INTRODUCTION

Mercury (Hg) and its main mineral, cinnabar (HgS), started to be known by humans in ancient times (ca. > 4000 BCE) and their applications still continue today. The unusual property of Hg (the only metal to be liquid at standard conditions) explains its large use in various sectors of human life, ranging from medicine, agriculture to scientific and industrial applications (Gray, 2003). After the 1970s, Hg production started to be regulated at least in industrialized countries (Pirrone et al., 2010), and the European Commission adopted a legislation to ban Hg exports between the EU countries and to store excess Hg (EU, 2008).

Mercury is a pervasive pollutant, accumulating in organisms, and being highly toxic (US EPA, 1997). Organic forms, particularly methyl-Hg and dimethyl-Hg have the highest toxicity, affecting mainly the human nervous systems (WHO, 1990). After the mass poisonings events of Japan in 1970s, the symptomatology associated with methyl-Hg poisoning is known as “Minamata disease”. Human exposure to methyl-Hg mainly relates to the ingestion of contaminated food, such as edible plants, rice and fish (Qiu et al., 2012). Actually, fish consumption advisories for Hg are set in order to protect humans from indirectly exposure to methyl-Hg.

Since the first industrial period (1870-1915), the increased use of Hg in different human activities has multiplied its emission sources to the environment. Globally, about 1 million tons Hg were extracted from various ore bodies in the world (Hylander & Meili, 2003), contributing to alter the global Hg cycle. Historically, three-fourths of the Hg global production has come from the following five mining regions (Rytuba, 2003): Almadén (Spain), Idrjia (Slovenia), Mt. Amiata (Italy), California Coast Range (USA) and Huancavelica (Peru). The release of Hg from mine impacted areas represents a serious risk for biota and humans, which may be exposed to high levels of Hg for long time after the operations have ceased (Gray et al., 2000). Moreover, Hg easily converts to methyl-Hg that accumulates in biotic compartments surrounding Hg mines, resulting in decades of persistent contamination after the end of mining (Gray et al., 2000; Gosar & Teršič, 2012).

The Mt. Amiata Hg district

This PhD project focuses on the Hg district of Mt. Amiata (southern Tuscany, Italy), and on the environmental consequences deriving from the mining activity occurred in this region. Here, mining exploitation for Hg started with the Etruscans (8th - 1st centuries BC), it was interrupted during the Romans, and started again in 1849 (Strappa, 1977). In the past millennium, the Mt. Amiata production of Hg develops at a larger scale, rapidly becoming the 4th in the world for global production. The Abbadia San Salvatore mine (ASSM) (1897-1982) was one of the largest in Europe and one of the most productive. After 30 years from the end of mining, residues of calcines and metallic Hg are widely observable in soils of ASSM, particularly in the proximity of the roasting plants and condensers, where Hg droplets are visible at naked eyes.

This research project, carried on with the partnerships of different national and international research centres, as U.S. Geological Survey and Regione Toscana, addresses to the Paglia River basin (PBR), which is the main natural collector of Hg drained by the east sector of the Mt. Amiata, and especially of Hg coming from the ASSM (Fig. 1). As the remediation project for this mine is begun just in 2012, this thesis may furnish useful comparison data for the future post-remediation monitoring, and could help to define updated remediation strategies for this mine. Moreover, as the Paglia River is a tributary of the Tiber River (Fig. 1), the principal river course of Central Italy discharging to the Mediterranean Sea, the interest of this work is not limited to the local scale but may extend to the regional one.
The main goals of the work are the following: i) to establish an updated the pictures of Hg and methyl-Hg diffusion and distribution in the PBR and to characterize the principal source of Hg contamination for the basin (part I); ii) to estimate the mass load of particulate and dissolved Hg transported by the Paglia River (part II); iii) to characterize the speciation of Hg (and hence its bioavailability) in different environmental matrices (part III); iv) to define the regional background for Hg (part IV), which is a crucial step to discriminate between natural and anthropogenic contaminated geological matrices in this perturbed environment.

RESULTS AND DISCUSSION

Mercury and methyl-mercury survey (part I)

Presently, detailed studies of Hg diffusion through abiotic and biotic compartments at Mt. Amiata district are scant and relatively old. Moreover, methyl-Hg production is unknown in the sediment and water column in this area, and the determinations of Hg in fish have not been made since the 1970s (Bacci & Renzoni, 1973). Therefore, the objective of this study is to evaluate (1) the 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) the potential exposure of Hg to humans in the area through the measurement of Hg and methyl-Hg concentrations in freshwater fish. In this survey, concentrations of Hg in mine-waste calcine (retorted ore), sediment, water, soil, and freshwater fish collected from the ASSM and downstream in the Paglia River. Local baselines were established by sampling sites distal from Hg mines in the region. The results of this work have been published in 2012 (Rimondi et al., 2012).

Concentrations of Hg in calcine samples range from 25 to 1,500 μg/g, all of which exceed the industrial soil contamination level for Hg of 5 μg/g used in Italy, and indicate the presence of high proportions of unconverted cinnabar and/or secondary formed Hg compounds. Stream and lake sediment samples collected downstream from the ASSM range in Hg concentration from 0.26 to 15 μg/g; more than 50% of these samples exceeds the probable effect concentration (PEC) 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 samples collected downstream from the ASSM contain methyl-Hg concentrations that vary from 0.20 to 8.7 ng/g, which are in the range of those common encountered in worldwide Hg mines (Gray et al., 2000).
Freshwater fish were collected from the Pagliola Creek, Paglia River (Fig. 1) and from a small lake located next to the mine area (Laghetto Verde). Mercury is elevated in fish muscle samples and ranges from 0.16 to 1.2 µg/g (wet weight), average 0.84 µg/g. Of the fish samples, 96% exceeds the 0.3 µg/g (methyl-Hg, wet weight) US EPA fish muscle standard recommended to protect human health (US EPA, 2009; Fig. 2a). Higher concentrations of Hg are not surprisingly reported for fish collected from Pagliola Creek, the site located most proximal to Hg mines (Fig. 1). A subset of 17 fish muscle was analyzed for methyl-Hg and these data confirm that ≥ 90% of the Hg in these fish is methyl-Hg (Fig. 2b), as common reported in literature for freshwater fish (Fitzgerald & Clarkson, 1991). Mercury concentrations in fish muscle analyzed in this study are then directly comparable to the 0.3 µg/g (methyl-Hg, wet weight) US EPA standard.

Concentrations of Hg found in the fish muscle samples collected in this study are among the highest found downstream from areas mined for Hg (Gray et al., 2003; Horvat et al., 2004; Qiu et al., 2009), and are particularly 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 have lower Hg concentrations compared to higher order, carnivorous fish (US EPA, 1997). Therefore, such elevated Hg in these small fish indicates a significant bioavailability of methyl-Hg in the Paglia River ecosystem.

**Mass loads of dissolved and particulate mercury (part II)**

Total dissolved and particulate Hg mass balances were estimated in different hydrological seasons in four sites along the Paglia River basin (Fig. 1): one site upstream ASSM that can be referred to a “background” site for the Amiata region (Pagliola Monte, PM); (2) two sites located just downstream ASSM, in order to quantify mass loads related to runoff from the ASSM (Pagliola Valle, PV, Paglia Casetta, PC), and (3) one site at the end of the Hg district (Paglia Allerona, PA), which was collected to account for all Hg runoff from the south-eastern part of the Mt. Amiata district. Quantification of Hg mass loads in this Hg mine district can allow to: (1) identify the contamination sources, (2) evaluate the effect of Hg on the environment, and (3) determine the processes that are able to affect Hg transport. Sampling campaigns were carried out in order to sample the most different water flow conditions (i.e., March 2011) after abundant rainfall occurred during winter and spring times, and at the beginning of September 2011, after almost three months of drought. The 2012 spring was characterized by a striking hydrological deficit for the Tuscany watersheds, due to the scarce precipitations occurred in the autumn-spring seasons. As a consequence, the last campaign was performed in March 2012 and it was aimed to define how mass loads varied as result of the prolonged absence of rainy events.
Mass loads were calculated as the product of metal concentration in water and water discharge. Different aliquots of filtered and unfiltered water were then sampled to study the spatial and temporal changes in concentrations, partitioning and load of Hg and other trace metals (such as As and Sb). Moreover, both flow meters and tracer dilution methods (Kite, 1993) were applied whenever possible to calculate flow discharges in order to prevent any possible underestimations of the real water flows by flow meters (Kimball, 1997). Dilution measurements were made through injection of a specific tracer (NaCl) as a slug or gulp injection (Hudson & Fraser, 2005).

The data indicate that up to 34 g d\(^{-1}\) of Hg is transported during the rainy season at PV site as a result of direct drainage of the ASSM (Table 1). Specifically, up to 99% of Hg is transported as particulate Hg, whereas dissolved Hg loads account only for 1-33% of the total Hg flux (Table 1). As previously reported for other mining districts worldwide (Faganeli et al., 2003; Hissler & Probst, 2006; Schäfer et al., 2006), the transport of Hg in the Paglia River occurs mainly as suspended particles. The decrease of particulate loads between sites PV and PC recorded in 2011 (Table 1) is likely due to the deposition of particulate Hg along this section of the river, which is largely influenced by the reduced water flow, resulting from the decreased hydrological gradient between these two sites. The generally positive correlation between Hg loads and seasonal discharge (i.e., loads are higher during the wet season; March 2011), suggests the importance of runoff associated with heavy winter rains as an important factor promoting the mobilization of Hg in the PRB. However, the similar Hg loads reported at PV during both wet and dry seasons in 2011 (i.e., 34 and 24 g d\(^{-1}\) respectively; Table 1), support the hypothesis that a water flow-independent source of Hg exists in the basin, which supplies a constant amount of Hg independently from the discharge. Such a source may be represented by the Hg deposited along the river reach, which acts as a sort of transient sink, from which Hg is re-suspended in low flow regimes.

Table 1 - Loads of Hg reported as total (F-MeT), dissolved (F-MeD) and particulate (F-MeP) loads and expressed as g d\(^{-1}\).

<table>
<thead>
<tr>
<th></th>
<th>F-Hg(_T)</th>
<th>F-Hg(_D)</th>
<th>F-Hg(_P)</th>
</tr>
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<tbody>
<tr>
<td><strong>March 2011</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM</td>
<td>0.6</td>
<td>0.09</td>
<td>0.5</td>
</tr>
<tr>
<td>PV</td>
<td>34</td>
<td>2</td>
<td>33</td>
</tr>
<tr>
<td>PC</td>
<td>18</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>PA</td>
<td>12</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td><strong>September 2011</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>PV</td>
<td>24</td>
<td>0.17</td>
<td>24</td>
</tr>
<tr>
<td>PC</td>
<td>3.4</td>
<td>0.10</td>
<td>3.3</td>
</tr>
<tr>
<td>PA</td>
<td>0.5</td>
<td>0.10</td>
<td>0.4</td>
</tr>
<tr>
<td><strong>March 2012</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM</td>
<td>0.005</td>
<td>0.003</td>
<td>0.002</td>
</tr>
<tr>
<td>PV</td>
<td>1.3</td>
<td>0.17</td>
<td>1.1</td>
</tr>
<tr>
<td>PC</td>
<td>0.9</td>
<td>0.15</td>
<td>0.7</td>
</tr>
<tr>
<td>PA</td>
<td>1.1</td>
<td>0.22</td>
<td>0.9</td>
</tr>
</tbody>
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* Total loads are intended as the sum of dissolved flux \(F-(Hg)_D\) plus particulate flux \(F-(Hg)_P\).

Studies on the dynamics of Paglia River bed have evidenced that human interventions have severely altered its morphology, resulting in bed narrowing and channel incision (Ciccacci et al., 1988). This would prevent a definitive stacking of Hg as river bed bottom deposit, promoting on the contrary Hg mobilization and transport downstream the Paglia River. Thanks to this effective mobilization, important quantities of Hg might be directly discharged into the Tiber River, provoking a contamination of its ecosystem.
As a result of the drought in March 2012, Hg loads measured during this period likely represent the lowest Hg transport in the PRB. The prolonged absence of rainfall in the PRB results in 20/30-fold lower Hg loads at PV that those observed during the previous year. The constancy of Hg loads among the studied sites, ~ 1 g d⁻¹, suggests that these hydrological conditions are able to prevent a marked deposition of Hg along the river course, which is then further transported downstream. This season could therefore depict the condition of a steady-state flow, which occurs when Hg introduced in the river exactly balances the quantity that can be transported in such hydrological conditions by the flow.

Preliminary estimation of yearly Hg flux shows that up to 11 kg of Hg can directly be related to downstream transport by ASSM during baseline conditions.

**Mercury speciation by XAS, SEC and SEM-EDS techniques (part III)**

A fundamental step in order to evaluate the biogeochemical and, in particular, the eco-toxicological significance of Hg diffusion in the environment is to determine its speciation in solid matrices rather than the absolute content. Various independent techniques, such as SEM-EDS, sequential chemical extractions (SCE), and X-Ray Absorption Spectroscopy (XAS), are applied in this study to unravel the Hg speciation in samples coming from the Hg mining district of Mt. Amiata. A comprehensive set of samples, ranging from 27-1,500 μg/g Hg, was analyzed in this work. They include: 1) waste calcines and soils of the mining area; 2) particulate dispersed in stream waters, and stream sediments collected from the watercourse receiving drainage from the Hg mines; 3) pre-industrial stream sediments. This dataset was chosen in order to delineate a complete picture of Hg speciation in different geological and mine samples, starting from the source of Hg to the environment (calcines and soils of the mining area) down to the targets of contamination (lake and stream sediments), whereas the present-day transport conditions are monitored by means of particulate matter in waters. The study of Hg speciation in sediments presumably deposited in pre-industrial times, and therefore not affected by anthropogenic activities, should provide an indication of the local natural background.

Speciation results obtained by the application of XAS, SCE and SEM-EDS generally show Hg sulphides (cinnabar and metacinnabar) as the dominant mineral phases in most of the analyzed samples, suggesting low availability of Hg in the Mt. Amiata. However, anthropogenic processes such as ore roasting have considerable effects on the speciation of the waste calcines, mainly resulting in: a) residual minor amount of Hg⁰; b) conversion of cinnabar to metacinnabar; c) secondary formation of moderately soluble Hg(I)-chlorides and Hg-N-Cl-compounds; d) formation of extremely soluble Hg species such as HgCl₂, HgO and HgSO₄, as common documented in other mining areas (Kim et al., 2003; Esbrí et al., 2010). In particular, mercuric-chloride, -oxide, and -sulphates, although do not represent more than ~ 10% of global Hg species, may result in the mobilization of environmentally significant amounts of Hg (up to 136 μg/g), as demonstrated by SCE.

Examples of some of the Hg phases detected in this work by SEM-EDS are reported in Fig. 3.

Ionic Hg compounds are moreover revealed in present day stream sediments of Paglia River, suggesting chemical leaching of these compounds from calcines, which are still improperly disposed at ASSM. The
The presence of ionic Hg compounds in the sediments of the Paglia River represents the main environmental concern for this basin because, differently from cinnabar, these minerals can solubilize and release free \( \text{Hg}^{2+} \), which may be readily available for methylation reactions. \( \text{Hg}_0 \) may moreover contribute to methyl-Hg formation by oxidation to Hg(II) (Ullrich et al., 2001).

A preliminary estimate of mercury background (part IV)

The present work attempts to finally assess the geogenic Hg background of the Mt. Amiata area, proposing Quaternary sediments as sample media. In particular, thanks to morphological and paleo-hydrographic reconstruction of the Pagliola drainage course, fossil stream sediments referred to the ancient Pagliola were considered as pristine (pre-date mining activity), and sampled. Two groups of sediments, related to different phases of paleo-Pagliola evolution, were distinguished: PS and TR. The dataset was then completed with present-day stream sediments (SS) and sediments from a recently formed fluvial terrace (BR), which are expected to represent the modern and the historical ambient background (i.e., coeval with mining activity), respectively. In order to investigate the geochemical relationships between these groups identified by stratigraphy, multivariate statistical analysis was applied to the database of this study.

Distribution of Hg among the four groups confirms the subdivision operated in the field, reporting elevated Hg in sediments that are coeval or subsequent to mining activity (BR: 290±3 \( \mu \text{g/g} \); SS: 32±1 \( \mu \text{g/g} \)), whereas, in pristine sediments, at least one order of magnitude lower Hg is reported (TR: 2±2 \( \mu \text{g/g} \); PS: 10±4 \( \mu \text{g/g} \)). As shown by the sub-composition CaO-Hg-Al_2O_3 (Fig. 4a), higher Hg concentrations (i.e., SS and BR groups) are associated to higher CaO% (Al_2O_3/Ca \approx 1), whereas lower Hg concentrations (i.e., TR and PS groups) are coupled with lower CaO% (Al_2O_3/Ca > 1), disclosing a quasi-linear data pattern in the ternary diagram (Fig. 4a), which was better clarified by the isometric log-ratio data transformation (Fig. 4b).

This linear trend (R^2 = 0.87) suggests that Hg content in the Mt. Amiata samples is not random distributed but can be modeled and predicted on the base of CaO%, indicating that this oxide may act as a proxy for Hg in this area. The natural chemical/physical weathering of calcines, where lime was added during roasting to recover the residues of metallic Hg (calcination), must be considered the cause of the CaO-enrichment of the recent and present-day stream sediments of Pagliola, which are directly impacted by the runoff from the waste calcines disposed at ASSM. On the contrary, the absence of calcine deposits at the time when the pre-mining sediments were formed, determines lower CaO% in the PS and TR groups and corresponding lower Hg. The linear trend...
observed in the ternary diagram, may then represent an indirect estimate of the mass of calcines being altered: the higher is the quantity of weathered calcines in sediments, the higher is the corresponding Hg contents.

The Hg-background defined on the base of PS and TR groups is estimated between 2 and 6 \( \mu \text{g/g} \). This range, although representing only a preliminary estimate as the number of samples must be further increased, is two orders of magnitude higher than the normal concentrations encountered in the crust (0.056 \( \mu \text{g/g} \); Rudnick & Gao, 2003), indicating the presence of a wide geogenic anomaly in the Mt. Amiata as a result of the natural denudation of Hg-rich rocks and the dismantling of cinnabar deposits. Moreover, this Hg-background values are much higher than the limit of 1 \( \mu \text{g/g} \) established by the Italian legislation for Hg in soils, suggesting that a most reliable contamination level should be then defined for this area in order to: a) properly discriminate between the natural baseline and the anthropogenic contamination; b) define reliable and cost-effectiveness thresholds for the remediation project carrying on at the ASSM.

CONCLUSIONS

After three decades from the end of mining, the results of this study indicate that a still pervasive Hg diffusion is present in all environmental compartments belonging to the Paglia River, mainly as a result of Hg runoff from contaminated waste calcines (up to 1500 \( \mu \text{g/g} \) Hg), piled up without proper disposal at Abbadia San Salvatore. Weathering/leaching of these deposits is highly impacting for the downstream environment of Paglia River, affecting: i) the major and minor chemical composition of sediments; ii) the speciation of Hg in geological matrices; iii) the formation of methyl-Hg. The extremely high bioavailability of methyl-Hg in the Paglia River basin is the most significant environmental concern for the Mt. Amiata region, which can relate to the presence of Hg soluble phases in calcines and river sediments, easily to methylate. Additional research on the factors influencing and enhancing methyl-Hg reactions in this area is required, due to the potential high impact of this compound to biota living in this ecosystem and to humans.

This project may represent a useful base to further investigate specific aspects of Hg geochemistry in the Mt. Amiata area and may help with the on-going remediation strategy that is carrying on at the Abbadia San Salvatore Hg mine. Moreover, as the importance of the Mt. Amiata district is not limited to the local scale but may extend far away from this region, a study focusing on the Tiber River abiotic and biotic compartments is projected in order to quantify the impact of Paglia River on this basin and then to the Mediterranean Sea.

REFERENCES


