

# Mercury methylation influenced by areas of past mercury mining in the Terlingua district, Southwest Texas, USA

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## Abstract

Speciation and microbial transformation of Hg was studied in mine waste from abandoned Hg mines in SW Texas to evaluate the potential for methyl-Hg production and degradation in mine wastes. In mine waste samples, total Hg, ionic Hg<sup>2+</sup>, Hg<sup>0</sup>, methyl-Hg, organic C, and total S concentrations were measured, various Hg compounds were identified using thermal desorption pyrolysis, and potential rates of Hg methylation and methyl-Hg demethylation were determined using isotopic-tracer methods. These data are the first reported for Hg mines in this region. Total Hg and methyl-Hg concentrations were also determined in stream sediment collected downstream from two of the mines to evaluate transport of Hg and methylation in surrounding ecosystems. Mine waste contains total Hg and methyl-Hg concentrations as high as 19,000 µg/g and 1500 ng/g, respectively, which are among the highest concentrations reported at Hg mines worldwide. Pyrolysis analyses show that mine waste contains variable amounts of cinnabar, metacinnabar, Hg<sup>0</sup>, and Hg sorbed onto particles. Methyl-Hg concentrations in mine waste correlate positively with ionic Hg<sup>2+</sup>, organic C, and total S, which are geochemical parameters that influence processes of Hg cycling and methylation. Net methylation rates were as high as 11,000 ng/g/day, indicating significant microbial Hg methylation at some sites, especially in samples collected inside retorts. Microbially-mediated methyl-Hg demethylation was also observed in many samples, but where both methylation and demethylation were found, the potential rate of methylation was faster. Total Hg concentrations in stream sediment samples were generally below the probable effect concentration of 1.06 µg/g, the Hg concentration above which harmful effects are likely to be observed in sediment dwelling organisms; whereas total Hg concentrations in mine waste samples were found to exceed this concentration, although this is a sediment quality guideline and is not directly applicable to mine waste. Although total Hg and methyl-Hg concentrations are locally high in some mine waste samples, little Hg appears to be exported from these Hg mines in stream sediment primarily due to the arid climate and lack of precipitation and mine runoff in this region.

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## 1. Introduction

Abandoned and inactive Hg mines worldwide are of environmental concern because of toxic Hg compounds present in discarded mine waste at these

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sites. Mercury mines in the United States are not presently in operation, primarily because of low demand for Hg, environmental concerns related to Hg toxicity, and significant recycling of Hg that reduces the demand for Hg (Sznopce and Goonan, 2000). Extraction of Hg during mining is generally carried out in a retort or a rotary furnace where Hg ore is heated to temperatures of about 600–700 °C, which easily converts the Hg ore cinnabar (HgS) to elemental Hg (Hg<sup>0</sup>), which is the final Hg product that is used for commercial trade (Bailey and Phoenix, 1944). This ease of conversion, combined with the unique properties of metallic Hg, is the reason why Hg has been mined for such a long time, more than 2 ka (Higuera et al., 2006). Because the process of Hg recovery involves roasting (calcination), the mine waste generated is referred to as calcine or mine waste calcine. The retorting of Hg-bearing ore is known to be an inefficient and incomplete process, and as a result, mine waste found at most Hg mines contains unconverted cinnabar, Hg<sup>0</sup>, and ionic Hg compounds formed during processing (Kim et al., 2003; Gray et al., 2004). Even after many years of inactivity, areas affected by Hg mining continue to be characterized by highly elevated Hg concentrations as a result of the inefficient and incomplete Hg extraction process (Gosar et al., 1997; Gray et al., 2000). Estimates from some studies indicate as much as 25% loss of Hg to surrounding environments during processing (Gosar et al., 1997). Several of the reactive Hg compounds found in mine waste may adversely affect the environment, especially Hg chlorides, oxychlorides and sulfates, which are water soluble and are potentially released to surrounding environments in mine runoff. Mine waste containing reactive ionic Hg compounds and Hg<sup>0</sup> have also been found to contain elevated methyl-Hg concentrations indicating significant Hg methylation at some mine sites (Gray et al., 2004).

The most significant environmental concerns associated with Hg mines include downstream transport of Hg and chemical and microbial Hg transformation to bioavailable organic compounds such as methyl-Hg (CH<sub>3</sub>Hg<sup>+</sup>), the most toxic of the Hg compounds. Methylation of Hg is primarily the result of microbial activity (Compeau and Bartha, 1985), which is magnified in organic-rich environments, although abiotic, chemical Hg methylation is also known (Ullrich et al., 2001). Methyl-Hg is of special concern because it is water soluble and readily transferred from the

sediment column, to the water column, and then to biota such as fish. Methyl-Hg bioaccumulates in living organisms, its concentration increases with increasing trophic position in the food chain (biomagnification), and it is a human neurotoxin. Conversion and transfer of methyl-Hg from active and inactive Hg mines to surrounding ecosystems is a potential concern worldwide (Gray et al., 2004).

Several inactive Hg mines constitute the Terlingua district, NW of Big Bend National Park (BBNP), Texas (Fig. 1). Another smaller Hg mine, Mariscal, is located in the southern part of BBNP, near the United States border with Mexico. Access to these mines is generally unrestricted, especially in BBNP, and as a result, tourists often visit these sites. The extent of human Hg exposure through inhalation or ingestion of airborne mine waste, a potential consequence of interacting with these sites, is unknown. More importantly, runoff and downstream transport of waste from the mines potentially contaminates streams and rivers in the area, which are located in, or flow through, BBNP and eventually into the Rio Grande (Fig. 1). This study was initiated to evaluate the nature and extent of potential Hg contamination in and around BBNP. Thus, to evaluate Hg contamination and processes of methyl-Hg transformation around these Hg mines, samples of mine waste calcine (retorted ore) from Hg mines in the Terlingua district (Mariposa, Study Butte, and Terlingua mines) and the Mariscal mine in BBNP were collected and analyzed and: (1) total Hg, methyl-Hg, Hg<sup>2+</sup> (ionic mercury), and Hg<sup>0</sup> concentrations, (2) the identity of various Hg compounds, and (3) the potential rate of methyl-Hg formation and destruction, were determined. In addition, because downstream Hg transport can adversely affect surrounding environments, the concentration of total Hg and methyl-Hg in stream sediment collected downstream from the Study Butte and Mariscal mine sites were also measured.

## 2. Study area

The study area is in SW Texas where large, inactive Hg mines of the Terlingua district are found in and around the town of Terlingua. Mercury was mined in the Terlingua region between about 1888 and 1973 (Sharp, 1980; Avery et al., 1996), but these mines are now inactive. Total production from this region was >5000 t of Hg (150,000 flasks of 34.5 kg each) (Sharp, 1980). Consequently, the Terlingua

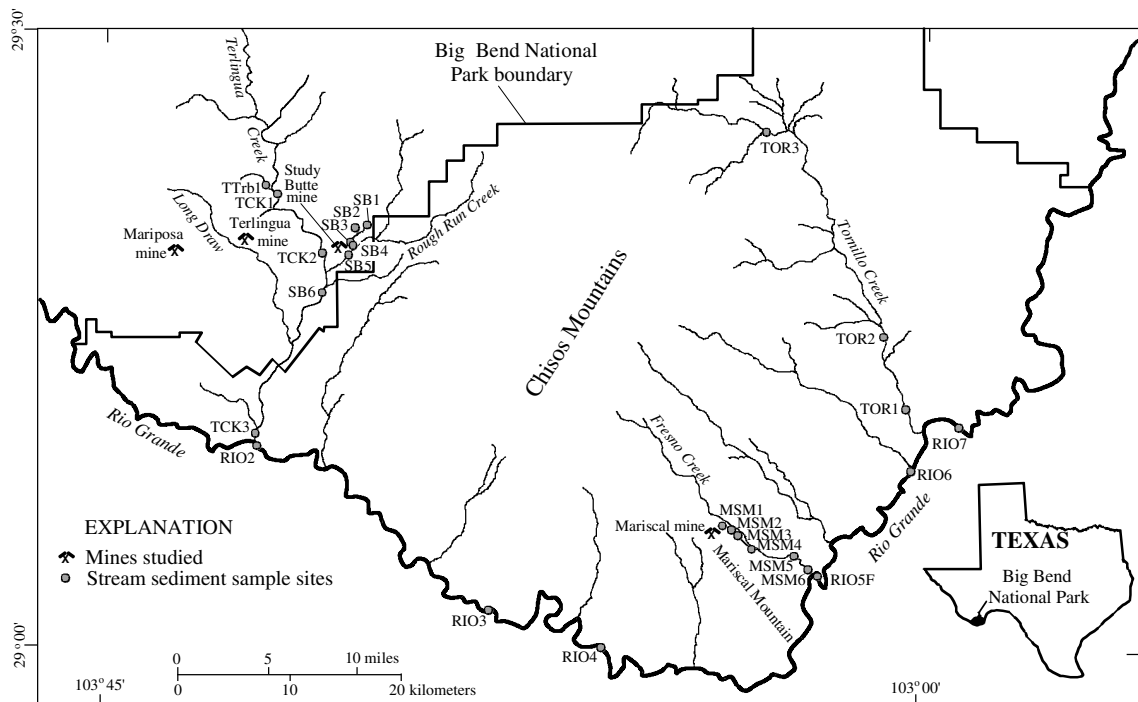


Fig. 1. Location of study area, mines studied, and stream sediment sample sites.

district ranks as the third largest Hg producing district in the United States and only Hg mines in the California Coast Ranges (120,000 t of Hg) and McDermitt, Nevada (10,000 t of Hg) are larger. The Terlingua district includes over 30 separate mines (Ross, 1941), but the present work focused on the Study Butte, Mariposa, and Terlingua (also known as the Chisos mine) mines (Fig. 1). The Mariscal mine, which is of particular importance because it is located in BBNP (Fig. 2), was also studied. Mariscal is a smaller mine and is separate from mines of the Terlingua district. Mariscal produced about 50 t (1400 flasks) of Hg between about 1900 and 1943 and it became part of BBNP when the park was established in 1944 (Avery et al., 1996).

Similar to most Hg mines worldwide, Hg ore at the studied mines is dominantly cinnabar (hexagonal, HgS), but metacinnabar (isometric, HgS), elemental Hg, Hg chlorides such as calomel ( $\text{Hg}_2\text{Cl}_2$ ), and oxychlorides such as Terlinguaite ( $\text{Hg}_2\text{ClO}$ ) and eglestonite ( $\text{Hg}_2\text{Cl}_2\text{O}$ ), were identified at some mines (Ross, 1941; Sharp, 1980). One consequence of Hg mining is that considerable mine waste is generated during ore processing, which is typically discarded on the mine site, and much of this mine waste contains toxic Hg compounds. The authors estimate that over  $2 \times 10^6 \text{ m}^3$  of mine waste remains



Fig. 2. View of the inactive Mariscal Hg mine located in Big Bend National Park. This was the smallest mine studied and it produced about 50 t of Hg during mining from 1900 to 1943. There are presently about  $3 \times 10^4 \text{ m}^3$  of mine waste calcine at the Mariscal mine.

in the Terlingua district and about  $3 \times 10^4 \text{ m}^3$  at the Mariscal mine. This mine waste contains elevated Hg concentrations due to incomplete Hg recovery and generation of Hg byproduct compounds during retorting, some of which are water soluble (Kim et al., 2003; Gray et al., 2004). Mine waste piles in the Terlingua district and at the Mariscal mine

contain significant amounts of Hg available for downstream transport, leaching and biogeochemical transformation to methyl-Hg. Runoff from mines in the Terlingua district (including the Study Butte, Terlingua, and Mariposa mines) potentially transports sediment and water containing various Hg compounds downstream into tributaries that flow into Terlingua Creek, which eventually flows into the Rio Grande. Similarly, runoff from the Mariscal mine is transported downstream into Fresno Creek that flows into the Rio Grande.

The mines studied are in the Chihuahuan desert and, during this study, the climate was hot (26–41 °C), dry, and sunny to partly cloudy. Rainfall in the area averages about 25 cm/a and principally occurs during storm events. Generally, most precipitation in this region falls from July to September, but this is highly variable in time and space. Most of the streams and smaller tributaries in the study area are ephemeral, flowing only for a short period after storm events, and watersheds downstream from both the Study Butte and Mariscal mine are also ephemeral. Neither rainfall nor surface water runoff from these mines was observed during the work. All stream sediment samples collected were dry (except for 01SB3ss that was collected from a damp area in the stream bed) and no water was collected as part of this study.

### 3. Methods

Samples of mine waste calcine were collected in April 2003 from the Mariscal, Mariposa, Study Butte and Terlingua mines (Fig. 1). Calcine was collected as grab samples about 25–50 cm below the surface to avoid the highly-oxidized near-surface environment, and generally at this depth, the calcines changed in color from red-brown to dark gray indicating less oxidized conditions. Calcine samples were collected deeper in the mine waste piles to access moist, potentially anoxic areas with generally more active anaerobic methylating bacteria, and therefore, the highest Hg methylation potential. Thus, the measurements of methyl-Hg concentrations and potential methylation should be considered the maximum at the sites studied. However, it is likely that the vast majority of the waste piles were similar to this deeper material because only surficial material was completely dry. Stream sediment samples were collected upstream and downstream from the Study Butte mine in November 2001, whereas sediment samples were collected

downstream from the Mariscal mine in April 2003 and 2004 (Fig. 1). Stream sediment was also collected from regional baseline sites from Terlingua Creek, Tornillo Creek, and the Rio Grande in April 2004 (Fig. 1). Stream sediment was collected from surface-layer, bed-load alluvium and composited from several localities at each site. All solid samples were stored in glass vessels and samples for Hg speciation analysis were immediately frozen until analysis. Samples for thermal desorption pyrolysis analysis were not preserved. Samples for Hg methylation and demethylation rate measurements were refrigerated, but not frozen. Prior to geochemical analysis, stream sediment and calcine samples were air dried, sieved to –80-mesh (0.18 mm), and pulverized.

The concentration of total Hg was determined in the calcine and sediment samples using an aqua-regia digestion and cold-vapor atomic fluorescence spectrometry (CVAFS) following EPA method 1631 (Bloom and Fitzgerald, 1988). Methyl-Hg analysis follows EPA draft method 1630 using CVAFS (Bloom, 1989). During methyl-Hg analysis, sediment and mine waste samples were extracted into methylene chloride during digestion to avoid possible methylation artifact effects (Bloom et al., 1997). Measurement of  $\text{Hg}^{2+}$  (ionic Hg) was also made using CVAFS following cold-leaching of calcine samples for 18 h with 1 N HCl using a liquid to solid ratio of 10:1 (Bloom and Fitzgerald, 1988). This operationally defined technique liberates most Hg not bound in cinnabar or elemental Hg. Concentrations of  $\text{Hg}^0$  were also determined by an operational method using CVAFS following leaching of calcine with 12 M  $\text{HNO}_3$  for 18 h using a liquid to solid ratio of 10:1; this method leaches both  $\text{Hg}^0$  and  $\text{Hg}^{2+}$ , and thus, concentrations obtained from the  $\text{Hg}^{2+}$  analyses were subtracted from the  $\text{Hg}^0$  determinations to obtain  $\text{Hg}^0$  concentrations. Quality control for total Hg and methyl-Hg analysis was established using method blanks, blank spikes, matrix spikes, standard reference materials (SRM's), and sample duplicates. Recoveries for total Hg on blank and matrix spikes were 93–107%. The relative percent difference in sediment and calcine sample duplicates was  $\leq 18\%$  for total Hg,  $\leq 21\%$  for methyl-Hg, and  $\leq 14\%$  for  $\text{Hg}^{2+}$  and  $\text{Hg}^0$ . For the SRMs, NIST 2704 and PACS-2 analyzed in this study, recoveries ranged from 96% to 107% of certified values for total Hg. Method blanks were below the limits of determination for total Hg and methyl-Hg. Total Hg,  $\text{Hg}^{2+}$ ,  $\text{Hg}^0$ ,



and methyl-Hg determinations in all mine waste calcine and stream sediment samples were performed by Battelle Marine Science Laboratory in Sequim, Washington. The lower limit of determination was 0.005  $\mu\text{g/g}$  for total Hg,  $\text{Hg}^{2+}$  and  $\text{Hg}^0$ , and 0.01  $\text{ng/g}$  for methyl-Hg.

The mine waste calcine and sediment samples were also analyzed for organic C and total S because the process of Hg-methylation depends on these constituents. Organic C was determined by subtracting carbonate C from total C concentrations. Total C was determined using an automated C-analyzer with an infrared detector that measures  $\text{CO}_2$  gas liberated as the sample is combusted at 1370  $^\circ\text{C}$ . Carbonate C was determined by liberating  $\text{CO}_2$  following treatment with 2 N  $\text{HClO}_4$ ; this  $\text{CO}_2$  was collected in a solution of monoethanolamine that is then coulometrically titrated using Pt and Ag/KI electrodes. Total S was determined using an automated S-analyzer equipped with an infrared detector that measures  $\text{SO}_2$  liberated from samples heated in a combustion tube in a stream of  $\text{O}_2$ . The relative percent difference in sediment and calcine sample duplicates was  $\leq 15\%$  for organic C and total S analysis and the lower limit of determination was 0.05%. Organic C and total S determinations in all mine waste calcine and stream sediment samples were performed at the US Geological Survey, Denver, Colorado.

Determination of various Hg compounds in mine waste samples was made by thermal decomposition or thermal desorption pyrolysis analysis (Biester et al., 2000; Sladek et al., 2002). Analysis was carried out at the University of Heidelberg using an electric furnace coupled with a Hg detection cell. Samples were heated continuously at a rate of 0.5  $^\circ\text{C/s}$  to 800  $^\circ\text{C}$  under  $\text{N}_2$ -gas flow of 300 mL/min, and as Hg compounds were converted to  $\text{Hg}^0$ , they were continuously detected by a connected atomic absorption spectrometer. Results for the mine waste samples were compared to those obtained from thermal desorption pyrolysis analysis of several natural and synthetic Hg compounds, including  $\text{Hg}^0$ , Hg sorbed to particulates, cinnabar, metacinnabar, and some Hg salts. The thermal desorption pyrolysis technique used in this study is a qualitative method in which it was possible to determine the presence of a particular compound, but not its relative abundance.

Potential rate constants of methyl-Hg production and destruction (%/day) were determined using radioisotopic methods (Hines et al., 2000). Sample

slurries consisting of 3.0 mL of wet sediment or 3.0 g of dry calcine or sediment diluted with 3.0 mL of water were injected (2.0  $\mu\text{L}$ ) with the radiotracers  $^{203}\text{HgCl}_2$  (0.5  $\mu\text{Ci}$  equivalent to 170 ng Hg) or  $^{14}\text{C}$ -methyl-Hg chloride (0.8  $\mu\text{Ci}$  equivalent to 2.8  $\mu\text{g}$  Hg) for methylation and demethylation, respectively, and incubated at room temperature in darkness for  $18 \pm 2$  h. The methylation/demethylation experiments were run aerobically on dry sediment, where water was added at the beginning of the incubation, because the authors wanted to simulate ambient arid conditions where dry sediment becomes wet during precipitation such as in short duration storm events. Slurries were also used to ensure that radiotracers were evenly mixed in samples. Although this may have influenced microbial processes, it is believed that the uniformity in incubation conditions allowed for a more thorough comparison of data among sites. In addition, preliminary experiments in which dry oxidized samples were incubated as anaerobic slurries revealed no significant changes in Hg transformation activities during the first day of incubation (M.E. Hines, unpublished data).

The amount of Hg added as  $^{203}\text{Hg}$  was several times less than ambient total Hg in samples. The  $^{14}\text{C}$ -methyl-Hg added was approximately equal to ambient methyl-Hg in some instances to over 1000-fold higher than ambient methyl-Hg in others. 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  $\text{CO}_2$  formation. Demethylation reactions in samples that exhibited significant  $\text{CO}_2$  formation were terminated using NaOH (3 N) instead of HCl. For methylation assays, radiolabeled methyl-Hg was extracted twice into toluene following treatment with  $\text{CuSO}_4$  and KCl in  $\text{H}_2\text{SO}_4$ . Pooled toluene extracts were dehydrated using anhydrous  $\text{NaSO}_4$  and radioactivity determined by scintillation counting. Methylation activity, determined from conversion of  $^{203}\text{HgCl}_2$  to methyl- $^{203}\text{Hg}$ , yielded a first order rate constant that is a measure of the percentage of inorganic  $^{203}\text{Hg}$  converted to methyl- $^{203}\text{Hg}$  per time (day). Demethylation was determined by measuring  $^{14}\text{C}$  in  $\text{CO}_2$  and  $\text{CH}_4$  using a gas-proportional counter after separation of gases on a chromatographic column.  $^{14}\text{CH}_4$  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  $^{14}\text{CH}_4$  measurements, these samples were slowly acidified with HCl and  $^{14}\text{CO}_2$  was stripped via a stream of  $\text{N}_2$  and trapped in a mixture of phenethylamine, methanol, and scintillation fluid (Hines et al., 2000) that was counted. Demethylation activity, determined from the conversion of  $^{14}\text{C}$ -methyl-Hg to  $^{14}\text{CO}_2$  and/or  $^{14}\text{CH}_4$ , yielded a first order rate constant that is a measure of the percentage of  $^{14}\text{C}$ -methyl-Hg degraded per time (day). The “first order rate constants” of Hg methylation or methyl-Hg demethylation were converted to “potential rates” by multiplying the potential methylation rate constant (%/day) by the measured  $\text{Hg}^{2+}$  concentration for that sample to obtain the methylation potential rate (ng/g/day) or by multiplying the potential demethylation rate constant (%/day) by the measured methyl-Hg concentration to obtain the demethylation potential rate (ng/g/day).

## 4. Results and discussion

### 4.1. Terlingua district

Total Hg concentrations in mine waste samples collected from the Terlingua district vary widely from 4.1 to 19,000  $\mu\text{g/g}$  and methyl-Hg concentrations vary from 0.051 to 1500 ng/g (Table 1). Mine waste calcine in the Terlingua district also contains highly variable concentrations of  $\text{Hg}^{2+}$ , ranging from 1.5 to 2100  $\mu\text{g/g}$ . Detectable concentrations of  $\text{Hg}^0$  were found in only 5 of 20 calcine samples analyzed (Table 1), the greatest of which is a calcine soot sample collected from inside an abandoned retort at the Terlingua mine with a  $\text{Hg}^0$  concentration of 3100  $\mu\text{g/g}$  (03TER1rt, Table 1). Four other mine waste samples collected from the Terlingua and Mariposa mines contain  $\text{Hg}^0$  concentrations ranging from 0.12 to 6.7  $\mu\text{g/g}$ . Thermal desorption pyrolysis analysis of mine waste from the Terlingua district indicates that samples commonly contain cinnabar, metacinnabar and Hg sorbed onto particulates (Table 1). Pyrolysis analysis of the Terlingua mine retort soot sample (03TER1rt, Table 1) indicated the presence of  $\text{Hg}^0$  (Fig. 3), and although the pyrolysis analysis is only qualitative, this result is consistent with the chemical extraction and resultant CVAFS analysis indicating a significant  $\text{Hg}^0$  concentration (3100  $\mu\text{g/g}$ ) in this sample.

Total Hg and methyl-Hg concentrations in mine waste calcine collected from the Terlingua district are highly elevated relative to those in stream sedi-

ment samples collected from regional baseline sites in this study; baseline samples contain total Hg ranging from <0.010 to 0.028  $\mu\text{g/g}$  and methyl-Hg ranging from <0.010 to 0.025 ng/g (Table 2 and Fig. 4). Total Hg concentrations in all mine waste samples also exceed the probable effect concentration (PEC) of 1.06  $\mu\text{g/g}$  in sediment, the Hg concentration above which harmful effects are likely to be observed in sediment dwelling organisms (MacDonald et al., 2000), although it should be noted that this concentration is a Hg sediment quality guideline and not directly applicable to mine waste. Highly elevated and variable total Hg concentrations in Terlingua calcine are similar to those found at other Hg mines worldwide, e.g., Almadén, Spain and western Nevada, USA (Fig. 4); this characteristic relates to the presence of cinnabar and other Hg compounds in calcine. Most of the analyzed mine waste samples also indicate the presence of significant ionic  $\text{Hg}^{2+}$  (Table 1 and Fig. 5). Due to the highly toxic nature of methyl-Hg, concentrations of this compound are most noteworthy. Concentrations of methyl-Hg in mine waste from the Terlingua district are also similar to those found in other Hg mines throughout the world (Fig. 4), although the methyl-Hg concentration of 1500 ng/g in the Terlingua retort soot (Table 1) is an order of magnitude higher than that observed at most Hg mines. Only methyl-Hg concentrations in calcine collected from the Almadén area, Spain (3100 ng/g; Gray et al., 2004) have been reported to exceed those for Terlingua. At present, it is unclear whether methyl-Hg concentrations in mine waste reported here would be adverse to human health if such mine waste were to be inhaled. Health guidelines for methyl-Hg in sediment have not been established.

Data for the calcine samples indicate a strong correlation between methyl-Hg and organic C concentrations (Fig. 6,  $r^2 = 0.995$ ), although this correlation is highly influenced by sample 03Ter1rt (methyl-Hg = 1500 ng/g, organic C = 7.7%). When sample 03Ter1rt is removed from the dataset, the remaining calcine samples show a much lower correlation of organic C with methyl-Hg concentration ( $r^2 = 0.162$ ,  $p = 0.046$ ), although still statistically significant. A strong correlation is also observed for methyl-Hg and S concentrations (Fig. 7,  $r^2 = 0.755$ ). These data suggest that calcine samples with elevated organic C and total S concentrations have enhanced Hg methylation (Table 1), which is a relationship that has been observed in many other environments (Ullrich et al., 2001). Some Hg ores in

Table 1  
Geochemical data for mine waste samples collected from the Mariscal, Study Butte, Terlingua, and Mariposa mercury mines, Texas

Sample #	Location/description	Total Hg ( $\mu\text{g/g}$ )	Methyl-Hg ( $\text{ng/g}$ )	Hg <sup>2+</sup> ( $\mu\text{g/g}$ )	Hg <sup>0</sup> ( $\mu\text{g/g}$ )	Organic C (%)	S (%)	Pyrolysis
03MSM1ca	Mariscal mine, calcine	6.9	0.055	6.2	<0.005	0.03, 0.05 <sup>a</sup>	1.8, 1.8 <sup>a</sup>	Matrix Hg
03MSM2ca	Mariscal mine, calcine	31	0.34	34	<0.005	<0.01	1.1	Matrix Hg + cinnabar + metacinnabar
03MSM3ca	Mariscal mine, calcine	44, 42 <sup>a</sup>	0.23	35, 34 <sup>a</sup>	<0.005, <0.005 <sup>a</sup>	0.08, 0.11 <sup>a</sup>	1.2, 1.2 <sup>a</sup>	Matrix Hg
03MSM4ca	Mariscal mine, calcine	110	0.22	78	<0.005	0.31	0.58	Matrix Hg
03MSM5ca	Mariscal mine, calcine	150	1.5	59	<0.005	0.25	1.5	Matrix Hg + cinnabar + HgSO <sub>4</sub> ?
03SB1rt	Study Butte mine, calcine from retort	5900	12	690	<0.005	0.36	4.5	Matrix Hg
03SB2ca	Study Butte mine, calcine	12	0.11	3.5	<0.005	0.12	3.4	Matrix Hg
03SB3ca	Study Butte mine, calcine	480	0.095	110	<0.005	0.16	1.5	Matrix Hg
03SB4soot	Study Butte mine, condenser soot	3000	0.28	170	<0.005	0.31, 0.28 <sup>a</sup>	2.4, 2.4 <sup>a</sup>	Matrix Hg + cinnabar + metacinnabar
03SB5ca	Study Butte mine, calcine	35	0.051	35	<0.005	0.04	1.2	Matrix Hg + cinnabar + metacinnabar
03TER1rt	Terlingua mine, retort soot	19,000	1500	2100	3100	7.7, 7.6 <sup>a</sup>	9.9, 9.9 <sup>a</sup>	Matrix Hg + Hg <sup>0</sup> + cinnabar
03TER2ca	Terlingua mine, calcine	14	0.042, 0.047 <sup>a</sup>	7.8	0.80	0.18	2.1	Matrix Hg
03TER3ca	Terlingua mine, calcine	16	0.27	13	1.4	0.29	0.19	Matrix Hg
03TER4ca	Terlingua mine, calcine	170	0.17	22	<0.005	0.33, 0.34 <sup>a</sup>	2.2, 2.0 <sup>a</sup>	Matrix Hg + cinnabar
03TER5ca	Terlingua mine, calcine	4.1	0.082	1.5	<0.005	0.08, 0.10 <sup>a</sup>	1.4, 1.4 <sup>a</sup>	Matrix Hg + cinnabar
03MAR1rt	Mariposa mine, calcine from retort	170	23	93	6.7	0.23	2.0	Matrix Hg + cinnabar
03MAR2ca	Mariposa mine, calcine	190	2.6, 3.1 <sup>a</sup>	86	<0.005	0.13	1.2	Matrix Hg + cinnabar
03MAR3ca	Mariposa mine, calcine	35, 34 <sup>a</sup>	0.52	33, 32 <sup>a</sup>	<0.005, <0.005 <sup>a</sup>	0.10	2.4	Matrix Hg
03MAR4ca	Mariposa mine, calcine	25	0.32	6.7	0.12	0.06	0.63	Matrix Hg
03MAR5ca	Mariposa mine, calcine	13	0.12	9.5	<0.005	0.06	0.52	Matrix Hg + cinnabar

<sup>a</sup> Replicate analyses shown for reference.

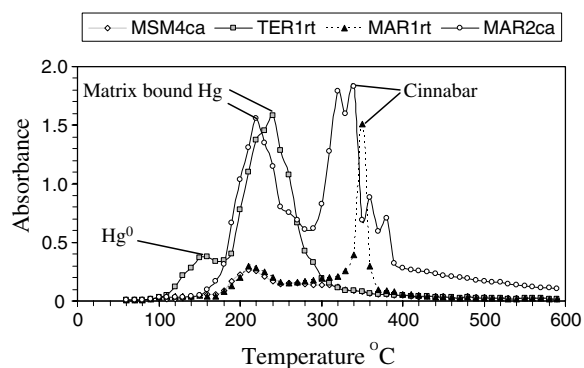


Fig. 3. Thermal desorption pyrolysis curves for four samples of mine waste calcine from the Mariscal (MSM4ca), Terlingua (TER1rt), and Mariposa (MAR1rt and MAR2ca) mines.

the Terlingua district were reported to have contained asphaltic material and hydrocarbons (Ross, 1941; Sharp, 1980). In addition, interior retort stacks at some Hg mines were coated with tar or asphalt to capture or clean Hg from stack emissions. The geochemistry and character of the Terlingua retort soot sample (03Ter1rt) suggest that Hg ore processed here may have contained significant hydrocarbons or that this retort may have been lined with an asphalt material during retorting. Methylation of Hg in this retort soot sample is clearly enhanced by the presence of elevated organic C and S concentrations, which is probably due to the presence of hydrocarbons in this sample. Conversely, most calcine samples collected in this study have generally low organic C (<0.36%, Table 1). Calcines, which are primarily crushed and retorted ore, typically contain low organic C concentrations, but the calcine data presented here generally show increasing Hg methylation with increasing organic C.

Stream sediment samples were collected near the Study Butte mine to evaluate downstream transport of Hg, as well as Hg methylation. At the Study Butte mine, there are no structures, trenches, or man-made construction features to prevent dispersal of mine waste from the site into surrounding watersheds. Consequently, some minor calcine material was observed in stream material proximal to the mine indicating downstream transport of mine wastes from this site. Two stream sediment samples collected from a tributary of Rough Run Creek  $\leq 0.5$  km downstream of the Study Butte mine (Fig. 1) contain slightly elevated total Hg concentrations (1.0 and 0.91  $\mu\text{g/g}$ , Table 2). However, sediment collected 3.5 km downstream from the

mine contains a total Hg concentration of only 0.026  $\mu\text{g/g}$ , which is lower than total Hg concentrations in two sediment samples collected upstream from the mine (0.040 and 0.050  $\mu\text{g/g}$ ) and similar to total Hg in sediment from regional baseline sites ( $n = 7$ ; average 0.020  $\mu\text{g/g}$ ; Table 2).

Furthermore, when compared to total Hg concentrations found in mine waste samples at the Study Butte mine ( $n = 5$ ; average 1900  $\mu\text{g/g}$ ), stream sediment collected downstream from the mine shows significant dilution of Hg, even those sediment samples collected within 0.5 km downstream from the mine (Tables 1 and 2). In addition, total Hg concentrations in all stream sediment samples collected from Rough Run Creek are also below the PEC sediment quality guideline of 1.06  $\mu\text{g/g}$ , which, as previously discussed, is the Hg concentration above which harmful effects are likely to be observed (MacDonald et al., 2000). Most stream sediment collected in and around the Study Butte mine contains methyl-Hg concentrations below the limit of determination of 0.010 ng/g, except for one wet stream sediment (01SB3ss) collected adjacent to the mine containing a methyl-Hg concentration of 0.014 ng/g (Table 2).

One dry stream sediment collected from near the Study Butte mine (Rough Run Creek) in 2003 was incubated with radiotracers to determine the potential for Hg methylation and methyl-Hg demethylation, with rate constants of 0.012% per day and 0.059% per day, respectively (03SB3ss, Table 3). The low methylation and demethylation rates underscore the finding that dry sediment samples do not generate significant methyl-Hg. However, sediment collected from this site (01SB3ss) in 2001 was water saturated, apparently by precipitation that fell prior to the fieldwork. When incubated with radiotracers, this sediment showed significant net Hg methylation (2.6 ng/g/day, Table 3) indicating a much higher potential for Hg methylation in wet stream sediment from near the Study Butte mine site.

Concentrations of organic C and total S in stream sediment samples from near the Study Butte site were generally low and similar to those in sediment collected from the regional baseline sites (Table 2). Methylation of Hg in sediments is dominantly an anaerobic microbial process, which is influenced by the interaction of several environmental factors such as biological activity, nutrient and organic matter availability, redox potential, pH, temperature, S chemistry (e.g., presence of



Table 2  
Geochemical data for stream sediment samples collected from near the Study Butte and Mariscal mercury mines, Texas

Sample #	Location/description	Hg ( $\mu\text{g/g}$ )	Methyl-Hg (ng/g)	Organic C (%)	S (%)
03MSM1ss	Tributary of Fresno Creek, 0.3 km downstream from Mariscal mine	7.9	0.62	0.61	0.54
03MSM2ss	Tributary of Fresno Creek, 0.7 km from Mariscal mine	0.87	0.33	0.41	0.09
03MSM3ss	Tributary of Fresno Creek, 1.2 km from Mariscal mine	0.82	0.19	0.23	<0.05
03MSM4ss	Tributary of Fresno Creek, 3.7 km from Mariscal mine	0.048	0.049	0.36	0.06
04MSM5ss	Fresno Creek, 9 km from Mariscal mine	0.018	0.060	0.14	<0.05
04MSM6ss	Fresno Creek, 10 km from Mariscal mine	0.014	0.028	0.20	<0.05
04RIO5Fss	Rio Grande, 10.3 km downstream from Mariscal mine (wet sediment)	0.018	0.033	0.22	<0.05
04RIO6ss	Rio Grande, 20 km downstream from Mariscal mine (wet sediment)	0.021, 0.021 <sup>a</sup>	0.041, 0.045 <sup>a</sup>	0.26	<0.05
04RIO7ss	Rio Grande, 29 km downstream from Mariscal mine (wet sediment)	<0.010	0.018	0.08	<0.05
04RIO4ss	Rio Grande, 40 km upstream from Mariscal mine (wet sediment)	0.028	0.036	0.25	<0.05
04RIO3ss	Rio Grande, 55 km upstream from Mariscal mine (wet sediment)	0.024	0.070	0.32	<0.05
04RIO2ss	Rio Grande, 85 km upstream from Mariscal mine (wet sediment)	0.023	0.012	0.22	<0.05
01SB1ss	Tributary of Rough Run Creek, 1.5 km upstream from Study Butte mine	0.040	<0.010	0.03	0.12
01SB2ss	Tributary of Rough Run Creek, 2 km upstream from Study Butte mine	0.050	<0.010	0.11	0.12
01SB3ss	Tributary of Rough Run Creek, adjacent to Study Butte mine (wet sediment)	0.11	0.014	0.13	0.06
03SB3ss	Tributary of Rough Run Creek, adjacent to Study Butte mine	0.14	<0.010	0.11	0.08
01SB4ss	Tributary of Rough Run Creek, 0.1 km downstream from Study Butte mine	1.0	<0.010	0.11	0.08
01SB5ss	Tributary of Rough Run Creek, 0.5 km downstream from Study Butte mine	0.91	<0.010	0.06	0.09
01SB6ss	Terlingua Creek, 3.5 km downstream from Study Butte mine	0.026	<0.010	0.11	0.06
<i>Regional baseline sites</i>					
04TOR1ss	Tornillo Creek, Big Bend National Park (wet sediment)	<0.010	<0.010	0.12	<0.05
04TOR2ss	Tornillo Creek, Big Bend National Park (wet sediment)	0.019	0.013	0.20, 0.20 <sup>a</sup>	0.06, 0.06 <sup>a</sup>
04TOR3ss	Tornillo Creek, Big Bend National Park (wet sediment)	0.013	<0.010	0.12	<0.05
04TCK1ss	Terlingua Creek, 10 km upstream from mines (wet sediment)	0.021	0.020	0.19	<0.05
04Trb1ss	Tributary of Terlingua Creek, 10 km upstream from mines (wet sediment)	0.019	0.017	0.30	0.06
04TCK2ss	Terlingua Creek, 2 km upstream of runoff from mines (wet sediment)	0.027	0.024	0.20, 0.24 <sup>a</sup>	0.05, 0.05 <sup>a</sup>
04TCK3ss	Terlingua Creek, 20 km downstream from known mines (wet sediment)	0.028	0.025	0.22	<0.05

Sediment collected was dry unless otherwise noted.

<sup>a</sup> Replicate analyses shown for reference.

SO<sub>4</sub>-reducing bacteria), and bioavailable Hg (NAS, 1978; USEPA, 1997; Ullrich et al., 2001). Rapid dilution of Hg and generally low concentrations of methyl-Hg, organic C and total S indicate that stream sediment samples collected downstream from the Study Butte mine generally have a low

potential for microbial Hg methylation. These sediments, particularly when dry, have a low capacity to generate methyl-Hg. Generally, the climate in this region is dry and hot, and the lack of rainfall limits Hg methylation and the transference of Hg and methyl-Hg from this mine site. However, as shown

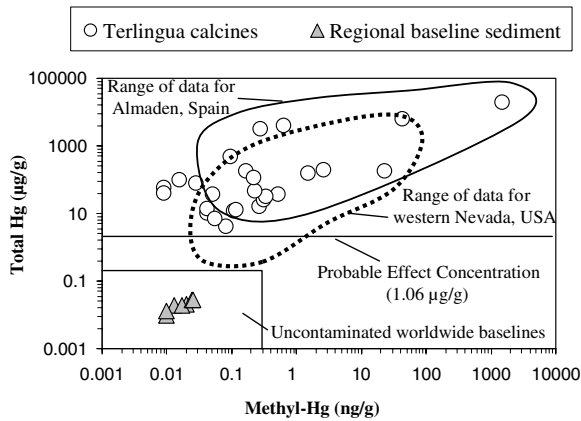


Fig. 4. Total Hg versus methyl-Hg concentrations in mine waste calcine and baseline sediment samples collected in this study. For comparison, the range of data is shown for Hg mines near Almadén, Spain (Gray et al., 2004) and in western Nevada, USA (Gray et al., 2002) as well as the probable effect concentration (MacDonald et al., 2000).

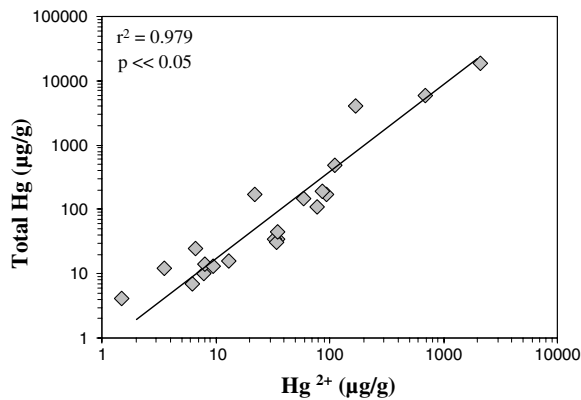


Fig. 5. Total Hg versus  $Hg^{2+}$  concentrations in mine waste samples.

by the higher net Hg methylation rate in the wet sediment, periods of high precipitation or spring runoff could yield conditions favorable for increased microbial methylation of Hg at this site.

Methylation rate data also indicate that few calcine samples in the Terlingua district mines have significant Hg methylation potential (Table 3). Calcine samples were dry and oxidizing, consequently methylation was not detected or was low in many cases because Hg methylation is an anaerobic process (Compeau and Bartha, 1985) and because the incubations were conducted under aerobic conditions. The only samples that display active methylation were soot collected from within the retort at the Terlingua mine (03TER1rt) and calcine samples collected from within the Mariposa retort (Table 3). These observations agree with high methyl-Hg

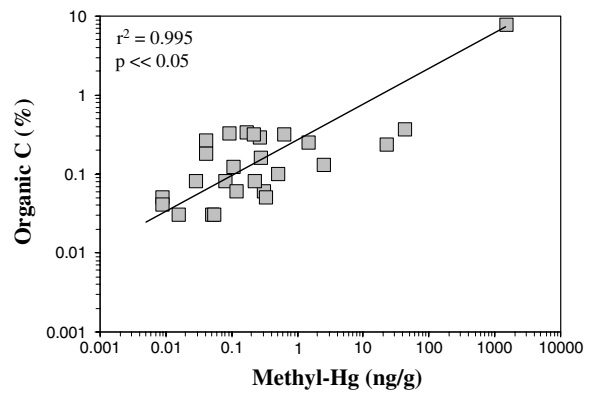


Fig. 6. Methyl-Hg versus organic C concentrations in mine waste samples.

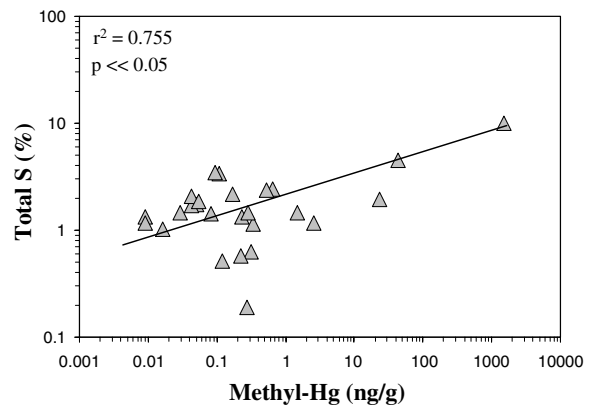


Fig. 7. Methyl-Hg versus total S concentrations in mine waste samples.

concentrations found at these sites (e.g., methyl-Hg concentrations of 1500 ng/g for 03TER1rt, and 23 ng/g for sample 03MAR1rt, Table 1). The product of the methylation rate constant and the  $Hg^{2+}$  concentration for the Terlingua soot sample yields an estimated potential rate of methyl-Hg formation of 11,000 ng/g/day (Table 3). As previously mentioned, this Terlingua retort sample has the highest concentration of  $Hg^{2+}$ , organic C and total S of the samples analyzed, all of which favor Hg methylation. The methylation potential rate for the Terlingua retort soot is high suggesting that methyl-Hg is being formed so quickly that its methyl-Hg concentration would increase by  $\sim 7$ -times per day, which is unlikely. The concentration of  $Hg^{2+}$  is high in this retort sample and it is probable that only a portion of this  $Hg^{2+}$  is readily available for methylating bacteria; consequently, the actual quantity of Hg methylated per day is probably much less than that calculated. High concentrations of inorganic Hg in this sample (2100  $\mu g/g$  of  $Hg^{2+}$ ) dilute the

Table 3

Methylation and demethylation rates for mine waste and sediment samples collected from the Mariscal, Study Butte, Terlingua, and Mariposa mercury mines, Texas

Sample #	Location/description	Methylation		Demethylation			Net methylation ng/g/day <sup>c</sup>
		Average <sup>a</sup> (%/day)	ng/g/day <sup>b</sup>	Average (%/day)	% CH <sub>4</sub>	ng/g/day <sup>b</sup>	
03MSM1ca	Mariscal mine, calcine	0	0	8.6	99	0.0047	−0.00047
03MSM2ca	Mariscal mine, calcine	0	0	3.7	100	0.013	−0.013
03MSM3ca	Mariscal mine, calcine	0	0	1.1	100	0.0025	−0.0025
03MSM4ca	Mariscal mine, calcine	0.0058	4.5	0.12	100	0.00026	4.5
03MSM5ca	Mariscal mine, calcine	0	0	1.6	100	0.024	−0.024
03SB1rt	Study Butte mine, calcine from retort	0	0	8.7	100	1.0	−1.0
03SB2ca	Study Butte mine, calcine	0	0	1.4	100	0.0016	−0.0016
03SB3ca	Study Butte mine, calcine	0	0	0	0	0	0
03SB4soot	Study Butte mine, condenser soot	0	0	0.15	100	0.00042	−0.00042
03SB5ca	Study Butte mine, calcine	0	0	0.30	0	0.00015	−0.00015
01SB3ss	Rough Run Creek, sediment, wet	26	2.6	1.5	0	0.00021	2.6
03SB3ss	Rough Run Creek, sediment, dry	0.012	0.0013	0.059	100	<0.0000059	0.0013
03TER1rt	Terlingua mine, calcine from retort	0.51	11,000	0	0	0	11,000
03TER2ca	Terlingua mine, calcine	0.0009	0.070	0.41	0	0.00017	0.070
03TER3ca	Terlingua mine, calcine	0	0	0.54	100	0.0015	−0.0015
03TER4ca	Terlingua mine, calcine	0	0	8.0	100	0.014	−0.014
03TER5ca	Terlingua mine, calcine	0.011	0.17	0.49	100	0.00040	0.17
03MAR1rt	Mariposa mine, calcine from retort	0.20	186	8.9	100	2.0	184
03MAR2ca	Mariposa mine, calcine	0.062	53	3.7	100	0.096	53
03MAR3ca	Mariposa mine, calcine	0.0041	1.4	2.5	100	0.013	1.4
03MAR4ca	Mariposa mine, calcine	0.0050	0.34	0	0	0	0.34
03MAR5ca	Mariposa mine, calcine	0.0007	0.067	2.2	6.7	0.0026	0.064

<sup>a</sup> First order rate constant ( $K_{met}$ ) expressed as a percentage of added radiotracer converted to methyl-Hg per day.

<sup>b</sup> Rate of Hg transformation calculated by multiplying rate constants times Hg<sup>2+</sup> concentrations from Table 1. Demethylation rates use methyl-Hg concentration data from Table 1.

<sup>c</sup> Methylation rate – demethylation rate.

<sup>203</sup>Hg radiotracer spike. Thus, the calculated methylation rate constant is high, even in the presence of this inorganic Hg diluent, confirming that this sample has a high potential to methylate Hg. The demethylation potential rate constant of the Terlingua retort soot sample was below the detection limit suggesting that this material is likely to accumulate methyl-Hg that will not be readily degraded by demethylation activity.

The Mariposa retort sample also exhibited high methylation activity, but its much lower Hg<sup>2+</sup> concentration (93 µg/g) relative to the Terlingua soot (2100 µg/g, Table 1), resulted in a gross methylation rate that was about 6-times lower than at Terlingua, 184 versus 11,000 ng/g/day (Table 3). As mentioned above, the Mariposa retort sample (03MAR01rt) contains much lower C (0.23%) and S concentrations (2.0%, Table 1), which inhibits the ability of bacteria to methylate Hg. Other calcine samples from Mariposa, especially sample 03MAR2ca, also displayed a net methylation activity (53 ng/g/day), which was

almost as active as the retort sample (184 ng/g/day, Table 3). Although methylation was detected in the Mariposa samples, the potential rate constants are generally low, ranging from 0.0007 to 0.20%/day.

Demethylation rate experiments indicated that methyl-Hg degradation was active in most of the mine waste samples in the Terlingua district with potential rate constants ranging from below detection (three samples) to nearly 9% per day in some cases (Table 3). Demethylation of methyl-Hg has been reported under oxidizing and anoxic conditions (Hines et al., 2000; Gray et al., 2004) and because Hg methylation is largely restricted to anoxic environments, Terlingua mine waste tends to favor demethylation of methyl-Hg over its formation. Since methylation activity was mostly below detection in these samples, net methylation (methylation – demethylation) was usually negative, resulting in net demethylation (Table 3). Similar findings have been reported for many calcine samples collected from the Almadén area in Spain, where

oxidized calcine samples exhibited demethylation, whereas methylation was not detected (Gray et al., 2004). Almadén calcine samples generally contain higher quantities of methyl-Hg and have lower demethylation potential rate constants than those reported here for the Terlingua district, so active demethylation may play a role in suppressing methyl-Hg accumulations at Terlingua. Despite a noted ability to degrade methyl-Hg, the Terlingua district calcines contain between 0.042 and 23 ng/g of methyl-Hg (Table 1), which suggests that (1) methyl-Hg formation increases when calcine becomes wet during periods of precipitation and (2) some of the methyl-Hg formed is not readily degraded and persists into dry periods.

The  $^{14}\text{C}$ -methyl-Hg radioisotope spike was added in a soluble, more easily biodegradable form than the ambient methyl-Hg that was likely associated with the calcine material. Hence, the calculated methyl-Hg degradation potential rate constants may be somewhat overestimated. It must be emphasized that these radiotracer methods yield a potential for Hg transformation activity and do not reflect actual in situ rates. When combined with concentrations of Hg species as outlined above, the rates calculated are still potential rates to some unknown degree. However, although these types of methods are relatively new, they have been employed in several types of habitats and the data obtained have agreed with other geochemical parameters known to strongly affect Hg transformations such as  $\text{SO}_4$ -reduction rates, S speciation, and Hg species distribution (Marvin-DiPasquale and Oremland, 1998; Marvin-DiPasquale et al., 2003; Stamenkovic et al., 2004).

In most cases,  $^{14}\text{C}$ -methyl-Hg added to calcine samples was degraded by the reductive pathway that liberates  $\text{CH}_4$  from the methyl group of methyl-Hg (Table 3). Only three samples yielded a significant amount of  $^{14}\text{CO}_2$  via demethylation, and two of those exhibited low rates of demethylation in which it was difficult to distinguish the relative distribution of  $^{14}\text{C}$  between the two products. The bacterial *mer* genetic system encodes for an enzyme that catalyzes the reductive cleavage of the methyl group of methyl-Hg with  $\text{CH}_4$  as the sole C product, as opposed to the oxidative pathway that yields  $\text{CO}_2$  (Barkay et al., 2003). Hence, the reductive demethylation pathway clearly dominated over the oxidative path in the Texas mine waste. The reductive path tends to dominate demethylation activity at high Hg concentrations since expression of the *mer* genes

is induced by ionic Hg (Barkay et al., 2003). However, the reductive pathway can also dominate at sites with low Hg concentrations under aerobic conditions, while the oxidative path is usually dominant under anaerobic conditions (Hines et al., 2000; Barkay et al., 2003). The formation of  $\text{CH}_4$  during reductive demethylation is due to the protonolysis of methyl-Hg by a thiol group situated on the bacterial *merB* enzyme, and the proton is replenished from a thiol redox buffer such as glutathione (Pitts and Summers, 2002). Hence, this reaction can occur under aerobic or anaerobic conditions, but seems to be favored in aerobic situations. The oxidative path is less well understood, but is thought to occur similarly to the oxidation of the other C-one compounds in which the methyl group is oxidized to  $\text{CO}_2$  and electrons are accepted by the most energetically favorable and available exogenous electron acceptor. Although the oxidative path can occur during  $\text{O}_2$  reduction, it tends to be favored in the absence of  $\text{O}_2$  when alternate electron acceptors are used such as  $\text{NO}_3^-$  or  $\text{SO}_4^{2-}$ . A similar situation was noted in samples from the Almadén mine where the reductive pathway dominated in aerobic calcines, whereas the oxidative path dominated in anaerobic sediment samples (Gray et al., 2004). The data reported here, and those for Almadén, indicate that the potential for demethylation is high in calcine and that bacteria harboring the *mer* enzyme systems mediate methyl-Hg degradation.

Unlike the Terlingua and Mariposa mine retort samples, the methylation potential rate constant in the Study Butte retort sample (03SB1rt) was below the detection limit, whereas demethylation potential was high (Table 3). This contrast with the Terlingua and Mariposa samples might explain why the methyl-Hg concentration in the Study Butte retort was more than 100-fold lower than that in the Terlingua retort even though the  $\text{Hg}^{2+}$  concentrations differed by only a factor of 3. The Mariposa retort sample exhibited active methylation (0.20%/day) and demethylation (8.9%/day, Table 3). In this Mariposa sample net methylation activity favors methylation and the methyl-Hg concentration (23 ng/g), although slightly higher than in the Study Butte retort (12 ng/g), was much lower than that in the Terlingua retort (1500 ng/g). Therefore, most retort material appears to support active populations of Hg-transforming bacteria, but the relative proportion of methylation to demethylation activity can vary greatly resulting in widely different methyl-Hg concentrations.

#### 4.2. Mariscal mine

As is true for calcine in the Terlingua district, mine waste calcine collected from the Mariscal mine also contains total Hg concentrations (6.9–150  $\mu\text{g/g}$ ) and methyl-Hg concentrations (0.055–1.5  $\text{ng/g}$ ) that are at least an order of magnitude higher than those found in stream sediment collected from regional baseline sites (Tables 1 and 2 and Fig. 4). Total Hg concentrations in the Mariscal mine waste samples also exceed the PEC of 1.06  $\mu\text{g/g}$ , the sediment quality guideline for Hg in sediment (MacDonald et al., 2000), which was also found for mine waste sampled in the Terlingua district (Fig. 4). Concentrations for  $\text{Hg}^{2+}$  vary from 6.2 to 78  $\mu\text{g/g}$  in mine waste samples collected from Mariscal, but  $\text{Hg}^0$  was not found in these samples (Table 1). Thermal desorption pyrolysis of Mariscal mine calcine indicated the presence of cinnabar, metacinnabar, Hg sorbed onto particulates, and one sample possibly contains  $\text{HgSO}_4$  (Table 1). Mine waste samples collected from the Mariscal mine contain significantly lower methyl-Hg concentrations ( $n = 5$ , ave. = 0.47  $\text{ng/g}$ ) than those in mine waste throughout the Terlingua district ( $n = 15$ , ave. = 103  $\text{ng/g}$ ). Lower methyl-Hg concentrations in Mariscal mine waste probably relate to generally low organic C and total S concentrations at this site (Table 1).

Mercury transformation activity data for Mariscal mine calcine is consistent with the finding of low methyl-Hg concentrations in these samples (Table 3). Methylation activity was essentially absent in four of the five Mariscal calcine samples, which is a finding similar to results for most calcine collected from the Study Butte and Terlingua mines. The oxidized nature of the Mariscal mine waste together with low C and S concentrations precludes any significant methylation activity by naturally occurring bacteria. Demethylation rate constants for the Mariscal calcine samples were also similar to those noted in the Terlingua District, which yielded net methylation rates that were negative in all but one sample, and demethylation occurred via the reductive pathway, i.e.,  $\text{CH}_4$  was the sole C product of methyl-Hg demethylation. Therefore, Mariscal calcine did not produce or accumulate significant methyl-Hg, at least during the dry periods that were investigated.

Stream sediment samples were collected downstream from the Mariscal mine to evaluate transport of Hg and methyl-Hg to the Rio Grande, about 10 km downstream Mariscal mine. These stream

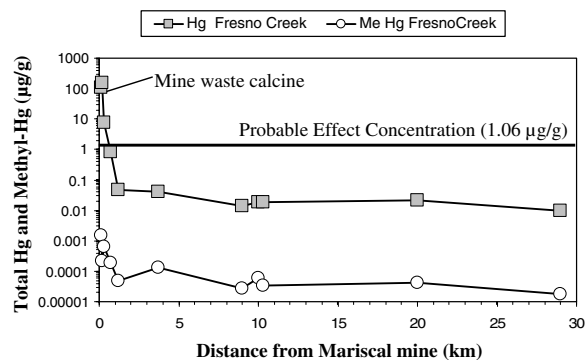


Fig. 8. Total Hg and methyl-Hg concentrations in stream sediments versus distance from the Mariscal mine.

sediment samples show a significant decrease in total Hg and methyl-Hg concentration with increasing distance from the mine (Fig. 8). As in the Terlingua district, no structures have been built at the Mariscal mine to prevent erosion and downslope transport of mine waste. Also similar to mines in the Terlingua district, some calcine material (although minor) is present in stream beds within a few m of the Mariscal mine. Concentrations of total Hg (7.9  $\mu\text{g/g}$ ) and methyl-Hg (0.62  $\text{ng/g}$ ) are elevated in stream sediment sample collected nearest the Mariscal mine, but within a few km, downstream concentrations are similar to those found in the regional baseline sites; organic C and total S in stream sediment samples show a similar downstream trend (Table 2). In addition, only the stream sediment sample collected nearest the Mariscal mine contains total Hg (7.9  $\mu\text{g/g}$ ) exceeding the PEC of 1.06  $\mu\text{g/g}$  in sediment, the Hg concentration above which harmful effects are likely to be observed in sediment dwelling organisms (MacDonald et al., 2000); total Hg in all other sediment samples are below the PEC (Fig. 8 and Table 2). Mariscal is the closest Hg mine to the Rio Grande, however, geochemical results in the Mariscal mine area suggest that processes favoring microbial Hg methylation are suppressed and transference of significant Hg and methyl-Hg from mine waste, to downstream sediment, and then to the Rio Grande is unlikely.

#### 5. Conclusions

Methylation of Hg is primarily a microbially mediated process enhanced in anaerobic, water saturated sediments. Environments favorable for Hg methylation generally include bioavailable Hg,  $\text{SO}_4$ -reducing bacteria, and high nutrient and



organic matter contents. Studies of Hg mine sites have indicated that Hg methylation is generally more efficient in humid climates where temperatures and precipitation are high, mine wastes are water saturated, and methylating bacteria are more active (Rytuba, 2000; Gray et al., 2003). Calcine samples collected from the mines studied here contain highly elevated total Hg concentrations, similar to other Hg mines worldwide, which mostly relate to the presence of cinnabar. Although cinnabar is the dominant Hg ore mineral and is the primary Hg compound present in mine waste, it is highly insoluble, resistant to weathering, and probably plays a minor role in Hg methylation. More important for Hg speciation and Hg methylation are ionic Hg compounds that generally constitute a smaller part of the total Hg present, but are more significant inorganic Hg sources that are converted to methyl-Hg by bacteria.

Data presented here indicate that calcine characterized by high  $\text{Hg}^{2+}$ , organic C and total S concentrations has a high potential for methyl-Hg production in this substrate given the right conditions. A soot sample collected inside an abandoned retort at the Terlingua mine exemplifies this relationship as it contains the highest methyl-Hg,  $\text{Hg}^{2+}$ , organic C and total S concentration and has the highest net Hg methylation rate (Tables 1 and 3). The soot collected from this retort was dry and oxidized, and the high methyl-Hg concentration in this material is unusual. The ability of this material to methylate  $^{203}\text{Hg}$  suggests that Hg methylation at this site is biologically mediated. Generally, calcine collected from inside abandoned retorts contained the highest methyl-Hg concentrations and Hg methylation potential rates in this study. Active methyl-Hg degradation potential by the *mer*-encoded reductive demethylation pathway was found in samples of mine waste, indicating that C-poor mine wastes do not accumulate high concentrations of methyl-Hg because ambient bacteria probably degrade a significant portion of any methyl-Hg formed.

Formation of methyl-Hg is most important because of its high toxicity and ability to biomagnify in food chains. Although total Hg and methyl-Hg concentrations in calcine are locally elevated, there is only minor transference of Hg to surrounding ecosystems based on the stream sediment samples analyzed. Concentrations of total Hg and methyl-Hg are low in most stream sediment samples collected downstream from the Mariscal and Study Butte

mine and exhibit significant dilution downstream. Several factors favor Hg methylation at the mine sites, however, the arid climate and lack of precipitation inhibit microbial Hg methylation downstream probably because methylating bacteria are dormant under such conditions. Data shown here for wet sediments indicate that, during periods of precipitation, the potential for increased Hg methylation is elevated in watersheds located downstream from the mines. The Rio Grande, the largest ecosystem in this region, is more conducive to Hg methylation because organic matter is readily available and because it is a perennial river with an abundance of associated water-saturated sediment. However, Hg mines in this region are distant from the Rio Grande (>10 km), and again, due to the lack of precipitation, mine runoff is transported and significantly diluted before it reaches the Rio Grande. This influence is corroborated by the fact that total Hg and methyl-Hg concentrations in Rio Grande sediment are similar to those found in regional baseline sediment.

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