greatly exceed several important water quality standards including: (i) the 1000 ng/L international drinking water standard for Hg (12), (ii) the 2000 ng/L drinking water standard for Hg used in the United States (38), and (iii) the 12 ng/L Hg standard recommended by the U.S. EPA to protect against *chronic* effects to aquatic wildlife (15) (Figure 5). Water from Azogado Creek or El Entredicho pit lake is not a source of public drinking water, but the total Hg concentrations are of great concern because they are several orders of magnitude higher than the standard recommended to protect against chronic effects to aquatic wildlife. Total Hg concentrations in water collected in the Almadén area are also some of the highest reported as compared to other Hg mines throughout the world (Table 1). Conversely, water collected from the baseline sites in the Almadén region contain much lower total Hg concentrations (upstream site on the Valdezogues River, 7.6 ng/L; Gargantiel Reservoir, 9.1 ng/L) and are below all of the water standards for Hg discussed above. One tap-water sample collected from the University of Castilla-La Mancha in the town of Almadén contained 43 ng Hg/L and is well below the 1000 ng/L drinking water standard for Hg established by the WHO. Water collected from the Valdezogues River from sites downstream from mines contain total Hg concentrations ranging from 43 to 161 ng/L and are much lower than those found in water from Azogado Creek (sites within 7 km of Almadén calcines),

FIGURE 5. Concentration of total Hg vs methyl-Hg in surface water collected in the Almadén area. The World Health Organization (WHO) drinking water standard and the U.S. Environmental Protection Agency (U.S. EPA) Hg standard to protect against adverse chronic effects to aquatic life are shown for reference. The range of data for worldwide uncontaminated baselines is also shown for reference.

FIGURE 6. Total Hg concentration in water and leachates of calcines vs distance from the Almadén mine. Sample site localities (e.g., V1, V2, etc.) are the same as shown in Figure 2. The pit-lake sample is from the El Entredicho mine. Negative distances are those downstream from the Almadén mine; positive distances are those upstream from Almadén.

but the concentrations significantly exceed the 12 ng/L U.S. EPA standard for Hg to protect against chronic effects to aquatic life (Figure 6). Total Hg concentrations in water from the Valdezogues River are below drinking water standards for Hg, but the Valdezogues River is also not generally used as a source of public drinking water.

There are no established standards for methyl-Hg in water, but concentrations of methyl-Hg in the water collected from Azogado Creek (4.9 and 30 ng/L) are highly elevated when compared to methyl-Hg in water collected from baseline sites (0.048 and 0.41 ng/L) in the Almadén area. Similar to the results for total Hg, concentrations of methyl-Hg found in water from Azogado Creek are among the highest reported for that observed in water samples collected near other Hg mines worldwide (Table 1). Methyl-Hg concentrations in water collected from the Valdezogues River varied from 0.39 to 1.8 ng/L (Figure 5) and are also generally higher than found in baseline sites in the Almadén area and higher than observed in uncontaminated baseline streams and lakes worldwide (Table 1). The Valdezogues River is a large and important watershed and fishery in the region. Our results suggest significant transference of methyl-Hg from the sediment to the water column in this important ecosystem. Recent studies in the Almadén area reported elevated

concentrations of total Hg and methyl-Hg in freshwater clams in the Valdezogues River and suggest that Hg contamination is being actively transferred to biota (6).

Water was not found flowing through, or directly from, mine-waste calcines at any of the sites studied in the Almadén area. Therefore, water leach experiments of mine-waste calcines were carried out to simulate mine-water runoff, which demonstrates the capacity of the calcines to release Hg into surrounding downstream watersheds during periodic high precipitation events. Some of the leachates produced during the leach experiments contained extremely high concentrations of Hg. For example, calcines collected from waste piles in the Almadén area ($n = 15$) produced leachate concentrations of total Hg varying from 2.2 to 1400 $\mu\text{g/L}$ in unfiltered samples and from 2.0 to 400 $\mu\text{g/L}$ in samples filtered to 0.45 μm . Leachates of calcine samples from the Almadenejos retort site ($n = 4$) were even higher in Hg, ranging from 5.2 to 120 000 $\mu\text{g/L}$ in unfiltered samples and from 3.6 to 120 000 $\mu\text{g/L}$ in samples filtered at 0.45 μm . These leachate concentrations are several orders of magnitude higher in total Hg than observed in natural streamwaters in the Almadén area (e.g., maximum total Hg concentration of 13 $\mu\text{g/L}$ in Azogado Creek water) and are 120 000 times higher than the 1000 ng/L WHO drinking water standard for Hg.

In the leachates from this study, as much as 70% of the total Hg was found in the filtered samples, indicating that water-soluble Hg compounds are present in the calcines or that finely particulate Hg-bearing material or colloids released during leaching passed through the 0.45- μm filter during filtration. Using scanning electron microscopy, we have observed calomel (Hg_2Cl_2), a soluble Hg salt, in mine-waste samples from the Almadenejos site; however, additional studies are needed to quantify the amount of these compounds present in these mine wastes. The total Hg concentrations in the leachates produced in this study probably represent the maximum concentrations that can be obtained by water leaching of the calcines because the U.S. EPA 1312 method is a very rigorous and thorough leaching method.

Over 1 000 000 m^3 of calcines are on the ground surface in the Almadén area, and much more additional mine waste has been back-filled into underground adits, shafts, and tunnels. Using an average Hg concentration for Almadén calcines of about 1000 μg of Hg/g, we calculate over 4000 t of Hg in the Almadén area (excluding the Almadenejos site) that is potentially available for surface leaching and runoff into the surrounding environment. In addition, significant quantities of cinnabar-bearing ore have been stockpiled at Almadén, which may add to the load of Hg available for surface leaching.

The Hg data for Valdezogues River water show natural dilution and attenuation (Figure 6), as do the Hg results for the stream sediments (Figure 3), which is to be expected. However, total Hg concentrations for all water samples collected downstream from mined areas on Azogado Creek and the Valdezogues River exceed the 12 ng/L U.S. EPA aquatic life standard. Methyl-Hg concentrations in these waters are also generally higher than those found in uncontaminated baseline sites worldwide (Figure 5). In addition, Hg contamination in the Valdezogues River and Azogado Creek is widespread, which may have significant adverse effects to biota in this important ecosystem. To further evaluate the potential effects to this aquatic ecosystem, Hg concentrations need to be measured in freshwater fish in the Valdezogues River, and presently, Hg data for such fish are lacking.

Acknowledgments

This study was jointly funded by the U.S. Geological Survey, the U.S. National Science Foundation Grants 99-75407 and 03-22022, and the Spanish Ministry of Science and Technol-

ogy Grant REN2002-02231. Phil Hageman (USGS) provided total Hg analyses of leachate samples. We thank Ian Ridley and Pat Shanks (USGS) and three anonymous *Environ. Sci. Technol.* reviewers for constructive comments that helped to improve the paper.

Literature Cited

- Peabody, C. E. The association of cinnabar and bitumen in mercury deposits of the California Coast Ranges. In *Bitumens in Ore Deposits*; Parnell, J., Kucha, J., Landais, P., Eds.; Springer-Verlag: Berlin, 1993; pp 179–209.
- Hernández, A.; Jébrak, M.; Higuera, P.; Oyarzun, R.; Morata, D.; Munhá, J.; *Miner. Deposita* **1999**, *34*, 539–548.
- Hines, M. E.; Horvat, M.; Faganeli, J.; Bonzongo, J. C.; Barkay, T.; Major, E. B.; Scott, K. J.; Bailey, E. A.; Warwick, J. J.; Lyons, W. B. *Environ. Res. Sect. A* **2000**, *83*, 129–139.
- Gray, J. E.; Theodorakos, P. M.; Bailey, E. A.; Turner, R. R. *Sci. Total Environ.* **2000**, *260*, 21–33.
- Gray, J. E.; Crock, J. G.; Fey, D. L. *Appl. Geochem.* **2002**, *17*, 1069–1079.
- Berzas Nevado, J. J.; Garcia Bermejo, L. F.; Rodriguez Martin-Doimeadios, R. C. *Environ. Pollut.* **2003**, *122*, 261–271.
- Bonzongo, J. C.; Lyons, W. B.; Hines, M. E.; Warwick, J. J.; Faganeli, J.; Horvat, M.; Lechler, P. J.; Miller, J. R. *Geochem. Explor. Environ. Anal.* **2002**, *2*, 111–120.
- Kim, C. S.; Bloom, N. S.; Rytuba, J. J.; Brown, G. E., Jr. *Environ. Sci. Technol.* **2003**, *37*, 5102–5108.
- Higuera, P.; Oyarzun, R.; Biester, H.; Lillo, J.; Lorenzo, S. J. *Geochem. Explor.* **2003**, *80*, 95–104.
- Lindberg, S. E.; Jackson, D. R.; Huckabee, J. W.; Janzen, S. A.; Levin, M. J.; Lund, J. R. *J. Environ. Qual.* **1979**, *8*, 572–578.
- U.S. EPA. *Mercury study report to Congress*, Vols. I–VIII; U.S. Environmental Protection Agency: Washington, DC, 1997; EPA-452/R-97-003.
- WHO. *Environmental Health Criteria No. 1: Mercury*; World Health Organization: Geneva, Switzerland, 1976.
- Clarkson, T. W. *Environ. Toxicol. Chem.* **1990**, *9*, 957–961.
- WHO. *International Standards for Drinking Water*, 3rd ed.; World Health Organization: Geneva, Switzerland, 1971.
- U.S. EPA. Water quality standards; establishment of numeric criteria for priority toxic pollutants; states' compliance; final rule. *Fed. Regist.* **1992**, *40 CFR Part 131*, 57/246, pp 60,847–60,916.
- U.S. EPA. *Test methods for evaluating solid waste, Vols. I and II (SW-846)*, 3rd ed.; U.S. Environmental Protection Agency: Washington, DC, November 1986.
- Bloom, N. S.; Fitzgerald, W. F. *Anal. Chim. Acta* **1988**, *208*, 151–161.
- Bloom, N. S. *Can. J. Fish. Aquat. Sci.* **1989**, *7*, 1131–1140.
- Bloom, N. S.; Colman, J. A.; Barber, L. *Fresenius J. Anal. Chem.* **1997**, *358*, 371–377.
- Biester, H.; Gosar, M.; Muller, G. *J. Geochem. Explor.* **1999**, *65*, 195–204.
- Rytuba, J. J. *Environ. Geol.* **2003**, *43*, 326–338.
- Bailey, E. A.; Gray, J. E.; Theodorakos, P. M. *Geochem. Explor. Environ. Anal.* **2002**, *2*, 275–286.
- Compeau, G. C.; Bartha, R. *Appl. Environ. Microbiol.* **1985**, *50*, 498–502.
- Gilmour, C. C.; Henry, E. A.; Mitchell, R. *Environ. Sci. Technol.* **1992**, *26*, 2281–2287.
- Gilmour, C. C.; Riedel, G. S. *Water Air Soil Pollut.* **1995**, *80*, 747–756.
- Matlock, M. M.; Howerton, B. S.; Atwood, D. A. *Adv. Environ. Res.* **2003**, *7*, 347–352.
- Barkay, T.; Miller, S. M.; Summers, A. O. *FEMS Microbiol. Rev.* **2003**, *27*, 355–384.
- Marvin-DiPasquale, M. C.; Oremland, R. S. *Environ. Sci. Technol.* **1998**, *32*, 2556–2563.
- Gosar, M.; Pirc, S.; Bidovec, M. *J. Geochem. Explor.* **1997**, *58*, 125–131.
- Horvat, M.; Jereb, V.; Fajon, V.; Logar, M.; Kotnik, J.; Faganeli, J.; Hines, M. E.; Bonzongo, J. C. *Geochem., Explor. Environ. Anal.* **2002**, *2*, 287–296.
- Rytuba, J. J. *Sci. Total Environ.* **2000**, *260*, 57–71.
- Gray, J. E.; Crock, J. G.; Lasorsa, B. K. *Geochem. Explor. Environ. Anal.* **2002**, *2*, 143–149.
- Gray, J. E.; Greaves, I. A.; Bustos, D. M.; Krabbenhoft, D. P. *Environ. Geol.* **2003**, *43*, 298–307.

- (34) Leermakers, M.; Meuleman, C.; Baeyens, W. Mercury distribution and fluxes in Lake Baikal. In *Global and Regional Mercury Cycles: Sources, Fluxes and Mass Balances*; Baeyens, W., Ebinghaus, R., Vasiliev, O., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1996; pp 303–315.
- (35) Gill, G. A.; Bruland, K. W. *Environ. Sci. Technol.* **1990**, *24*, 1392–1400.
- (36) Painter, S.; Cameron, E. M.; Allan, R.; Rouse, J. J. *Geochem. Explor.* **1994**, *51*, 213–246.
- (37) Lyons, W. B.; Welch, K. A.; Bonzongo, J. C. *Geophys. Res. Lett.* **1999**, *26*, 2235–2238.
- (38) U.S. EPA. *List of Drinking Water Contaminants and Their Maximum Contaminant Levels*; EPA 816-F-02-013; 2002; www.epa.gov/safewater/mcl.html (accessed 10/20/03).
- (39) Marvin-DiPasquale, M. C.; Agee, J. L.; Bouse, R. M.; Jaffe, B. E. *Environ. Geol.* **2003**, *43*, 260–267.

Received for review February 13, 2004. Revised manuscript received May 6, 2004. Accepted May 25, 2004.

ES040359D