

# Chemical availability of mercury in stream sediments from the Almadén area, Spain

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The chemical speciation, fractionation and availability of mercury in sediments from a cinnabar mining area (Almadén, Spain) was studied with different extraction and analytical procedures, in order to determine the degree to which the ecosystem is harmed by this pollutant. Three total extraction procedures, a sequential extraction and the speciation of organo-mercury compounds were performed in nine sediment samples. In the study area, although concentrations of mercury can be extremely high (up to 1000 mg kg<sup>-1</sup>), no organo-mercury compounds were detected (<2 µg kg<sup>-1</sup>) and the availability of this element seems restricted. One of the methods for total extraction presented considerably lower recovery in Almadén's sediments, yet the results were controlled with certified reference materials. This disagreement was attributed to the fact that the mercury is in a refractory form. Sequential extraction was able to show that most of the mercury is associated with sulfides (probably as metacinnabar) or in the residual refractory phase (probably as red cinnabar).

## Introduction

The contamination of the environment by mercury has been a matter of large concern throughout the world for the last four decades. Since the early 1960s, the growing awareness of mercury pollution has stimulated the development of more accurate, precise and efficient methods to determine mercury and its compounds in a wide range of matrices. Special attention has been paid to the determination of mercury in aquatic systems, because this element tends to accumulate in bottom anaerobic sediments.<sup>1</sup>

The determination of total mercury content is frequently carried out to assess the extent of the contamination accumulated and to provide an overview of the long-term potential toxicity effects. However, the determination of total mercury concentrations is not sufficient for an understanding of the environmental impact and pathways of the different species.<sup>2</sup> Transport, bioavailability, and physico-chemical and toxicological properties are highly dependent on the chemical form that is present in the environment.<sup>2-4</sup> Mercury can exist in a large number of different physical and chemical forms with a wide range of properties. These forms can be defined (a) functionally, *e.g.*, as “available species”, (b) operationally, according to the reagent or procedure used for their isolation or (c) as a specific chemical compound or oxidation state, for instance as “methylmercury” or “mercuric mercury”.<sup>5,6</sup> In this work, the procedures for the determination of the first and second families of forms are going to be referred to as fractionation. The third family is going to be referred to as speciation.

Operationally defined metal fractionation procedures often involve the use of extractants that release species associated with particular sediment phases. In sequential extractions, the sample is treated with a succession of reagents intended to dissolve different sediment phases.<sup>7</sup> One of the first sequential

extraction methods was developed by Tessier *et al.*<sup>8</sup> and has been thoroughly applied since then, with minor modifications, to a wide range of samples and elements.<sup>9</sup> Due to its specific chemical characteristics, the fractionation procedures applied to other metals have shown to be unsuitable for mercury. For instance, Wallschläger *et al.*<sup>10</sup> and Barrocas and Wasserman<sup>11</sup> have shown that very little mercury is present in very labile phases (exchangeable). On the other hand, the humic/fulvic phase constitutes an important carrier for mercury and deserves a specific step.<sup>10-12</sup> An important phase is sulfide,<sup>13,14</sup> which renders mercury unavailable for methylation.<sup>15,16</sup> Mercury sulfide has been extracted by a number of different partial procedures,<sup>17,18</sup> and Barrocas and Wasserman<sup>11</sup> included a specific step for mercury sulfide (based on ref. 17) in a sequential extraction procedure. Finally, the mercury from the residual phase does not need to be extracted with hydrofluoric acid as stated for other metals, because it is seldom associated with silicate minerals.

Because of the varying levels of toxicity of the different forms of mercury, knowledge of the speciation is crucial for establishing environmental issues. The most common chemical forms are: elemental mercury (Hg<sup>0</sup>), divalent inorganic mercury (Hg<sup>2+</sup>), methylmercury (CH<sub>3</sub>Hg<sup>+</sup>) and dimethylmercury ((CH<sub>3</sub>)<sub>2</sub>Hg). Of particular interest is methylmercury, not only because it is highly toxic but also because this element can be methylated in the environment (specifically marine and freshwater sediments) and accumulated in the tissues of fish, with extensive bio-magnification along the food chain.<sup>2,19</sup>

Almadén is located in Central Spain and forms part of a large geological anomaly of the Mediterranean basin (Fig. 1). The Almadén is the largest (1/3 of the total world production) and the oldest (more than 2000 years) mercury mine/refining operation in the world. Almadén's extensive cinnabar deposits and mining activities constitute a “natural laboratory” for the study of mercury exchanges and transformations. The long-term mercury permanence in the Almadén's environment probably yielded a steady state cycling and methylation rate. In spite of the importance of this area, only a few studies have

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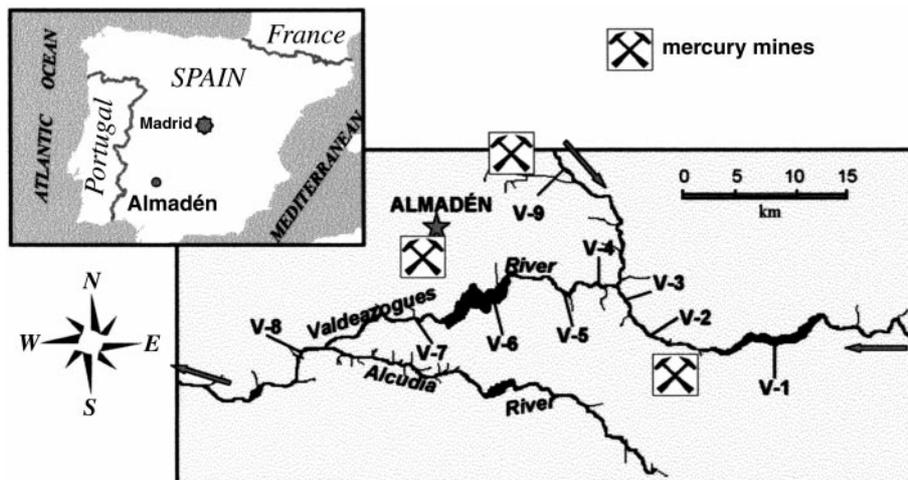


Fig. 1 Location of sampling sites in the Valdeazogues River (Almadén area, Spain).

been conducted to evaluate the environmental impact and potential hazards of mercury contamination.<sup>20-23</sup> Recently, an integrated program to assess the distribution of total and mono-methylated mercury in the aquatic environment of the Almadén area was carried out.<sup>24</sup> The present work is included in this program and reports the results obtained in the sediments, aiming at the evaluation of mercury availability and harm for the environment in Almadén.

Three different methods of total extraction were applied to sediment samples from the Almadén area (Valdeazogues River) with the aim of inter-comparing their results and establishing if they are suitable for specific samples from cinnabar mining areas. The next step was to identify which geochemical phase would bear mercury and if this mercury was available to be remobilized into the environment. In this case, a sequential extraction was applied. Finally, the speciation of mercury was performed in order to identify which forms were present, and to quantify the extent of mercury methylation in the sediments.

## Experimental

### Sample collection, storage and preservation

Nine sediment samples were collected from Valdeazogues River (Fig. 1). Station V-1 was the only one located upstream from the mercury mines, stations V-2 to V-9 were subject to mining wastes. Station V-9 is located on a tributary to the Valdeazogues River and is also influenced by mine wastes. In September 1997, samples were grab collected from the top 5 cm of the sediment. Five sub-samples were homogenised in a stainless-steel and plastic mixer to constitute each composite sample. The sediment samples were transported to the laboratory under refrigeration and air-dried for 3 d, large aggregates were broken up and small pebbles were discarded. The samples were ground with an agate mortar to a particle size smaller than 63  $\mu\text{m}$  (in a Nylon sieve). The samples were placed in hermetic polyethylene bags at 4 °C until analysis. Organic matter content was estimated by loss on ignition at 500 °C.

### Instrumentation

**Open focused microwave oven.** A commercial open focused microwave digester (Model 301, Prolabo, Fontenay-sous-bois, France) with a frequency of 2450 MHz and a maximum power of 200 W, equipped with a TX32 programmer (Prolabo) was used for the total mercury and speciation extractions. The samples for extraction were placed in a 50 mL round-bottomed open borosilicate vessel (150 mm, 35 mm id). A refluxing condenser unit on the top of the extraction vessel prevented possible losses of the analyte by volatilisation.

**Closed microwave acid digestion bomb.** The bomb (Parr 4782 microwave digestion bomb) consisted of a sealable 45 ml PTFE beaker, within a polymer resin shell. The bomb has a pressure indicator and an internal pressure release mechanism for safety. This safety mechanism operates when the pressure in the bomb reaches 1200 psi. A domestic microwave oven (800 W) was used for the extraction.

**Flow-injection inductively coupled plasma mass spectrometry (FI-ICP-MS).** An Elan 6000 inductively coupled plasma mass spectrometer (Perkin-Elmer, Überlingen, Germany) equipped with a Scott-type double-pass spray chamber and a Perkin-Elmer FIAS 400 flow injection system and an AS 91 auto-sampler was used for total mercury analysis. Optimisation was carried out daily with a normal verification solution (10 ng ml<sup>-1</sup>, Rh, Mg, Pb, Ce, Ba). Two isotopes of mercury (<sup>200</sup>Hg, <sup>202</sup>Hg) were measured in each determination, in order to avoid possible interferences. Concentrations based on the mean intensity of the mass spectrometry signal were automatically calculated by the Sciex software installed on a personal computer (IBM PS/2 Model 70). To avoid memory effects a 200 ng ml<sup>-1</sup> gold solution (1% HNO<sub>3</sub> + 0.5% HCl) was added to all samples, standards, blanks and the rinse solution.<sup>25</sup>

**Cold-vapour atomic absorption spectrometry.** An atomic absorption spectrometer (Varian, Mulgrave, Australia) Model Spectraa-300, equipped with a mercury hollow cathode lamp operated at 4 mA (spectral band-pass, 0.5 nm) and a resonance wavelength of 253.7 nm was used for total mercury analyses. A Varian Model VGA-96 mercury/hydride system was connected to the spectrometer to generate the mercury vapour.

**Ethylation, cryofocusing, gas chromatography, quartz furnace atomic absorption spectrometry (Et-CT-GC-QFAAS).** The determination of mercury species was performed by an automated in-line ethylation system with pre-concentration and separation by cryofocusing/thermal desorption followed by detection in a quartz furnace atomic absorption spectrometer. All the analytical steps are controlled by the BORWIN software (JMBS Developments, Grenoble, France). A detailed description of the system can be found elsewhere.<sup>26-32</sup> An approximately 0.01% m/v solution of sodium tetraethylborate (NaBEt<sub>4</sub>) was prepared in a glove-bag, filled with N<sub>2</sub>, by dissolving the reagent in water. The NaBEt<sub>4</sub> solution was purged with helium for 30 min in order to eliminate volatile mercury compounds that contaminate the reagent.<sup>33</sup>

### Standard stock solutions and certified reference materials

Standard stock solutions (1000 µg ml<sup>-1</sup>) of Hg<sup>2+</sup> and methylmercury were prepared by dissolving mercury(II) chloride in 1% HNO<sub>3</sub> and methylmercury chloride in methanol, respectively. All stock solutions were stored in a refrigerator and protected against light. Working standard solutions were prepared by appropriate dilution in water from the stock solutions and were stored for not more than 1 week.

Certified reference materials were used to validate the applied methods (Table 1). IAEA 356 is a reference sediment from the International Atomic Energy Agency, certified for total mercury. PASC-1 is a certified reference sediment from the National Research Council of Canada. GBW 08301 (river sediment), GBW 07311 (stream sediment) and GBW 07301 (stream sediment) were purchased from the National Research Centre for CRM (China) and are certified for total mercury. CRM 580 is certified for methylmercury and was obtained from the BCR (Community Bureau of Reference) and BCR 320 is a river sediment that was also obtained from the Community Bureau of Reference. BCR S19 (Estuarine sediment) is a candidate reference material which was used in an intercomparison exercise organised by the BCR.<sup>34</sup>

### Procedures for mercury analysis

A scheme of the extraction procedures used in this work is presented in Fig. 2. The experimental procedures are summarised below and have been fully described in previous publications.

**Total mercury analysis using an open-focused microwave system (TMOM)<sup>35</sup>.** A 0.25 g sediment sample was extracted in the open microwave digester at 20 W for 5 min. After cooling, the extracts were stored in a refrigerator until analysis. Strong memory effects have been reported in the literature when mercury is determined by ICP-MS. A previously proposed method, adapted from Rosland and Lund,<sup>25</sup> using a gold solution (200 ng ml<sup>-1</sup>) in an acid medium (1% HNO<sub>3</sub>+0.5% HCl) was applied. Linear calibration curves ( $r=0.9997-1.0000$ ) were obtained and the sensitivity did not change when repeated calibration curves were executed, indicating that problems emerging from memory effect of mercury were completely overcome. Under these conditions IAEA 356, PASC-1 and BCR S19 reference material analyses were carried out (Table 1), showing that this digestion procedure was long enough to recover 100% of the mercury, as previously stated in the literature by Woller *et al.*<sup>35</sup>

**Total mercury analysis using a closed microwave system (TMCM)<sup>36</sup>.** Approximately 0.5 g of dry sediment was weighed in a Parr 4782 bomb and microwave extracted (Fig. 2). The bomb was then cooled and the sample filtered through an Albet (Rosello, Barcelona, Spain) type 1238 paper into a calibrated flask and made up to a volume of 10.0 or 25.0 ml with Milli-Q water (Millipore, Milford, MA, USA). Measurements were carried out by cold-vapour atomic absorption spectrometry. The accuracy of the method for total mercury determination was tested by analysing three reference materials. Eight aliquots of each material were mineralised, measured, and the results are shown in Table 1.

**Total mercury analysis using conventional heating (TMCH)<sup>37,38</sup>.** A 2 g dry sediment sample was weighed into a PTFE bomb and dampened with 5 ml water. Five ml of *aqua regia* (3:1 conc. HCl–conc. HNO<sub>3</sub>) were added to this solution. As soon as no further effervescence remained the bomb was sealed, heated in a water bath for 5 min at 60 °C and allowed to cool to room temperature. Then 50 ml of water and 15 ml of potassium permanganate (5% m/v) were added and digestion continued by heating to 60 °C in a water bath for 30 min. The extract was separated by centrifugation and measured by ICP-MS. The BCR 320 reference material was analysed to control the quality of the results. Sediment residues from the sequential extraction (“residual phase”) procedure were digested the same way.

**Mercury sequential extraction (MSE).** A sequential extraction procedure proposed by Barrocas and Wasserman<sup>11</sup> was applied in order to evaluate the geochemical partitioning of mercury in sediments. The procedure consisted of four partial extraction steps and one residual digestion step (Fig. 2). Two grams of dry sediment samples were extracted in 50 ml polyethylene centrifuge tubes with screw caps. A mechanical orbital shaker regulated at a speed adapted to ensure continuous suspension of the mixture was used. Each phase was then separated by centrifugation at 3000 rpm for 15 min. All final extracts were stored in pre-cleaned Sterilin (Bellefonte, PA, USA) bottles for mercury determination. In a preliminary assay it was observed that a viscous phase appeared in the extracts of the step 4 (‘sulfides’), probably due to the formation of sulfides. This viscous phase considerably interfered in the analyses by ICP-MS and probably constituted a strong trap for mercury. To overcome this problem the residue in step 3 (‘organic matter’) was washed with 20 ml of water and shaken for 15 min. The wash solution was discarded before sodium sulfide addition.

The material remaining after sequential extraction was

**Table 1** Results of the determinations of mercury in certified reference materials

Reference material	Certified/mg kg <sup>-1</sup>	Determined/mg kg <sup>-1</sup>	Detection limits/µg kg <sup>-1</sup>
<i>TMOM method<sup>a</sup>—</i>			
BCR S19	91.07 ± 7.03	95 ± 3	1.0
IAEA 356	7.62 ± 0.62	7.3 ± 0.1	
PASC-1	4.57 ± 0.16	4.7 ± 0.3	
<i>TMCM method<sup>b</sup>—</i>			
GBW 08301	0.22 ± 0.04	0.21 ± 0.02	12.0
GBW 07311	0.072 ± 0.006	0.074 ± 0.005	
GBW 07310	0.280 ± 0.018	0.274 ± 0.033	
<i>TMCH method<sup>c</sup>—</i>			
BCR 320	1.03 ± 0.23	1.027 ± 0.141	5.0
	MeHg/µg kg <sup>-1</sup>	MeHg/µg kg <sup>-1</sup>	
<i>MOC method<sup>d</sup>—</i>			
BCR S19	53.1 ± 8.6	50.6 ± 4.5	2.0
CRM 580	75.4 ± 5.0	73.3 ± 5.7	

<sup>a</sup>Four independent experiments. <sup>b</sup>Eight independent experiments. <sup>c</sup>Five independent experiments. <sup>d</sup>Six independent experiments. (Standard deviations within the 95% confidence interval.)

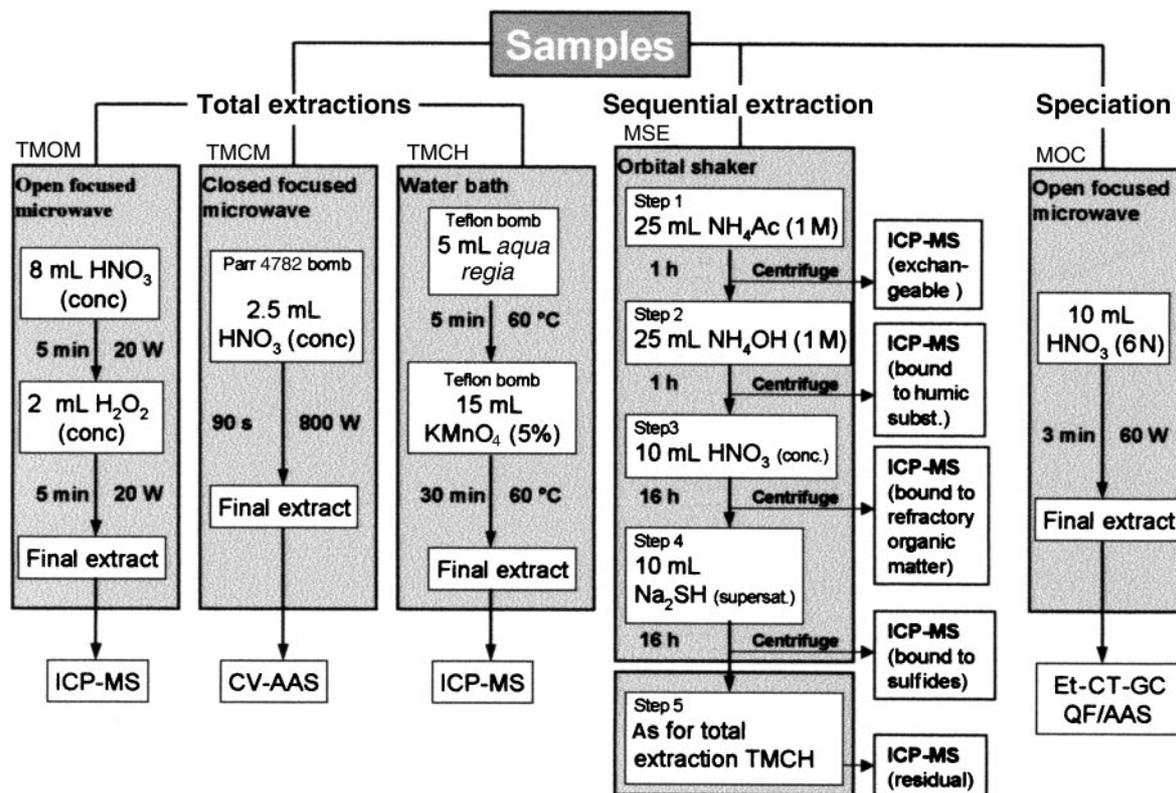


Fig. 2 Scheme summarising the extraction procedures applied in this work.

digested with *aqua regia*+potassium permanganate as in TMCH.

**Mercury organo-compounds analysis (MOC).** Mercury species, were quantitatively extracted from sediments with a microwave technique, previously described in the literature<sup>29–32</sup> (Fig. 2). The extracts were stored in the refrigerator until analysis. An aliquot of 1 ml of the extract was analysed in a hyphenated Et-CT-GC-QFAAS system.<sup>26–32</sup>

## Results

### Total mercury

The results of three total digestion procedures for mercury (TMOM, TMCM, and TMCH) are presented in Table 2.

TMOM extraction presented values that are surprisingly low when compared to the other procedures. From these results arose the suspicion that the TMOM procedure was not suitable for the type of sediment sampled in Almadén. Nonetheless, it has to be underlined that the same procedure applied to the reference materials did show a good recovery rate (Table 1).

The extraction using Parr bombs (TMCM), yielded mercury concentrations ranging between 5.5 and 1005.0 mg kg<sup>-1</sup> (Table 2). These results are much higher than those previously obtained using the TMOM method, but they are more consistent with the degree of contamination of the studied environment. The satisfactory results obtained by these two methods (TMOM and TMCM) for reference materials (Table 1) indicate that the choice of a reliable procedure must be influenced by the type of sediment.

Further confirmation of the reliability of the TMCM method is achieved when comparing these results with the results of the method of conventional heating (TMCH, Table 2). The agreement obtained between TMCM and TMCH concentrations in real samples (Table 2) indicates that these two procedures are effective. The unexpected low values obtained when TMOM method was used suggest that this method is not appropriate for the mercury analyses of this type of sediments. Considering that the volatilisation of mercury compounds has been shown to be a minor source of error (this would yield bad results for certified reference materials), incomplete extraction of the mercury present in these sediments must explain the low results. At this point, fractionation and speciation procedures

Table 2 Comparison of the different analytical methods for mercury determination in Almadén's sediments

Sediment No.	Method				
	TMOM <sup>a</sup> /mg kg <sup>-1</sup>	TMCM <sup>b</sup> /mg kg <sup>-1</sup>	TMCH <sup>c</sup> /mg kg <sup>-1</sup>	MOC <sup>d</sup> /mg kg <sup>-1</sup>	Σ Seq. extr. <sup>e</sup> /mg kg <sup>-1</sup>
V-1	1.31	15.90	15.80	6.31	13.40
V-2	0.21	60.00	74.10	11.40	51.60
V-3	0.84	68.60	90.00	6.35	73.20
V-4	1.69	65.60	79.40	10.70	54.30
V-5	2.67	78.90	110.00	6.03	79.90
V-6	0.15	5.53	5.42	2.06	4.82
V-7	1.75	74.40	92.20	4.97	74.90
V-8	22.80	107.20	130.00	19.10	72.90
V-9	276.10	1005.00	930.00	15.40	1260.00

<sup>a</sup>HNO<sub>3</sub>(c)+H<sub>2</sub>O<sub>2</sub>; ICP-MS. <sup>b</sup>HNO<sub>3</sub>(c); CVAAS. <sup>c</sup>*Aqua regia*+KMnO<sub>4</sub>; ICP-MS. <sup>d</sup>HNO<sub>3</sub> (6N); Et-CT-GC-QFAAS. <sup>e</sup>Sum of the sequential extraction fractions.

were applied to the Almadén's sediments in order to establish in which form the mercury is present in this environment: sequential extraction (MSE) and mercury organo-compound analyses (MOC). It was expected that speciation and sequential extraction (fractionation) would furnish explanation for the bad results obtained using the TMOM method.

### Mercury fractionation by sequential extraction

The results presented in Table 2 show that the sum of the amount removed by each extractant was similar to the amount released by the total extractions with TMCM and TMCH methods. Once more the comparison of the sum of sequential extraction and TMOM method concentrations yielded very different results, confirming that this mild extraction is not able to leach all of the mercury present in the sample. Further calculations show that TMOM can hardly extract the first three operationally defined phases of the sequential extraction (Table 3).

The operationally defined mercury partitioning in the sediment is shown in the Fig. 3. A feature deserving attention is the strong association of mercury with the "sulfidic phase" in all nine samples. Mercury is also strongly associated with the "residual phase". With few exceptions, the amounts of mercury in the "exchangeable phase" and "bound to humic acids phase" remain beyond the detection limits of the applied procedure. The organic matter content obtained by loss on ignition is presented on Table 4.

Mercury bound to the "sulfidic phase" is increasingly more important as total concentrations increase. More than 50% of the mercury is bound to sulfides in most of the samples, reaching 95% in sample 9, which has a total mercury concentration of more than 1000 mg kg<sup>-1</sup>.

With respect to the sampling points, there is a marked similarity of phase distribution patterns in sites 1 and 8, whereas the other sites behaved differently. Sites 1 and 8 have a higher percentage of mercury bound to the "refractory organic phase" that can be attributed to the greater distance from the mining area. In these two sites there is less residual mercury and probably more available mercury, that is trapped in organic matter. On the other hand, the "sulfidic phase" plays a significant role in the retention of mercury in the sediments of Almadén and generally the availability of mercury in these sediments seems to be reduced.

### Mercury speciation

Previous investigations on the methylation of mercury have shown that methylmercury is produced in the sediment<sup>39-41</sup> as well as in the water column.<sup>40</sup> Even though the degree to which the sediment acts as a source of methylmercury to aquatic biota is unknown, both inorganic mercury and methylmercury are mainly concentrated in sediments relative to the water column. In our study, no organic mercury could be detected in the sediment samples (detection limit = 2 µg kg<sup>-1</sup>), therefore, the organic species constitutes less than 1.5% of the total, a

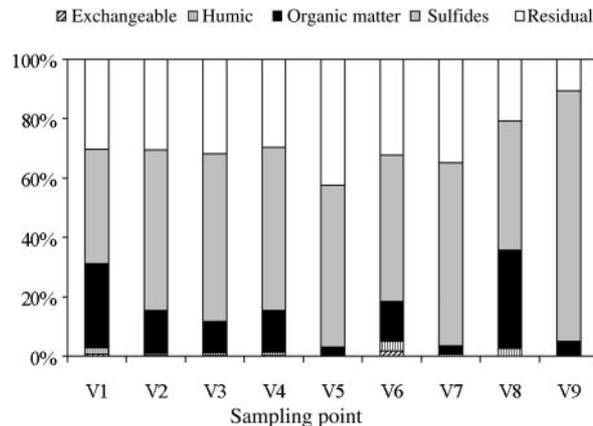


Fig. 3 Operationally defined partitioning of mercury between sediment phases.

Table 4 Organic matter content of the sediment samples obtained by loss on ignition

	V-1	V-2	V-3	V-4	V-5	V-6	V-7	V-8	V-9
Loss on ignition (%)	5.42	3.46	5.82	2.90	4.42	1.26	4.89	4.89	6.24

percentage that was reported in the literature for other sediments with lower total mercury concentrations.<sup>19</sup> Even though the values of inorganic mercury (Hg<sup>2+</sup>) using this procedure are elevated, ranging between 2.06 and 19.1 µg g<sup>-1</sup> (Table 2), they were considerably lower than those obtained by total extraction, further showing that mercury is relatively unavailable in the Almadén's sediments.

### Discussion

It can be seen from the results that one of the applied methods for total mercury analyses fails to adequately recover mercury in the Almadén's sediments, probably because it is present as insoluble mercury sulfide (metacinnabar and cinnabar). The solubility product of cinnabar is as low as 10<sup>-36.8</sup>, and even though, to our knowledge, the metacinnabar solubility product has not yet been measured, it is also insoluble.<sup>42</sup>

The sequential extraction results indicate that forms associated to the "sulfidic phase" and "residual phase" play a significant role in the chemical immobilisation of mercury in these sediments. It is probable that cinnabar and metacinnabar are present in our samples, but differently from the results of Barnett *et al.*,<sup>43</sup> who observed neo-formed metacinnabar in the Oak Ridge (TN) flooded soils, in Almadén, we suggest that metacinnabar (extracted in the "sulfidic phase") is a result of a partial degradation of red cinnabar (extracted in the "residual phase"). The presence of elevated concentrations of cinnabar is attributed to the fact that in Almadén, most of the mercury has

Table 3 Comparison of the results of TMOM procedure with the sum of the two first steps of the sequential extraction (exchangeable + bound to humic substances) and with the sum of the three first steps (exchangeable + bound to humic substances + bound to refractory organic matter)

Sediment No.	TMOM/mg kg <sup>-1</sup>	Σ 2 phases/mg kg <sup>-1</sup>	Σ 3 phases/mg kg <sup>-1</sup>
V-1	1.31	0.27	4.28
V-2	0.21	0.52	6.71
V-3	0.84	0.73	8.05
V-4	1.69	0.54	8.15
V-5	2.67	Nd <sup>a</sup>	3.20
V-6	0.15	0.29	0.96
V-7	1.75	0.75	3.75
V-8	22.80	2.92	28.43
V-9	276.10	Nd	63.00

<sup>a</sup>Nd = not detectable (< 5.0 mg kg<sup>-1</sup>).

**Table 5** Total mercury and methylmercury concentrations in some contaminated mining areas compared to our results

Location	Total Hg/mg kg <sup>-1</sup>	MeHg/μg kg <sup>-1</sup>	MeHg (%)	Reference
Carson River (Western Nevada, USA)	2–156	2–28	<3	52
Berry's Creek (NJ, USA)	9–450	<1–8	—	49
Kuskokwim Mountains Region (South-western Alaska)				51
Cinnabar Creek mine soil	0.13–1500	5.07–133		
Red Devil mine soil	0.05–1200	2.7–3.2		
Monte Amiata (Italy)	—	—	1–2	50
Valdeazogues River	5–1000	<2	<0.1	This study

been extracted before the 20<sup>th</sup> century (mainly between the 15<sup>th</sup> and the 19<sup>th</sup> centuries). In this period, the roasting techniques used did not involve very high temperatures and most of the mercury remains in the environment as the sulfide mineral. This was observed in the Idrija mine (Slovenia), where older tailings presented mercury as cinnabar while in more recent tailings this element is more bioavailable.<sup>44</sup>

It is interesting to note that when Barrocas and Wasserman applied this same sequential extraction procedure to sediment samples from a tropical coastal environment, they obtained very different results.<sup>11</sup> In their study area, a degraded environment (Guanabara Bay, Rio de Janeiro) with profuse organic matter (reaching 10% organic carbon), they found more than 95% of the mercury bound to the “refractory organic matter phase” and the “sulfidic phase” constituted a minor carrier for this metal. On the other hand, Biester and Scholz<sup>18</sup> tested a similar sequential extraction procedure<sup>12</sup> and observed, in a wide range of samples (comprising Idrija mine samples) the predominance of the residual phase, which led them to conclude that the sequential extraction procedure was not reliable. The later results of Barrocas and Wasserman<sup>11</sup> did show that the lack of a sulfidic phase in the DiGiulio and Ryan<sup>12</sup> scheme explains the absence of specificity as observed by Biester and Scholz.<sup>18</sup>

The relationship between organic matter and mercury is widely reported in the literature.<sup>2,45,46</sup> Further, mercury forms stable complexes with humic acids,<sup>47</sup> amino acids<sup>48</sup> and hydroxycarboxylic acids.<sup>2</sup> However, in the present work the correlation of mercury concentration with the organic matter content was insignificant ( $r=0.48$ ,  $n=9$ ,  $p>0.1$ ), which could be attributed to the lack of mobility of mercury (associated with refractory sulfides). These results are also confirmed by the sequential extraction procedure.

There is relatively little information on the levels of methylmercury in Hg-contaminated freshwater sediments from mining areas for comparison. Table 5 shows a comparison between our results and those from other works in areas that are affected by cinnabar mining operations.<sup>49–52</sup> The methylmercury concentration for these highly polluted areas are not very far from the average background concentration of 2.5 μg kg<sup>-1</sup> reported for sediments world-wide by Baeyens,<sup>53</sup> showing that in this type of environment, despite the high levels of total mercury, methylation is reduced.

## Conclusions

The total extraction results and the sequential extraction procedure have shown that mercury in the Almadén's sediments is quite stable and presents low chemical availability. This lack of availability renders inorganic mercury methylation difficult. The results are consistent with mineralogy of the mercury deposits, since cinnabar has an extremely low solubility in water,<sup>43,54</sup> is resistant to physical and chemical weathering and is hardly leached under acid drainage. The conversion of the mercury present in cinnabar crystals (HgS) to methylmercury has been reported to be low,<sup>55</sup> though it can also be increased by organic matter.<sup>42</sup>

Although it has been demonstrated that mercury in

Almadén's sediments is not available, and no methylation at a detectable level was found, the elevated concentrations of mercury in the sediments of the Valdeazogues River confirm that this stream is a major source for the aquatic environment in the region. The water from the Valdeazogues River flows into a large dam recently constructed, which presently is the natural terminus of the river after 15 km. The water from this reservoir will be utilised for irrigation of agricultural lands and for fishing. Inorganic mercury from Valdeazogues River sediments can be re-suspended during winter and spring flooding events and provide an intermittent source of inorganic mercury. In such conditions, suspended particulate matter can carry a significant load of mercury, thereby spreading this contaminant widely throughout the region.

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## References

- 1 U. Förstner, in *Chemical methods for Assessing Bio-available Metals in Sludges and Soil*, ed. R. Leschber, R. D. Davis and P. L'Hermite, Elsevier, London, 1985, pp. 1–30.
- 2 J. W. Moore and S. Ramamoorthy, in *Heavy Metals in Natural Waters, Applied Monitoring and Impact Assessment*, ed. J. W. Moore and S. Ramamoorthy, Springer-Verlag, New York, 1984, pp. 125–160.
- 3 M. Hempel, Y. K. Chau, B. J. Dutka, R. MacInnis, K. K. Kwan and D. Liu, *Analyst*, 1995, **120**, 721.
- 4 R. P. Mason, J. R. Reinfelder and F. M. M. Morel, *Environ. Sci. Technol.*, 1996, **30**, 1835.
- 5 A. M. Ure, in *Metals in Soils*, ed. B. J. Alloway, Blackie and Son, Glasgow, 1990, pp. 30.
- 6 A. M. Ure, P. Quevauviller, H. Muntau and B. Griepink, *Int. J. Environ. Anal. Chem.*, 1993, **51**, 135.
- 7 C. M. Davidson, P. T. Rhodri, S. E. McVey, R. D. L. Perala and A. M. Ure, *Anal. Chim. Acta*, 1994, **291**, 277.
- 8 A. Tessier, P. G. C. Campbell and M. Bison, *Anal. Chem.*, 1979, **51**, 844.
- 9 N. Meguellati, D. Robbe, P. Marchandise and M. Astruc, *International Conference on Heavy Metals in the Environment, Toronto, Canada 1983*, CEP Consultants, Edinburgh, pp. 1095–1097.
- 10 D. Wallschläger, M. V. M. Desai, M. Spengler and R. D. Wilken, *International Conference on Heavy Metals in the Environment, Hamburg, September 1995*, ed. R. D. Wilken, U. Förstner and A. Knöchel, CEP Consultants, Edinburgh, pp. 52–56.
- 11 P. R. G. Barrocas and J. C. Wasserman, in *Environmental Geochemistry in the Tropics*, ed. J. C. Wasserman, E. V. Silva-Filho and R. Villas-Boas, Springer-Verlag, Heidelberg, 1998, vol. 72, pp. 171–184.
- 12 R. T. DiGiulio and E. A. Ryan, *Water Air Soil Pollut.*, 1987, **33**, 205.
- 13 M. A. Huerta-Diaz and J. W. Morse, *Geochim. Cosmochim. Acta*, 1992, **56**, 2681.
- 14 C. Gagnon, E. Pelletier and A. Mucci, *Mar. Chem.*, 1997, **59**, 159.
- 15 P. J. Craig and P. A. Moreton, *Water Res.*, 1986, **20**, 1111.

- 16 J. M. Benoit, R. P. Mason and C. C. Gilmour, *Abstracts of the 5<sup>th</sup> International Conference Mercury as a Global Pollutant, Rio de Janeiro, May, 23–28 1999*, CETEM, Rio de Janeiro p. 527.
- 17 N. W. Revis, T. R. Osborne, D. Sedgley and A. King, *Analyst*, 1989, **114**, 823.
- 18 H. Biester and C. Scholz, *Environ. Sci. Technol.*, 1997, **31**, 233.
- 19 P. J. Craig, in *Organometallic Compounds in the Environment, Principles and Reactions*, ed. P. J. Craig, Longman, Harlow, Essex, 1986, pp. 65–101.
- 20 S. E. Lindberg, D. R. Jackson, J. W. Huckabee, S. A. Janzen, M. J. Levin and J. R. Lund, *J. Environ. Qual.*, 1979, **8**, 572.
- 21 S. G. Hildebrand, J. W. Huckabee, F. Sanz Diaz, S. A. Janzen, J. A. Solomon and K. D. Kumar, *Distribution of mercury in the environment at Almadén, Spain*, Oak Ridge National Laboratory, Oak Ridge, TN, 1980, ORNL/TM-7446.
- 22 J. W. Huckabee, F. Sanz-Diaz, S. A. Janzen and J. Solomon, *Environ. Pollut., Ser. A*, 1983, **30**, 211.
- 23 R. Ferrara, B. E. Maserti, M. Andersson, H. Edner, P. Ragnarson, S. Svanberg and A. Hernandez, *Atmos. Environ.*, 1998, **32**, 3897.
- 24 R. C. Rodríguez-Martín-Doimeadios, PhD Thesis, University of Castilla de la Mancha, 1999.
- 25 E. Rosland and W. Lund, *J. Anal. At. Spectrom.*, 1998, **13**, 1239.
- 26 O. F. X. Donard, L. S. R. Randall and J. H. Weber, *Int. J. Environ. Anal. Chem.*, 1986, **27**, 55.
- 27 O. F. X. Donard, S. Rapsomanikis and J. H. Weber, *Anal. Chem.*, 1986, **58**, 772.
- 28 S. Rapsomanikis, O. F. X. Donard and J. H. Weber, *Anal. Chem.*, 1986, **58**, 35.
- 29 C. M. Tseng, A. de Diego, F. M. Martin, D. Amouroux and O. F. X. Donard, *J. Anal. At. Spectrom.*, 1997, **12**, 743.
- 30 C. M. Tseng, A. de Diego, F. M. Martin and O. F. X. Donard, *J. Anal. At. Spectrom.*, 1997, **12**, 629.
- 31 C. M. Tseng, A. de Diego, H. Pinaly, D. Amouroux and O. F. X. Donard, *J. Anal. At. Spectrom.*, 1998, **13**, 755.
- 32 C. M. Tseng, H. Garraud, D. Amouroux, O. F. X. Donard and A. de Diego, *J. Autom. Chem.*, 1998, **20**, 99.
- 33 C. M. Tseng, A. de Diego, J. C. Wasserman, D. Amouroux and O. F. X. Donard, *Chemosphere*, 1999, **39**, 1119.
- 34 P. Quevauviller, *J. Anal. At. Spectrom.*, 1996, **11**, 1225.
- 35 A. Woller, H. Garraud, F. Martin, O. F. X. Donard and P. Fodor, *J. Anal. At. Spectrom.*, 1997, **12**, 53.
- 36 J. J. Berzas, L. F. Garcia and R. C. Rodríguez, *Vth International Symposium on Analytical Methodology in the Environment Field, La Coruña, Spain*, Universidad de La Coruña, La Coruña, 1998, p. 283.
- 37 W. R. Hatch and W. L. Ott, *Anal. Chem.*, 1968, **40**, 2085.
- 38 P. R. Barrocas, J. C. Wasserman, T. Jennerjahn and F. Pivetta, *International Symposium on Perspectives for Environmental Geochemistry in the Tropical Countries, Niterói (Brazil), September 1993*, ed. J. J. Abrão, J. C. Wasserman, E. V. Silva-Filho, Universidade Federal Fluminense, Rio de Janeiro, pp. 143–148.
- 39 G. C. Campeau and R. Bartha, *Appl. Environ. Microbiol.*, 1985, **50**, 498.
- 40 L. Xun, N. E. R. Campbell and J. W. M. Rudd, *Can. J. Fish. Aquat. Sci.*, 1987, **44**, 750.
- 41 C. C. Gilmour and E. A. Henry, *Environ. Pollut.*, 1991, **71**, 131.
- 42 M. Ravichandran, G. R. Aiken, M. M. Reddy and J. N. Ryan, *Environ. Sci. Technol.*, 1998, **32**, 3305.
- 43 M. O. Barnett, L. A. Harris, R. R. Turner, R. J. Stevenson, T. J. Henson, R. C. Melton and D. P. Hoffman, *Environ. Sci. Technol.*, 1997, **31**, 3037.
- 44 H. Biester, M. Gosar and G. Müller, *Vth International Conference on Mercury as a Global Pollutant, Rio de Janeiro 1999*, CETEM/UFF, Rio de Janeiro, p. 169.
- 45 E. A. Jenne, in *Mercury in the Environment*, US Geological Survey, Washington, DC, 1970, vol. 713, pp. 40–45.
- 46 M. A. Trent, J. D. Trent, J. M. Roberston and J. G. Laguros, *VIIth International Conference on Heavy Metals in the Environment, Geneva 1989*, ed. J. P. Vernet, CEP Consultants, Edinburgh, pp. 118–127.
- 47 H. Kerndorff and M. Schmitzer, *Geochim. Cosmochim. Acta*, 1980, **44**, 1701.
- 48 M. A. Rashid, *24th International Geological Congress, Montreal 1972*, ed. R. W. Boyle and D. M. Shaw, Geological Society of America, Boulder, Co, pp. 346–352.
- 49 M. Berman and R. Bartha, *Environ. Pollut., Ser. B*, 1986, **11**, 41.
- 50 C. Barghigiani and T. Ristori, in *Mercury Pollution: Integration and Synthesis*, ed. C. J. Watras and J. W. Huckabee, Lewis Publishers, Boca Raton, FL, 1994, pp. 41–49.
- 51 E. A. Bailey and J. Gray, in *Geological Studies in Alaska by the U.S. Geological Survey, 1995*, US Geological Survey, Washington, DC, 1996, vol. 2068, pp. 41–56.
- 52 Y. Chen, J. C. Bonzongo and G. C. Miller, *Environ. Pollut.*, 1996, **92**, 281.
- 53 W. Baeyens, *Trends Anal. Chem.*, 1992, **11**, 1131.
- 54 J. D. Hem, in *Mercury in the Environment*, US Geological Survey, Washington, DC, 1970, vol. 713, pp. 19–24.
- 55 J. E. McLean and B. E. Blesoe, *Behaviour of metals in soils. EPA Ground Water Issues*, Environmental Protection Agency, Washington, DC, 1992, EPA/540/3-92/018.