Concentration, distribution, and translocation of mercury and methylmercury in mine-waste, sediment, soil, water, and fish collected near the Abbadia San Salvatore mercury mine, Monte Amiata district, Italy

Valentina Rimondi [a], John E. Gray [b,*], Pilario Costagliola [a], Orlando Vaselli [a], Pierfranco Lattanzi [c]

[a] Dipartimento di Scienze della Terra, Università di Firenze, Via G. La Pira 4, Firenze, 50121 Italy
[b] U.S. Geological Survey, MS 973, Federal Center, Denver, CO 80225 USA
[c] Dipartimento di Scienze della Terra, Università di Cagliari, Via Trentino 51, Cagliari, 09127 Italy

ABSTRACT

The distribution and translocation of mercury (Hg) was studied in the Paglia River ecosystem, located downstream from the inactive Abbadia San Salvatore mine (ASSM). The ASSM is part of the Monte Amiata Hg district, Southern Tuscany, Italy, which was one of the world's largest Hg districts. Concentrations of Hg and methyl-Hg were determined in mine-waste calcine (retorted ore), sediment, water, soil, and freshwater fish collected from the ASSM and the downstream Paglia River. Concentrations of Hg in calcine samples ranged from 25 to 1500 μg/g, all of which exceeded the industrial soil contamination level for Hg of 5 μg/g used in Italy. Stream and lake sediment samples collected downstream from the ASSM ranged in Hg concentration from 0.26 to 15 μg/g, of which more than 50% exceeded the probable effect concentration for Hg of 1.06 μg/g, the concentration above which harmful effects are likely to be observed in sediment-dwelling organisms. Stream and lake sediment methyl-Hg concentrations showed a significant correlation with TOC indicating considerable methylation and potential bioavailability of Hg. Stream water contained Hg as high as 1400 ng/L, but only one water sample exceeded the 1000 ng/L drinking water Hg standard used in Italy. Concentrations of Hg were elevated in freshwater fish muscle samples and ranged from 0.16 to 1.2 μg/g (wet weight), averaged 0.84 μg/g, and 96% of these exceeded the 0.3 μg/g (methyl-Hg, wet weight) USEPA fish muscle standard recommended to protect human health. Analysis of fish muscle for methyl-Hg confirmed that >90% of the Hg in these fish is methyl-Hg. Such highly elevated Hg concentrations in fish indicated active methylation, significant bioavailability, and uptake of Hg by fish in the Paglia River ecosystem. Methyl-Hg is highly toxic and the high Hg concentrations in these fish represent a potential pathway of Hg to the human food chain.

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1. Introduction

Active and inactive Hg mines represent a significant source of Hg to the environment as a result of the disposal of Hg-rich mine-waste calcine at these sites. Extraction of Hg during mining is generally carried out in retort or rotary furnace, where ore is heated to 600–700 °C, leading to the thermal decomposition of cinnabar (HgS) to elemental mercury (Hg^0). Condensation of Hg vapor to liquid elemental Hg is the final step in ore processing and elemental Hg is the product sold on the international market. Retorting of cinnabar ore is an incomplete process, and thus, mine-waste calcines generally contain elevated Hg concentrations due to the presence of unconverted cinnabar as well as the formation of metacinnabar, Hg^0, and other insoluble and soluble Hg salts during retorting (Gray et al.,

2010; Kim et al., 2003, 2000). Runoff from mine-waste calcines produces adverse effects to ecosystems located downstream from Hg mines (Gosar et al., 1997; Gray et al., 2002, 2003; Higueras et al., 2006). Stream sediment and water downstream from Hg mines are characterized by anomalously high Hg concentrations even decades after the end of Hg mining (Gosar et al., 1997; Gray et al., 2000; Hines et al., 2000; Qiu et al., 2005).

The most significant environmental concern associated with downstream transportation of Hg is potential conversion of inorganic Hg to methyl-Hg (CH$_3$Hg$^+$), the most toxic form of Hg (Eisler, 1987). Methyl-Hg is a neurotoxin (Clarkson, 1990; WHO, 1976) and high intake of Hg by humans may result in health effects such as deafness, blindness, and even death (Eisler, 1987; NAS, 1978; USEPA, 1997). In natural environments, methylation of inorganic Hg is mainly the result of microbial activity, primarily by the action of sulfate reducing bacteria (Compeau and Bartha, 1985), which is particularly enhanced in organic-rich sediment (Hines et al., 2000; Ullrich et al., 2001). Despite the generally

low organic contents in mine-waste calcine, areas mined for Hg have been shown to have active formation of methyl-Hg resulting in high concentrations of methyl-Hg in calcine in some instances (e.g., up to 11,100 ng/g, Almadén, Spain; (Gray et al., 2004)), thus, representing a potentially significant source of methyl-Hg to ecosystems surrounding Hg mines. Methyl-Hg formation is potentially hazardous because it is water soluble, bioavailable, and bioaccumulates in living organisms (NAS, 1978; USEPA, 1997), and generally increases in the food chain with increasing trophic levels (biomagnification) (NAS, 1978). Consumption of fish containing high concentrations of Hg is the dominant exposure pathway of methyl-Hg to humans because generally more than 90% of Hg in fish is methyl-Hg (Bloom, 1992; Fitzgerald and Clarkson, 1991; Mason et al., 2000). In order to limit exposure of methyl-Hg to humans, the USEPA established a consumption advisory for methyl-Hg in fish muscle of 0.3 μg/g (wet weight) (USEPA, 2009b).

The Monte Amiata Hg district, Southern Tuscany, Italy, is part of the large circum-Mediterranean Hg belt, which hosts 65% of the world's cinnabar deposits (Bargagli et al., 1986). Monte Amiata was one of the five mining regions (Almadén, Spain; Idrija, Slovenia; California Coast Ranges, USA; Monte Amiata, Italy; Huancavelica, Peru) that dominated the historical global production of elemental Hg (Ferrara et al., 1999). Several presently inactive mines constitute the Monte Amiata district (Fig. 1).

Presently, detailed study of Hg diffusion through abiotic and biotic compartments at the Monte Amiata district is lacking. Speciation of Hg is unknown in the sediment and water column in the Monte Amiata area, and in addition, determinations of Hg in fish have not been made since the 1970s (Bacci and Renzoni, 1973). Significant production of Hg from the Monte Amiata Hg district and the presence of large deposits of mine-waste calcine containing high concentrations of Hg potentially contaminate surrounding streams and rivers located downstream from Hg mines. Furthermore, potential conversion of inorganic Hg to methyl-Hg leads to increased bioavailability of Hg and potential exposure of Hg to the food chain in this area. This study focuses on the largest mine in the Monte Amiata district, the ASSM. The objective of this study was to evaluate (1) environmental effects of past Hg mining to the ecosystem downstream from the ASSM, (2) the source of Hg to the surrounding environment, (3) the distribution and transport of Hg and methyl-Hg in sediment and water runoff from the ASSM, and (4) potential exposure of Hg to humans in the area through the measurement Hg and methyl-Hg concentrations in freshwater fish. Samples of calcine, soil, stream sediment, stream and lake water, and fish were analyzed for both Hg and methyl-Hg in this study.

2. Study area

The area of study was the ASSM of the Monte Amiata Hg district, Italy (Fig. 1). Similar to most Hg deposits worldwide, cinnabar is the dominant ore mineral at mines in the Monte Amiata district, but minor pyrite, and rare stibnite, marcasite, and arsenic-bearing sulphides have also been reported (Falini, 1960; Morteani et al., 2011). Typical gangue is microcrystalline calcite and quartz, and rare celestite, gypsum, native sulphur, and hydrocarbons (Klemm and Neumann, 1984). The Amiata district deposits were originally worked by the Etruscans (about 800 BC), but these mines were later closed by the Romans (Barghigiani and Ristori, 1994; Ferrara et al., 1991). Deposits in the area were rediscovered in 1868 (Barghigiani and Ristori, 1994) and mines were thereafter reopened. About 102,000 t of Hg (3 million flasks of Hg of 34.5 kg each) were produced from the 1860s to 1980 (Ferrara et al., 1998), ranking it as the 4th largest Hg producing district worldwide. Samples for this study were collected in and

around the town of Abbadia San Salvatore (42°53′ N, 11°40′ E) (Fig. 1), where the retort for the majority of the mines of the Monte Amiata district was located. Precipitation averages about 85 cm per year at Abbadia San Salvatore and temperatures during field work varied from about 19–27 °C. During sample collection from September 2 to 8, 2010, weather was generally dry, and sunny to partly cloudy. Streams sampled in the area were generally perennial.

3. Methods

3.1. Sample collection and preparation

Samples of mine-waste calcine ($n = 15$) were collected from waste piles of the ASSM, which are located adjacent to the town of Abbadia San Salvatore (Fig. 1, Table 1). Calcine was collected as grab samples about 50 cm below the surface to avoid the highly-oxidized near-surface environment. Calcine samples were collected at this depth in the mine waste piles to access moist, potentially anoxic areas with generally more active anaerobic methylating bacteria, and therefore, the highest possibility of Hg methylation. Soil samples were collected from near the ASSM retort emission stack ($n = 4$) and from other sites ($n = 6$) located more distal from the retort area east and north of ASSM (Fig. 1, Table 1). Soil was collected from a 2–5 cm depth from the A-horizon following the removal of the forest floor O-horizon leaves and tree litter.

Sediment ($n = 17$) and various water samples ($n = 20$) were collected from sites in and around the ASSM and from regional baseline sites (Fig. 1, Table 2). Stream and lake sediment was collected from surface-layer, bed-load alluvium, and composited from several localities at each site. There were 15 stream sediment samples collected in this study, but only 2 lake sediment samples. The lake sediment samples were collected from two small lakes, which are located along Acqua Gialla Creek (Fig. 1b). These lakes are open systems where sediment and water flows in and through these lakes and eventually exits at the downstream point of discharge. All calcine, soil, and stream and lake sediment samples for Hg and methyl-Hg analysis were stored in amber glass vessels with Teflon-lined lids and were frozen until analysis. Solid samples for additional geochemical analysis were air dried, sieved to minus-80-mesh (0.18 mm), and pulverized.

Unfiltered water samples were collected from the same sites as stream and lake sediment samples, with the exception of one stream drainage (10MTA17) where there was no water, and four additional sites where only water was collected from mine adits and a drainage tunnel (10MTA03, 04, 05, and 09; Table 2). These unfiltered water samples were collected primarily to evaluate the variation of Hg and methyl-Hg concentrations in water downstream from the ASSM and throughout the region. Water samples for Hg and methyl-Hg analysis were collected in Teflon bottles pre-cleaned by boiling in concentrated HNO_3 for 48 h. Within 8 h of collection, the water samples were acidified with ultra-pure HCl using a final acid concentration of 0.5% v/v.

Freshwater fish were collected from the Paglia River and Pagliola Creek (Fig. 1) and from a small lake located next to the mine area (Laghetto Verde) using dip nets or rod and reel angling techniques. Fish were chilled in a cooler during the day and frozen within 4 h of collection. Species of fish collected included barbel (*Barbus plebejus*), chub (*Leiciscus cephalus*), roach (*Chondrostoma genei*), and carp (*Cyprinus carpio*). A total of 86 fish were caught and analyzed for Hg in this study. Seventy of the fish were later thawed and dissected with a stainless steel knife to obtain muscle samples (fillet) for analysis. From Pagliola Creek (site 10MTA11, Fig. 1) there were 59 fish collected, 52 roach and 7 barbel, from which muscle was removed and analyzed for Hg; an additional 5 barbel and 2 roach were collected from this site that were analyzed as whole fish. From the site on the Paglia River (near site
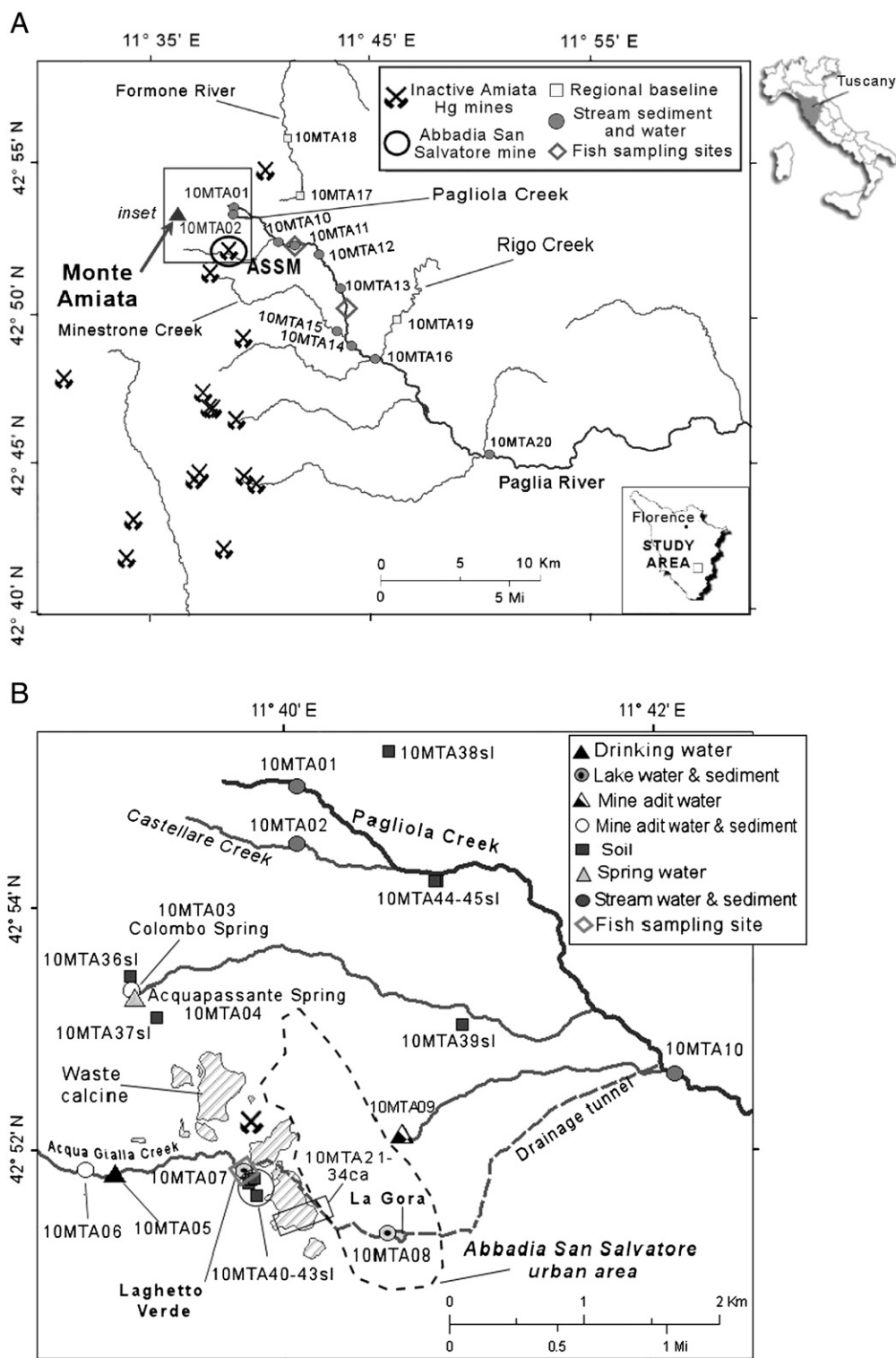
Fig. 1. A and 1B (inset). Location of sediment, water, mine-waste calcine, soil, and fish samples collected in this study.

10MTA13, Fig. 1) 9 fish were collected, 6 chub, 2 barbel, and 1 roach, which were dissected and muscle was collected for analysis; an additional 9 fish were collected from this site, 7 roach and 2 barbel, and analyzed as whole fish. There was one carp collected from Laghetto Verde, from which muscle was removed and analyzed. Fish collected were small, ranging from 8 to 19 cm in fork length and weighing between 7 and 90 g, with the exception of the carp collected from Laghetto Verde that was 25 cm in length, weighing 320 g. All fish samples were freeze dried,

pulverized, and then analyzed dry. Fish were weighed prior to freezing and then again after freeze drying to obtain wet weight conversions.

3.2. Chemical analysis

3.2.1. Mine waste calcine, stream sediment, and soil

The concentration of Hg was determined in the calcine, soil, and sediment samples using an aqua-regia digestion following EPA

Table 1
Geochemical data for calcine and soil collected in the Monte Amiata area.

Calcine				
Sample	Location	Hg (μg/g)	Methyl-Hg (ng/g)	TOC (%)
10 MTA 21ca	Abbadia San Salvatore mine	130	11	2.9
10 MTA 22ca	Abbadia San Salvatore mine	120	26	3.7
10 MTA 23ca	Abbadia San Salvatore mine	48	4.5	1.1
10 MTA 24ca	Abbadia San Salvatore mine	76	6.8	2.3
10 MTA 25ca	Abbadia San Salvatore mine	25	0.34	0.23
10 MTA 26ca	Abbadia San Salvatore mine	74	1.7	0.55
10 MTA 27ca	Abbadia San Salvatore mine	110	1.1	n.d.
10 MTA 28ca	Abbadia San Salvatore mine	32	0.39	0.11
10 MTA 29ca	Abbadia San Salvatore mine	1200	11	0.16
10 MTA 30ca	Abbadia San Salvatore mine	1500	8.8	0.44
10 MTA 31ca	Abbadia San Salvatore mine	110	0.60	0.030
10 MTA 32ca	Abbadia San Salvatore mine	130	2.3	0.35
10 MTA 33ca	Abbadia San Salvatore mine	380	11	0.20
10 MTA 34ca	Abbadia San Salvatore mine	250	3.6	0.27
10 MTA 35ca	Abbadia San Salvatore mine	140	0.86	0.10
Soil				
Mining area				
10 MTA 40sl	Abbadia San Salvatore mine	400	29	7.5
10 MTA 41sl	Abbadia San Salvatore mine	280	44	8.1
10 MTA 42sl	Abbadia San Salvatore mine	150	45	14
10 MTA 43sl	Abbadia San Salvatore mine	170	7.9	7.0
Baseline areas				
10 MTA 36sl	North Abbadia San Salvatore	2.4	18	6.5
10 MTA 37sl	North Abbadia San Salvatore	14	29	9.1
10 MTA 38sl	North Abbadia San Salvatore	0.64	0.95	2.2
10 MTA 39sl	East Abbadia San Salvatore	1.0	1.5	2.5
10 MTA 44sl	East Abbadia San Salvatore	2.7	1.4	4.8
10 MTA 45sl	East Abbadia San Salvatore	1.9	1.2	4.8

method 1631(Bloom and Fitzgerald, 1988). The Hg ions in the digestate were reduced by acidic $SnCl_2$ to Hg^0 and purged from the sample with argon. The released Hg was measured by cold vapor atomic absorption (using a Leeman Hydra AF instrument, Leeman Labs Inc., Hudson, New Hampshire). Methyl-Hg analysis follows EPA method 1630 using cold-vapor atomic fluorescence spectrometry (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). An ethylating agent was then added to the extract to form a volatile methyl-ethyl mercury derivative, and then purged onto graphitized carbon traps as a means of preconcentration and interference removal. The samples were then isothermally chromatographed, pyrolitically broken down to Hg^0 and detected using CVAFS (using a Tekran Model 2500, Tekran Instruments, Toronto, Canada). All Hg and methyl-Hg determinations were performed by Battelle Marine Science Laboratory, Sequim, Washington. Quality control for Hg and methyl-Hg analysis was established using blank spikes, matrix spikes, standard reference materials (SRM's), and/or sample replicates. Blank and matrix spikes were run as 1 per every 10 samples, replicates and SRM's were run as 1 per every 20 samples, and 3 method blanks were run every 20 samples. Recoveries for Hg on blank spikes were 99–100% and were 75–125% on matrix spikes. There were no blank spikes for methyl-Hg. Recoveries for methyl-Hg matrix spikes varied from 74 to 101%. The relative percent difference in sample replicates was ≤17% for Hg and was ≤20% for methyl-Hg. Replicates used were separate laboratory aliquots of individual samples (field samples). For the SRM, IAEA-405 analyzed in this study, recoveries ranged from 94–98% and 74–95% of the certified values for Hg and methyl-Hg, respectively. Method blanks contained Hg and methyl-Hg below the lower limit of determination, which was 0.01 μg/g for Hg and 0.04 ng/g for methyl-Hg.

The mine-waste calcine, soil, and sediment samples were also analyzed for total organic carbon (TOC) because the process of Hg-methylation depends on organic matter. Determination of TOC was by subtracting carbonate C from total C concentrations. Total C was determined using an automated C-analyzer with an infrared detector that measures CO_2 gas liberated as the sample is combusted at 1370 °C. Carbonate C was determined by liberating CO_2 following

Table 2
Geochemical data for sediment and water collected in the Monte Amiata area.

Stream/lake sediment					Stream/lake water	
Sample	Location	Hg (μg/g)	Methyl-Hg (ng/g)	TOC (%)	Hg (ng/L)	Methyl-Hg (ng/L)
	Upstream from mine					
10 MTA 01	Pagliola Creek	0.27	1.2	0.62	3.2	0.29
10 MTA 02	Castellare Creek	0.10	0.041	0.44	4.3	0.33
	Mine adits					
10 MTA 03	Colombo Spring	1.3	1.5	2.1	130	1.7
10 MTA 06	Acqua Gialla Creek	1.8	0.94	0.79	390	0.39
10 MTA 09	Galleria Italia	n.a	n.a	n.a	170	0.15
	Spring water					
10 MTA 04	Acquapassante Spring	n.a	n.a	n.a	2.6	<0.02
	Drinking water					
10 MTA 05	Galleria XXII	n.a	n.a	n.a	2.8	<0.02
	Downstream from mine					
10 MTA 10	Pagliola Creek	10	6.4	2.1	450	1.3
10 MTA 11	Pagliola Creek	13	5.8	0.50	1400	3.0
10 MTA 12	Paglia River	14	5.2	1.0	450	1.2
10 MTA 13	Paglia River	0.79	0.20	0.23	140	1.0
10 MTA 14	Paglia River	0.35	0.36	0.27	21	0.26
10 MTA 15	Minestrone Creek	0.26	0.22	0.24	3.7	0.15
10 MTA 16	Paglia River	0.42	0.25	0.29	11	0.14
10 MTA 20	Paglia River	10	0.69	0.25	3.8	0.10
	Lakes					
10 MTA 07	Laghetto Verde	15	8.7	2.4	55	0.67
10 MTA08	La Gora	11	4.7	1.0	290	0.77
	Regional baselines					
10 MTA 17	Formone River	0.023	0.068	0.42	n.a	n.a
10 MTA 18	Formone River	0.057	3.0	0.55	1.9	0.19
10 MTA 19	Rigo Creek	0.034	0.089	0.24	1.5	0.045

Table 3

Comparative Hg and methyl-Hg data for Hg mines, baselines, and standards worldwide. References are: 1 This study, 2 (Gray et al., 2004), 3 (Berzas Nevado et al., 2003), 4 (Higueras et al., 2003), 5 (Higueras et al., 2006), 6 (Hines et al., 2000), 7 (Gosar et al., 1997), 8 (Horvat et al., 2004), 9 (Rytuba, 2000), 10 (Rytuba, 2003), 11 (Davis et al., 2008), 12 (Gray et al., 2000), 13 (Bailey et al., 2002), 14 (Gray et al., 2002), 15 (Gray et al., 2003), 16 (Qiu et al., 2005), 17 (Qiu et al., 2009), 18 (Leermakers et al., 1996), 19 (Gill and Bruland, 1990), 20 (Painter et al., 1994), 21 (Lyons et al., 1999), 22 (MacDonald et al., 2000), 23 (USEPA, 2008), 24 (IME, 2006), 25 (WHO, 2005), and 26 (USEPA, 2009b).

Location	Unfiltered water		Sediment, calcine, or soil		Fish (muscle)	References
	Hg (ng/L)	Methyl-Hg (ng/L)	Hg (µg/g)	Methyl-Hg (ng/g)	Hg (µg/g, wet weight)	
Monte Amiata, Italy						1
Downstream from mines	3.8–1400	0.14–3.0	0.26–14	0.20–8.7	0.16–1.2	
Mine-waste calcine	–	–	25–1500	0.34–26	–	
Soil	–	–	0.64–400	0.95–45	–	
Lakes	55–290	0.66–0.77	11–15	4.7–8.7	0.49	
Almadén Hg district, Spain						2, 3, 4, 5
Downstream from mines	7.6–13,000	0.41–30	3.0–2300	0.32–82	0.72–1.9	
Mine-waste calcine	–	–	160–34,000	< 0.2–3100	–	
Soil	–	–	6–8889	–	–	
Mine-pit lake	2200–2800	0.040–0.065	935	3	–	
Idrija Hg mine, Slovenia						6, 7, 8
Downstream from mines	6.0–322	0.01–0.6	0.77–1347	0.01–11	1.1–1.8	
Mine-waste calcine	–	–	10–727	6.5–14	–	
Hg mines, California, USA						9, 10, 11
Downstream from mines	1400–19,000	0.96–4.5	0.4–220	1.1–150	0.1–2.1	
Mine-waste calcine	2–450,000	<0.003–47	10–1500	–	–	
Hg mines, southwest Alaska, USA						12, 13
Downstream from mines	1.0–2500	0.01–1.2	0.90–5500	0.05–31	0.03–0.62	
Mine-waste calcine and soil	–	–	3.5–46,000	0.2–41	–	
Hg mines, Nevada, USA						14
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						15
Downstream from mines	170–330	<0.02–0.33	3.7–15	0.28–3.9	0.03–1.1 [a]	
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	–	
Wanshan Hg mine, China						16, 17
Downstream from mines	15–9300	0.31–22	90–930	3.0–20	0.061–0.68	
Mine-waste calcines	–	–	5.7–400	0.17–1.1	–	
Soil	–	–	5.1–790	0.13–15	–	
Comparative baselines distal from mines						
Lake Baikal, Russia	0.14–2.02	0.002–0.161	0.005–0.072	–	–	18
Lake San Antonio, Calif., USA	0.6–1.8	–	–	–	–	19
Baselines streams, SW Alaska, USA	0.1–1.4	0.04–0.2	0.02–0.78	0.1–0.3	–	12
Uncontaminated streams, Canada	–	–	0.01–0.7	–	–	20
Streams and lakes, Antarctica	0.27–1.9	0.02–0.33	–	–	–	21
Comparative standards						
Probable effect concentration, sediment	–	–	1.06	–	–	22
USEPA, residential soil screening level	–	–	23	7.8	–	23
USEPA, industrial soil screening level	–	–	310	100	–	23
Italian industrial soil limit	–	–	5.0	–	–	24
Italian residential soil limit	–	–	1.0	–	–	24
Italian drinking water standard	1000	–	–	–	–	24
International drinking water standard	6000	–	–	–	–	25
USEPA drinking water standard	2000	–	–	–	–	26
USEPA, acute aquatic life water standard	2400	–	–	–	–	26
USEPA, chronic aquatic life water standard	770	–	–	–	–	26
USEPA, methyl-Hg standard fish muscle	–	–	–	–	0.3	26

[a] Marine fish.

treatment with 2 N $HClO_4$; this CO_2 was collected in a solution of monoethanolamine that is then coulometrically titrated using platinum and silver/potassium-iodide electrodes. The relative percent difference in sediment, soil, and calcine sample replicates was ≤ 15% for TOC and the lower limit of determination was 0.05%. Determinations for total C and carbonate C in all mine-waste calcine and sediment samples were performed at SGS Laboratory, Toronto, under a contract with the U.S. Geological Survey.

3.2.2. Water

Concentrations of Hg were determined in the water samples using cold-vapor atomic fluorescence spectrometry (CVAFS) following EPA method 1631e (USEPA, 2002). The water samples were digested for Hg by subjecting them to BrCl oxidation for a minimum of 24 h.

Mercuric ions in the oxidized sample were reduced to Hg^0 with $SnCl_2$, and then purged onto gold-coated sand traps as a means of preconcentration and interference removal. Mercury vapor was thermally desorbed to a second analytical gold trap and then sent to a fluorescence cell and analyzed by CVAFS (Bloom, 1989). For methyl-Hg analysis, the water samples were processed following a distillation and ethylating method (Horvat et al., 1993). An ethylating agent was added to each sample to form a volatile methyl-ethyl mercury derivative, and then purged onto graphite carbon traps as a means of preconcentration and interference removal. The sample was then isothermally chromatographed, pyrolitically broken down to Hg^0, and detected using a CVAFS detector (Bloom, 1989). Sample results were corrected for distillation efficiency. All Hg and methyl-Hg determinations in water were made using a Tekran Model 2500. Recoveries for Hg on blank spikes were 99–103% and for methyl-Hg were

94–105%. Recoveries for Hg on matrix spikes were 98–102% and for methyl-Hg were 75–102%. The relative percent difference in water sample replicates varied from 3–11% for Hg and 2–26% for methyl-Hg. The SRM, NIST 1641d was analyzed with the samples and Hg recovery was 97–101% of the certified value. Although no SRM for methyl-Hg is available for water, the SRM tissue sample DORM-3 was analyzed along with the water samples to evaluate method accuracy and methyl-Hg recovery was 93–100% of the certified value. Method blanks were below the lower limit of determination of 0.1 ng/L for Hg and 0.02 ng/L for methyl-Hg in water samples.

3.2.3. Fish

Fish muscle and whole fish samples were analyzed for the concentration of Hg following USEPA Method 7473 (USEPA, 2009a) using a direct mercury analyzer (DMA-80, Milestone Inc., Shelton, Connecticut) at the U.S. Geological Survey, Denver. Recoveries of Hg in blank spikes varied from 101 to 102% and Hg recoveries in matrix spikes varied from 93 to 100%. Analysis of sample replicates indicated a relative percent difference of 4–10% for Hg. The SRM's, DORM-2, DORM-3 and TORT-1 were analyzed with the samples and recoveries of Hg were 94–104% of the certified values. Method blanks were below the lower limit of determination, which was 0.001 μg/g for Hg during the analysis of fish.

A subset of 17 fish muscle samples were analyzed for methyl-Hg concentrations by using a modification of EPA Method 1630 with the digestion as described in (Bloom, 1989) by CVAFS (using a Tekran Model 2500) at Battelle Marines Sciences Laboratory (Sequim, Washington). Recoveries of methyl-Hg in blank spikes varied from 96–103% and from 91–114% in matrix spikes. The relative percent difference in sample replicates was ≤5% for methyl-Hg. The SRM, DORM-3 was analyzed with the samples and recovery of methyl-Hg was 97% of the certified value.

4. Results and discussion

Comparative standards for Hg (Table 3) are used throughout the text for comparison to solid, water, and fish samples collected in this study. The probable effect concentration (PEC) for Hg of 1.06 μg/g in sediment is the concentration above which harmful effects are likely to be observed in sediment dwelling organisms (MacDonald et al., 2000). A similar Hg concentration of 1.0 μg/g has been established as the limit in Italy for public and residential use of soil (commonly referred to as the "green limit"). For industrial use of soil, Italy has established a Hg limit of 5.0 μg/g (IME, 2006), while no national standards have been set for methyl-Hg. The recommended USEPA Soil Screening Levels (SSL) for inorganic Hg are 310 μg/g in industrial soil and 23 μg/g for residential soil (USEPA, 2008). Limits recommended by the USEPA for methyl-Hg in industrial and residential soil are 100 and 7.8 μg/g, respectively. Comparative standards are also shown for water and fish (Table 3).

4.1. Calcine

Concentrations of Hg in mine waste calcine samples collected in the Amiata district ranged from 25 to 1500 μg/g, whereas methyl-Hg varied from 0.34 to 26 ng/g (Table 1). All concentrations of Hg found in ASSM calcine exceeded the PEC for Hg (MacDonald et al., 2000), but the PEC is established for sediment and is not directly applicable to mine waste calcine, thus, the industrial SSL is a more appropriate point of comparison for calcine. Only three ASSM calcine samples exceeded the USEPA industrial Hg SSL of 310 μg/g (USEPA, 2008) (Table 1; Fig. 2), whereas all ASSM calcine samples exceeded the much lower industrial SSL for Hg of 5 μg/g used in Italy (IME, 2006) (Fig. 2). More important than the concentration of Hg is the concentration of methyl-Hg, which is highly toxic to all organisms
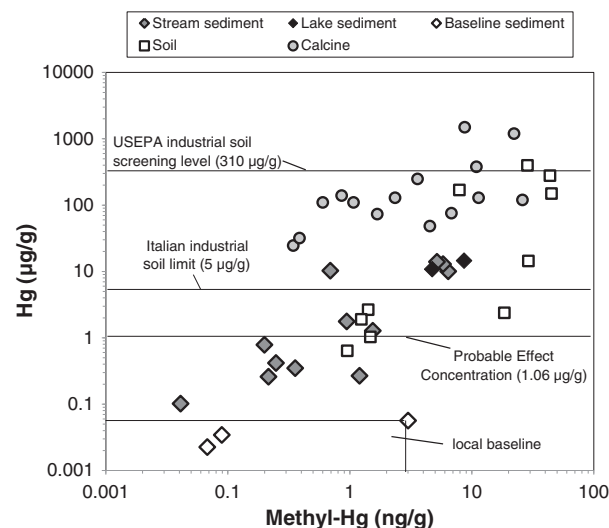

Fig. 2. Concentrations of methyl-Hg versus Hg in calcine, sediment, and soil samples collected in the Monte Amiata area. The limit in Italy for industrial soil, the USEPA screening level for industrial soil, and the Probable Effect Concentration (PEC) are shown for reference. Local baseline data are also shown for reference (Table 1).

(Eisler, 1987; USEPA, 1997). All concentrations of methyl-Hg in the ASSM calcine samples were lower than the USEPA methyl-Hg industrial SSL of 100 μg/g (USEPA, 2008) (Table 3).

Concentrations of Hg in the ASSM calcine samples were elevated compared to sediment collected from local baseline sites in this study and worldwide Hg baselines (Table 3; Fig. 2). Elevated Hg concentrations in mine-waste calcine worldwide is generally a result of the incomplete process of Hg ore retorting, thus, minor unconverted cinnabar is present in calcine. In addition, Hg^0 and other insoluble and soluble Hg compounds, formed during ore retorting, are also found in calcine (Esbrí et al., 2010; Gray et al., 2010; Kim et al., 2003, 2000). Concentrations of Hg and methyl-Hg in calcine from the ASSM were similar to those reported in calcine collected from other Hg mines worldwide (Table 3). Much higher concentrations of Hg have been reported for the Hg districts of Almadén, Spain, and Alaska and Nevada in the USA (Table 3). Furthermore, the ASSM calcine samples contained lower methyl-Hg concentrations than calcine at Almadén, and Hg mines in California and Nevada, USA (Table 3). Methylation of Hg is generally enhanced in anaerobic, organic-rich environments (Compeau and Bartha, 1985; Ullrich et al., 2001). In the Monte Amiata area, calcines were low in TOC and such low organic matter contents likely limit the ability of bacteria to methylate Hg.

4.2. Stream and lake sediment

Sediment samples were collected from the Paglia River and its tributaries and from lakes located along the Paglia River (Fig. 1) to evaluate Hg contamination and Hg methylation as result of past Hg mining and ore retorting. Local baselines were established by sampling sites distal from Hg mines in the region, although collection of uncontaminated baseline samples was difficult in this area as there are many Hg mines and potentially undiscovered Hg deposits (i.e., the region has high "geogenic" Hg). Stream sediment samples collected downstream from the ASSM ranged in Hg concentration from 0.26 to 14 μg/g (Table 2). The two lake sediment samples contained among the highest concentrations of Hg for sediment in this study (11 and 15 μg/g; Table 2), but similar Hg concentrations (10 to 14 μg/g, 10MTA10-12; Table 2) were found for Paglia River sediment samples collected from sites 3 to 5.5 km downstream from the ASSM (Fig. 3). The two lake sediment samples contained highly elevated Hg compared to the sediment sample collected upstream (10MTA06, 1.8 μg/g, Table 2), but the lakes are located downstream, and receive runoff,
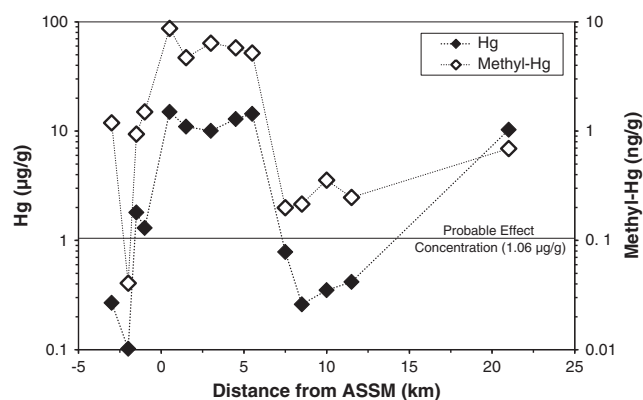
Fig. 3. Concentration of methyl-Hg and Hg in sediment versus distance from the ASSM. The Probable Effect concentration (PEC) is shown for reference (MacDonald et al., 2000). Positive distances shown are downstream from the ASSM, negative distances are upstream from the ASSM.

from calcine piles containing high Hg concentrations, whereas site 10MTA06 is upstream from these calcines (Fig. 1b). In addition, sediment collected at a site 20 km downstream from the ASSM contained a Hg concentration of 10 μg/g, but this site clearly receives sediment runoff from additional Hg mines of the Monte Amiata district located at the headwaters of other tributaries of the Paglia River (Fig.1). Sediment collected upstream from the ASSM and from regional baseline sites contained significantly lower Hg concentrations (0.023–0.27 μg/g), but were generally higher than the range of concentrations of Hg of 0.0123 to 0.056 μg/g reported for upper continental crust (Rudnick and Gao, 2003). Not all samples collected upstream from the ASSM are uncontaminated with respect to Hg, for example, mineralized rocks were observed in bed load sediment at site 10MTA01 and this sample contained a Hg concentration of 0.27 μg/g, which was higher than the baseline sites (Table 2; Fig. 3). This site likely receives runoff from undiscovered Hg-bearing mineralized rock, which is to be expected in an area with abundant mines and Hg-rich mineral deposits. Higher baseline concentrations of Hg are common in areas of past Hg mining worldwide as there are many Hg deposits, rocks, and minerals in these areas that contain elevated Hg (Berzas Nevado et al., 2003; Gray et al., 2004).

High concentrations of Hg found in stream sediment samples collected downstream from the ASSM were mainly the result of runoff from deposits of ASSM calcines, which were discarded on site and continue to erode into local streams. Although minor calcine was observed in streams proximal to the ASSM, Hg concentrations in stream sediment samples were generally lower than those found in ASSM calcine (Tables 1 and 2; Fig. 2). Concentrations of Hg in sediment samples collected from the Paglia River remained high and relatively constant for about 6 km (Fig. 3), followed by a sharp decrease, and an increase at site 10MTA20 about 20 km downstream from the ASSM (as previously noted, site 10MTA20 receives runoff from other Hg mines of the Monte Amiata district). The sharp decrease in the sediment Hg concentration at about 6 km downstream (0.79 μg/g, 10MTA13) is a result of increased dilution as several tributaries flow into the Paglia River from the east and north upstream from this site and the river at this site is larger with higher discharge than the sites upstream.

Concentrations of Hg in stream sediment samples collected in this study were similar to, but generally lower than, those reported in streams and rivers near other Hg districts worldwide (Table 3). More than 50% of stream and lake sediment samples collected downstream from the ASSM (Fig. 2) exceeded the PEC for Hg of 1.06 μg/g (MacDonald et al., 2000).

Stream and lake sediment samples collected downstream from the ASSM contained methyl-Hg concentrations that ranged from 0.20 to 8.7 ng/g (Table 2). Concentrations of methyl-Hg in sediment
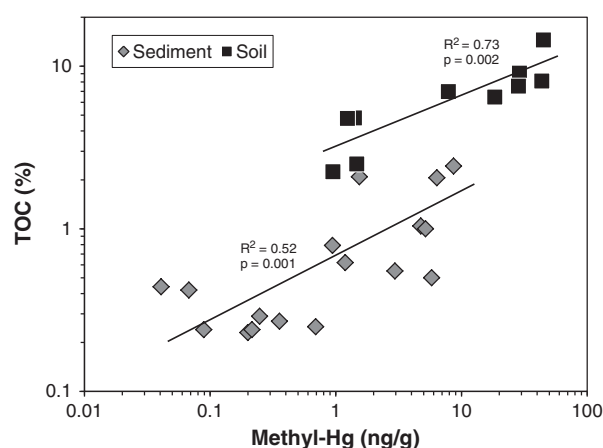
showed a similar trend to that of Hg (Fig. 3). Concentrations of methyl-Hg in sediment showed a significant correlation with TOC ($r^2 = 0.52$, $p = 0.001$; Fig. 4), a finding which has been observed in other studies (Gray and Hines, 2009; Olson and Cooper, 1976). Data for stream sediment samples also indicated a strong correlation between concentrations of Hg and methyl-Hg ($r^2 = 0.73$, $p \ll 0.01$, Fig. 5). These correlations indicated significant methylation of Hg and potential bioavailability of Hg in sediment of the Paglia River downstream from the ASSM, especially at sites with higher TOC.

4.3. Soil

Soil was sampled inside the abandoned ASSM next to retort stacks in order to evaluate the extent of Hg contamination and methylation of Hg in the area. Additional soil samples were collected from sites located more distal from the mine north and south of the town (Fig. 1), where there has not been any retorting activity. Concentrations of Hg in soil ranged from 0.64 to 400 μg/g (Table 1) and were highly elevated in samples collected proximal to the retort stack (150 to 400 μg/g). High concentrations of Hg in soil around other Hg mines worldwide are common (Table 3). For example, Hg concentrations were found to be as high as 8889 μg/g in soil near the Almadén retort, Spain and were as high as 790 μg/g in soil in the Wanshan district, China (Table 3). Soil collected near the abandoned ASSM retort were enriched in Hg by several orders of magnitude when compared to the 0.01 to 0.50 μg/g range of Hg concentration reported for uncontaminated soil worldwide (Senesi et al., 1999). In addition, concentrations of Hg in soil collected north and east of the ASSM decreased consistently with increasing distance from the retort, decreasing from 14 to 0.64 μg/g at distances of 1 to 3 km. Such high Hg
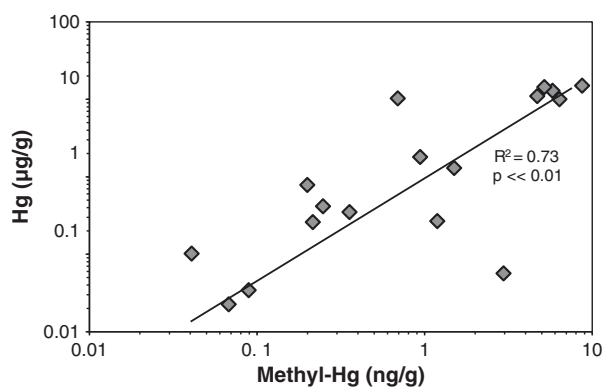

Fig. 4. Concentration of methyl-Hg versus TOC for sediment and soil samples.

Fig. 5. Concentration of methyl-Hg versus Hg in sediment samples.

concentrations in soil were the result of local Hg deposition from emission of Hg via retort stacks during cinnabar ore retorting. Previous research has estimated that more than 10,000 t of Hg[0] escaped during the century-long activity at the ASSM (Ferrara et al., 1998).

Soil samples collected distal from the ASSM contained Hg concentrations less than the USEPA residential and industrial SSL's (USEPA, 2008) (Table 3). Conversely, concentrations of Hg in all soil samples collected proximal to the ASSM retort stack exceeded the 23 µg/g USEPA residential SSL, but only one soil sample (10MTA40) exceeded the 310 µg/g USEPA industrial SSL for Hg (Tables 1 and 3). In addition, concentrations of Hg in all soil samples collected near the ASSM retort exceeded the 5 µg/g Italian SSL for Hg for industrial use (Tables 1 and 3). Soil samples collected distal from the ASSM contained Hg concentrations that exceeded the Italian residential SSL of 1 µg/g, with the exception of 10MTA38 that contained a Hg concentration of 0.64 µg/g (Table 1).

Methyl-Hg concentrations in soil in this study ranged from 0.95 to 45 ng/g (Table 1) and were higher than those reported in soil in Wanshan, China, and Southwestern Alaska, USA (Table 3). Methyl-Hg in soil collected near the ASSM was generally higher than that found in the sediment and calcine samples in this study, which was likely due to the generally higher TOC in these samples (Table 1). There is a significant correlation between methyl-Hg and TOC ($r^2 = 0.73$, $p = 0.002$; Fig. 4) in the collected soil samples that is consistent with the strong affinity of methyl-Hg for organic matter (Ullrich et al., 2001). Despite these high methyl-Hg concentrations, all soil samples in this study were considerably below the USEPA methyl-Hg SSL of 100 (Table 3) for industrial soil, but 60% of the soil samples exceeded the 7.8 µg/g residential SSL (USEPA, 2008).

4.4. Water

Water was collected from streams, lakes, springs, local baselines, mine adits, and from a town drinking water supply in and around the ASSM area (Fig. 1, Table 2). Water collected from streams draining the ASSM was elevated in Hg and methyl-Hg and ranged from 3.7 to 1400 ng/L and 0.10 to 3.0 ng/L, respectively (Table 2). Water with highly elevated Hg and methyl-Hg concentrations was collected at site 10MTA10 (Fig. 1)—this site is 300 m downstream from the point at which a drainage tunnel discharges water from underground workings of the ASSM (Fig. 1). However, the sample (10MTA11) with the highest Hg (1400 ng/L) and methyl-Hg concentration (3.0 ng/L) was located about 1.5 km downstream from the ASSM discharge tunnel, and in addition, this site is located near the point where sewage water from the town of Abbadia San Salvatore is discharged into the Paglia River. Such sewage potentially influences Hg in water at this site. Stream water at site 10MTA11 was also unusually turbid, with abundant suspended particulates visible in the stream, and was likely affected by Hg attached to particulates derived from sewage discharge or by runoff from calcines in and around in the town of Abbadia San Salvatore, or both. Additional water samples collected from sites downstream from 10MTA11 on the Paglia River generally contained decreased concentrations of Hg and methyl-Hg with increasing distance (data not shown).

Water collected from two lakes and three mine adits was also elevated in Hg and methyl-Hg and varied from 55 to 390 ng/L and 0.15 to 1.7 ng/L, respectively (Table 2; Fig. 6). Spring water (Hg = 2.6 ng/L, methyl-Hg ≤ 0.02 ng/L) and water used as a source of drinking water (Hg = 2.8 ng/L, methyl-Hg ≤ 0.02 ng/L) for the town of Abbadia San Salvatore was also collected and these samples contained Hg and methyl-Hg similar to the local baseline water samples (Table 2; Fig. 6). Concentrations of Hg in all water samples collected in this study were below established drinking water standards for Hg including (1) the 2000 ng/L drinking water standard used in the USA (USEPA, 2009b) and (2) the 6000 ng/L international drinking water standard (WHO, 2005) (Table 3). Only one sample (10MTA11)
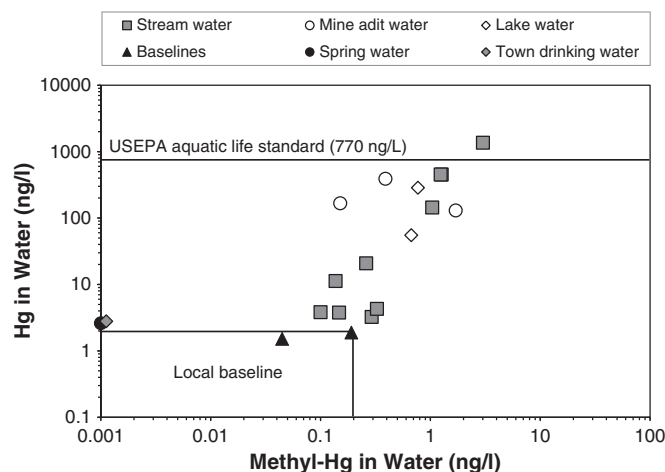

Fig. 6. Concentration of Hg versus methyl-Hg in water samples collected in this study.

exceeded the Hg concentration of 770 ng/L (Fig. 6), the standard recommended to protect against chronic effects to aquatic wildlife in the USA (USEPA, 2009b) and this sample also exceeded the 1000 ng/L Hg concentration used as the drinking water standard of Italy (IME, 2006)—all other water samples contained Hg below these two standards (Table 3). There is no recommended standard for methyl-Hg in water. Concentrations of Hg and methyl-Hg in water collected from adits, lakes, and streams in and around the ASSM were elevated when compared to local baselines (Fig. 6) and these water data indicate significant runoff of Hg from the ASSM and transference of methyl-Hg from the sediment column to water in the area.

4.5. Fish

Concentrations of Hg were elevated in fish muscle collected in this study and ranged from 0.16 to 1.2 µg/g (wet weight), and averaged 0.84 µg/g (Fig. 7). Of the fish muscle samples, 96% (66 of 70 samples) exceeded the 0.3 µg/g (methyl-Hg, wet weight) USEPA fish muscle standard recommended to protect human health (USEPA, 2009b). A subset of 17 fish muscle samples were analyzed for methyl-Hg and these data confirmed that ≥90% of the Hg in these fish was methyl-Hg (Fig. 8), which is consistent with the conclusion of other studies indicating that 90 to 100% of Hg in freshwater fish is methyl-Hg (Fitzgerald and Clarkson, 1991). Thus, Hg concentrations in fish muscle analyzed in this study were directly comparable to the 0.3 µg/g methyl-Hg USEPA standard.

Concentrations of Hg found in the fish muscle samples collected in this study are among the highest found downstream from areas
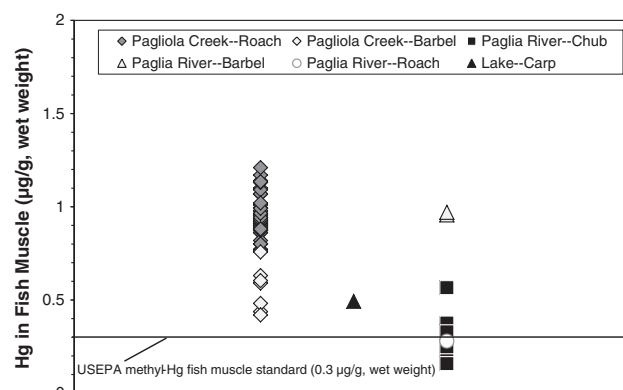

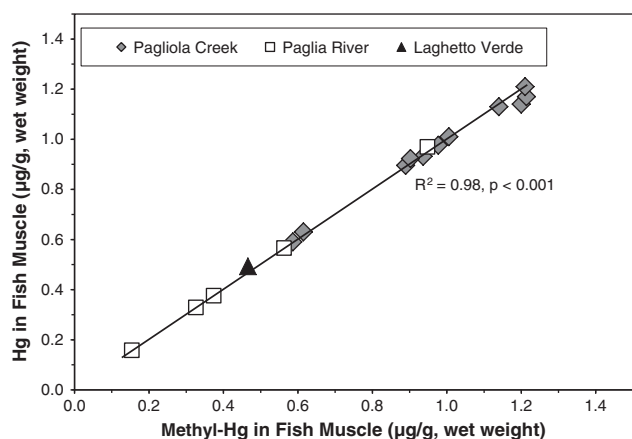Fig. 7. Concentration of Hg in fish muscle collected in the study area.

Fig. 8. Concentration of Hg versus methyl-Hg in fish muscle samples collected in this study.

mined for Hg (Table 3). The fish muscle Hg results found in the ASSM area were surprising considering that the fish collected are small and that they are bottom feeding, herbivorous fish. Generally, bottom feeding fish are low in the fish order and generally have lower Hg concentrations compared to higher order, carnivorous fish (USEPA, 1997). No correlation was found for Hg in fish versus fish wet weight or Hg in fish versus fish length (data not shown).

Elevated Hg concentrations in freshwater fish collected from areas downstream from Hg mines is not unusual (Table 3), e.g., fish collected downstream from Hg mines in California, USA, have been reported to contain Hg concentrations as high as 2.1 μg/g (muscle, wet weight) (Davis et al., 2008). In addition, elevated concentrations of Hg in fish were reported from the ASSM area as early as 1973, when Hg in fish muscle was reported to be as high as 8.0 μg/g in fish collected from the Paglia River (Bacci and Renzoni, 1973). The Bacci and Renzoni (1973) study was carried out during the time of active Hg mining and Hg retorting at the ASSM and these fish muscle data suggest that there was much higher Hg bioavailability at that time. Presently, in the ASSM area, exposure of Hg to humans consuming fish caught from Pagliola Creek and Paglia River is potentially high, although fish collected from this area are small and unlikely to be typically

used for food sources. In the United States, water bodies with average fish muscle Hg concentrations exceeding 0.3 μg/g are posted with an advisory recommending against human consumption of fish caught in these water bodies (USEPA, 1997). There is presently no human health advisory posted recommending against consumption of fish contaminated with Hg for either Pagliola Creek or the Paglia River.

5. Conclusions

Downstream transport of Hg from the ASSM is the dominant source of Hg to the Paglia River ecosystem. Concentrations of Hg and methyl-Hg were elevated in sediment and water in the Paglia River downstream from the ASSM, which is a result of runoff from mine-waste calcine and active methylation of Hg in this ecosystem. More than 50% of the stream sediment samples collected downstream from the ASSM in this study exceeded the PEC for Hg of 1.06 μg/g. Correlation of methyl-Hg concentrations with TOC in sediment indicates active methyl-Hg formation at sites with abundant organic matter. Concentrations of Hg in soil ranged from 0.64 to 400 μg/g and were highly elevated in samples collected proximal to the retort stack (150 to 400 μg/g). All soil samples collected near the ASSM retort exceeded the 5.0 μg/g Italian SSL for Hg for industrial soil use, whereas most soil collected distal from the ASSM contained Hg below this industrial limit. Water collected from streams draining the ASSM was elevated in Hg and methyl-Hg and varied from 3.7 to 1400 ng/L and 0.10 to 3.0 ng/L, respectively, whereas the local baseline stream water sites varied in Hg and methyl-Hg from 1.5 to 1.9 ng/L and 0.045 to 0.19 ng/L, respectively. Of the freshwater fish collected, 96% of the fish muscle samples exceeded the 0.3 μg/g (methyl-Hg, wet weight) USEPA fish muscle standard. Data show a progressive increase in the ratio of methyl-Hg/Hg in calcine (0.001–0.022%), sediment and soil (0.01–5.3%), water (0.04–10%), and fish muscle (90–100%) (Fig. 9). Concentrations of Hg and methyl-Hg in fish indicate significant bioavailability and transference of Hg to fish in the Paglia River ecosystem downstream from the ASSM.

Fig. 9. Ratio of methyl-Hg/Hg for calcine, sediment, soil, water, and fish of the Monte Amiata Hg district.

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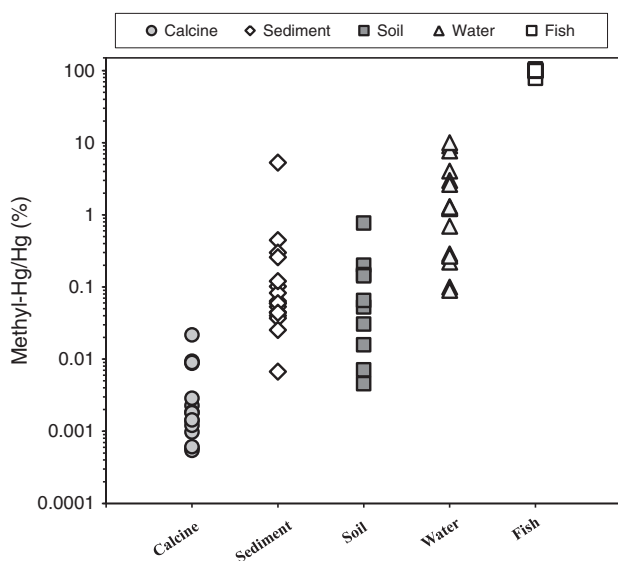
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