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Extractability and mobility of mercury from agricultural soils surrounding industrial and mining contaminated areas

Authors: Ana Teresa Reis¹, Sónia Morais Rodrigues¹, Christine M. Davidson², Eduarda Pereira¹; Armando C. Duarte¹

Address: ¹CESAM and Chemistry Department, University of Aveiro, Campus de Santiago, 3810-193, Aveiro (Portugal)
²WestCHEM, Department of Pure and Applied Chemistry, University of Strathclyde, 295 Cathedral Street, Glasgow G1 1XL, UK

Corresponding Author: Ana Teresa Reis (teresareis@ua.pt) Phone: +351 234 370 737 Fax: +351 234 370 084

Abstract

This study focussed on a comparison of the extractability of mercury in soils with two different contamination sources (a chlor-alkali plant and mining activities) and on the evaluation of the influence of specific soil properties on the behaviour of the contaminant. The method applied here did not target the identification of individual species, but instead provided information concerning the mobility of mercury species in soil. Mercury fractions were classified as mobile, semi-mobile and non-mobile.

The fractionation study revealed that in all samples mercury was mainly present in the semi-mobile phase (between 63 and 97%). The highest mercury mobility (2.7 mg kg⁻¹) was found in soils from the industrial area. Mining soils exhibited higher percentage of non-mobile mercury, up to 35%, due to their elevated sulfur content.

Results of factor analysis indicate that the presence of mercury in the mobile phase could be related to manganese and aluminum soil contents. A positive relation between mercury in the semi-mobile fraction and the aluminium content was also observed. By
contrary, organic matter and sulfur contents contributed to mercury retention in the soil matrix reducing the mobility of the metal.

Despite known limitations of sequential extraction procedures, the methodology applied in this study for the fractionation of mercury in contaminated soil samples provided relevant information on mercury’s relative mobility.

**Keywords:** mercury; mobility; sequential extraction; soils

### 1 Introduction

Soil mercury contamination is a problem found at many industrial and mining sites both active and inactive (Biester and Scholz, 1997; Fernández-Martínez et al., 2005; Inácio et al., 1998). Soil concentrations of a few to several thousand mg of mercury per kg of soil were found in the vicinity of industrial facilities where mercury and its compounds are or were used (Biester and Scholz, 1997; Reis et al., 2009). At mining sites, the weathering of chemical forms, facilitate dispersion in watersheds or through atmospheric emissions, and increase its bioavailability for organisms (Brown et al., 1999).

Soils play an important role in the biological cycle of mercury acting both as a sink and a source of this metal to biota, atmosphere and hydrological compartments (Oliveira et al., 2007). However, the speciation and the way the different species interact with the soil matrix cause changes in solubility, toxicity and bioavailability of the metal (Biester et al., 2002). Han et al. (2003) highlighted the high mobility and toxicity of alkyl species, such as methylmercury (MeHg+) and ethylmercury(II) (EtHg+), and the high solubility and mobility of soluble inorganic species, such as mercuric chloride (HgCl2), in comparison with other inorganic mercury species. As observed by the same authors, alkyl and inorganic soluble mercury species contribute to the major portion of potential mercury toxicity in soil (Han et al., 2003). On the other hand, chemically stable species, such as mercuric sulfide (HgS) are considerably less mobile, and, therefore, less toxic (Han et al., 2003).

As different species exhibit different behavior, mobility and availability, measurements
of total mercury in soils do not provide enough information on the potential toxicity of the soil (Beckvar et al., 1996; Biester et al., 2002). Knowledge of the chemical forms of mercury in soil can be accomplished by the application of sequential extraction methods (Bloom and Preus, 2003; Fernández-Martínez and Rucandio, 2003; Han et al., 2003) and is critical to evaluate its environmental risk. These sequence of extractions are used to subdivide the mercury content of soil samples into several operational defined groups of more or less soluble species (Rubio and Rauret, 1996). The fractions extracted early in the process are generally most weakly bound to the solid phase and have greater potential mobility and toxicity.

There are different protocols available for mercury speciation and fractionation in literature (see review of Issaro et al., 2009, and references therein). However, the chemistry of mercury requires the development of specific extraction schemes, specifically dedicated to this element (Bacon and Davidson, 2008). Difficulties in the comparison of sequential extraction results for mercury fractionation relate particularly to inconsistencies between different extraction protocols (Bacon and Davidson, 2008). It must be underlined that a limitation to the use of sequential extraction procedures in general is the lack of validation and reference materials for checking the performance both of method and the laboratory. Bacon and Davidson (2008) provided a comprehensive critical review on sequential extraction procedures, considering its limitations such as the lack of selectivity, the non-specificity and re-adsorption. The use of the remaining soil matrix for the next step may have influence on further steps of chemical extraction, since substrate composition has been altered. Therefore, establishing easy-to-use protocols is key to successful assessment of risk and contaminant-soil interaction in contaminated areas.

The present work will focus on a method for sequential extraction of mercury in soils and sediments validated by Han et al. (2003). Although we recognize the limitations associated with sequential chemical extraction procedures, we believe that the application of this procedure to contaminated soil samples provides valuable information on mercury mobility in contaminated areas. The method is based on the sequential extraction of different operationally defined fractions and provides detailed information about the potential mobility of mercury in the samples. Mercury mobility is defined in terms of the mercury leached in the following three fractions: mobile (M), semi-mobile (SM), and non-mobile (NM) (Fernández-Martínez et al., 2005; Han et al., 2003), with toxicity decreasing
in that order. The operationally-defined mercury fractions are summarized in Table 1.

In order to assess the dynamics of mercury within the soil system it is of paramount importance better to understand the relationships between mercury species and soil properties. The fractionation of mercury in soils can be affected by clay minerals, metal oxides, organic matter and pH (Sánchez et al., 2005). Complexes formed by divalent mercury with soluble organic matter, chlorides and hydroxides may contribute to its mobility (Millán et al., 2006; Sánchez et al., 2005). Organic matter presence in soils can also lead to the formation of mercury complexes and inhibit mercury biomethylation processes (Bloom and Preus, 2003). The pH affects the speciation of mercury in the soil solution and influences the mobility and availability of mercury in soil (Yin et al., 1996).

According to Jing et al. (2007) and Miretzky et al. (2005) desorption of inorganic divalent mercury species from soil components increases with decreasing pH since, in general, trace element cations become more soluble and therefore more mobile as pH decreases (Chopin and Alloway, 2007). The presence of sulfur is very important in the chemistry of mercury, as in the presence of sulfides mercury becomes tightly bound to them, forming the insoluble HgS (Boszke et al., 2003). Because HgS is not very reactive or mobile, the formation of this compound allows mercury to be retained; it therefore becomes less available for methylation and potentially less harmful to the environment.

The main objective of this work was to assess mercury extractability and mobility in agricultural soils from two locations, with different sources of mercury contamination (industrial and mining activities). The study also focussed on the evaluation of the influence of specific soil properties on the distribution and behaviour of the contaminant. Improved understanding of these relationships will allow more effective prediction of how changes in environmental conditions and soil characteristics (e.g. due to processes associated with climate change) may affect the mobility of mercury in contaminated soils, its potential availability to plants and toxicity to organisms.

2 Material and methods

2.1 Study site description
The first set of samples was collected in the vicinity of the industrial complex of Estarreja, North-Western coast of Portugal (Figure 1). This complex dates back to 1950 (Inácio et al., 1998) and is home to a large chlor-alkali plant which used to produce chlorine and caustic soda by the mercury cell process, where liquid elemental mercury is utilized as a cathode in the electrolysis of a saturated brine solution (Ullrich et al., 2007). As other studies show (Lacerda and Salomons, 1998; Ullrich et al., 2007) mercury-cell chlor-alkali plants have been identified as major sources of mercury to the environment. Although the plant started to change the production process in 1994 and completely ceased the use of mercury in 2002 (Ospar Commission, 2006), mercury emitted from the existing plant still remains significant in the surrounding environment. Until 1975 the liquid effluents from this plant, containing many different types of contaminants (Batista et al., 2002), including mercury, were discharged directly into man-made effluent streams. Consequently, the pollutants were transported for several kilometres through the agricultural fields surrounding the chlor-alkali plant (Costa and Jesus-Rydin, 2001). Although after 1975 impermeable pipes were constructed, and the streams are no longer used for effluent transport, these are still present in fields.

A second set of samples was collected in the Caveira sulfide mine, which is located in Grândola, in the North-West region of the Iberian Pyrite Belt (IPB, Figure 1). The IPB is a well known mining district of worldwide significance, due to its unusual concentration of large and medium sized mineral deposits, including ores of copper, iron, lead, sulfur and zinc. Antimony, arsenic, cadmium, cobalt, gold, mercury, selenium and silver can also be found in soils from the IPB (Barriga, 1990). Past mining activities at the Caveira mine included pyrite (FeS₂) and Cu extraction. From 1936 until the 1970’s Caveira massive sulfides were exploited for sulfur. Although the mine is now closed, soil metal contamination and acid mine drainage still pose severe environmental problems at the site. Large volumes of waste were produced by the mining activities and various types of tailings deposited in the area (the amount of waste stored on the site is estimated to be higher than 2 Mt) (Cardoso Fonseca e Ferreira da Silva, unpublished results). Rainwater circulates and percolates easily over and through these tailing materials causing significant erosion and transport of tailings debris to areas nearby and downstream.
2.2 Sampling

Seven soil samples were collected from fields within a radius of < 1 km from the industrial complex of Estarreja, close to a former effluent stream (Figure 1). These fields are used mainly for agricultural and cattle grazing purposes. A second set of seven soil samples was collected from fields surrounding the Caveira mine (South-West Portugal) within a radius of < 2 km from the mine. The Caveira samples 7, 11, 13, and 14 were collected from pasture fields located at 0.5-1.0 km from the mine tailing deposits. Ryegrass (Lolium perenne) was the predominant plant species at these fields. Sample 3 was collected from a tailing deposit. Finally, samples 5 and 9 were collected at an agricultural field located at approximately 1.7 km from the mine pit (Figure 1).

Sampling at both sites was performed using a plastic spatula and samples were placed in plastic bags during transport to the laboratory, where they were pre-treated within one hour. The soil sampling depth was 0–15 cm. Once in the laboratory, soil samples were air dried at room temperature to constant weight. Stones were removed and soil aggregates were crushed and homogenised, during the drying stage. The dried samples were sieved to < 2 mm using a nylon sieve. The air-dry soil, < 2 mm fraction, was used both for general characterization of these soils and for mercury extraction.

2.3 Soil samples characterisation

The soil pH (CaCl₂) was determined using a WTW pH meter-538, according to the ISO 10390:1994 method. Total carbon (TotC) percentages were measured on an Elemental Analysis instrument (LECO CNH-2000), according to ISO 10694:1995. For the determination of organic carbon content (OrgC), an excess of solution of 4 M of hydrochloric acid (HCl) was added to a crucible containing a weighed quantity of soil. The crucibles were left to stand for 4 h and then were dried for 16 h at 60-70 °C. The analysis of carbon content after the removal of carbonates (organic carbon) was performed using the same procedure of total carbon determinations.

The particle size distribution and clay contents of the soil samples were determined using a Coulter LS230 laser diffraction particle size analyzer. The classification of soils followed the USDA Texture Classes: sand fraction (0.050<%<2mm), silt fraction (0.002<%<0.050mm), and clay fraction (%<0.002mm). Classification of samples was achieved by using the Talwin 42® classification software program.
The pseudo-total contents of aluminium (Al), iron (Fe), manganese (Mn) and sulfur (S) were extracted by *aqua regia* (according to ISO 11466:1995) and analysed by ICP-MS (ICP-MS THERMO X Series, Peltier Nebulizing Camera, Burgener Nebulizer; CETAC AS510 auto-sampler; the CeO+/Ce+ ratio was optimized at < 2%; Internal standard: In). The instrument was tuned using a 10 µg kg⁻¹ multi-element tuning solution. The operational conditions used are summarized as follow: RF power: 1400 W; plasma gas flow (argon): 13 L min⁻¹; auxiliary gas (argon): 0.90 L min⁻¹; nebulizer flow (argon): 0.95 min⁻¹.

Amorphous iron (Fe_ox) and aluminium oxides (Al_ox) were determined by the extraction of 2.50 g of soil with 50 mL of a 0.1 M oxalic acid solution (buffered to pH 3 by ammonium oxalate) and shaken mechanically in the dark for 2 h. Aluminium and iron contents in the filtered extracts were analysed by ICP-MS. Two replicate extractions were performed for each sample. Two extraction blanks were included in each batch of 20 bottles. The filtered extracts were analysed by ICP-MS, according to ISO 17294-1:2005 and ISO 17294-2:2003, with operational conditions as previously described.

### 2.4 Sequential extraction procedure

The study of mercury fractionation was performed by the application of the “Kingston method” as described by Han *et al.* (2003) and Fernández-Martínez *et al.* (2005).

#### 2.4.1 Extraction of the mobile fraction (M)

Extraction of mobile and toxic mercury species involves the use of a solution of 1:1 (v/v) 2% HCl + 10% ethanol.

A sample (1.0–2.0 g) was weighed and added to a 10 mL centrifuge tube with 2.5 mL of the extract solution. The sample and the extract solution were mixed well by vigorous shaking for 2 min. The pH was checked and, when necessary, concentrated HCl was added drop-wise until the pH of the mixture was between 1.5 and 3. The sample was then sonicated at room temperature (not at 60±2 °C, as referred in Han *et al.* 2003) for 7 min, and centrifuged (3200 rpm, 5 min) to separate the supernatant from the soil matrix. The supernatants were collected using a Pasteur pipette and transferred to a vial. This extraction
was repeated three more times. The residue was then rinsed by adding 2.5 mL of DDI water, shaken for 2 min and centrifuged. All the extraction supernatants and the water rinse were combined. This final solution was kept at 4 ºC and analysed within 48 hours.

2.4.2 Extraction of the semi-mobile fraction (SM)

Before proceeding to the extraction of the semi-mobile phase, the residue was tested for the presence of chloride ions because their presence can promote the solubility of non-mobile mercury species (e.g., HgS) into the semi-mobile extract solution and consequently must be avoided. Because all samples revealed the presence of chloride ions, a procedure was undertaken to remove them, according to Fernández-Martínez et al. (2005). This consists of washing the residue with 5 mL DDI water, until the addition of 0.1 M AgNO₃ causes no turbidity. This procedure should not be applied more than 3 times, which was never necessary in any of the samples analysed.

For the extraction of semi-mobile species, a solution of 1:2 (v/v) HNO₃:DDI water is required. A 5 mL aliquot of this solution was added to the residue and mixed by shaking it vigorously. The mixture was heated to 95±2 ºC for 20 min in a sand bath. To avoid losses of volatile mercury species, cap tubes were replaced by glass spheres during the heating step, providing both sufficient cover and reflux. After cooling to room temperature, samples were centrifuged (3200 rpm, 5 min), the supernatant was collected, and the extraction was repeated. The remaining soil residue was washed with 5 mL DDI water. The rinse water was combined with both supernatants and the solution stored at 4 ºC until analysis.

2.4.3 Extraction of the non-mobile fraction (NM)

The procedure for the extraction of the non-mobile phase was similar to the one used for the semi-mobile phase except that the extraction solution was 1:6:7 (v/v/v) HCl:HNO₃:DDI water. The remaining residue (RES) was dried at 40 ºC and analysed for mercury content.

2.4.4 Mercury analysis

Total mercury contents in all samples, extracted solutions and residual matrix were
determined by thermal decomposition atomic absorption spectroscopy (AAS) with gold amalgamation (LECO model AMA-254), a rapid total mercury determination method (Costley et al., 2000). The system consists of a nickel boat in a quartz combustion tube containing a catalyst in which the solid sample is initially dried (30 s) prior to combustion at 750 °C (150 s) in an oxygen atmosphere. The mercury vapor which is produced is trapped on the surface of a gold amalgamator. After a pre-specified time interval (120–150 s), the amalgamator is heated to 900 °C to quantitatively release the mercury which is transported to a heated cuvette (120 °C) prior to analysis by AAS using a silicon diode detector, at 253.6 nm.

2.4.5 Quality control and quality assurance

All solutions were prepared from reagent-grade chemicals and were tested and found to be sufficiently low in mercury (less than 10 ng L⁻¹) before use. Analytical procedures were conducted using ultra-clean glassware (Derquin 5%, 24 h; HNO₃ 25%, 24 h), to avoid contaminating sample extracts. Care was taken to avoid cross-contamination of the samples. Each set of samples extracted included one blank, to check if both material and reagents were mercury free, and a certified reference material.

Three replicates of each sample were taken for sequential extraction, as well as blanks. The accuracy of Leco AMA-254 was assessed daily by the analysis of certified reference material BCR-142R. Recoveries were within the range 80–120%.

The sequential extraction method was also tested by applying the procedure to CRM-021 Dry soil No. 3 (sandy loam) from RTC (USA). Although this reference material is not certified for the mercury fractions targeted by the Kingston method, the sum of the three fractions was compared to the certified value for total Hg (4.7 mg kg⁻¹). The mean results found for the 8 replicate samples analyzed were 0.0199 mg kg⁻¹ and 4.5 mg kg⁻¹, for mobile and semi-mobile fractions, respectively. Mercury levels for the non-mobile and residual fractions were below the detection limit (0.05 ng). The mean sum (4.5 – 5.1 mg kg⁻¹) and, as a recovery of 96% was obtained, the extraction efficiency was found acceptable.

The quality control concerning total carbon determination included two replicate measurements performed in each sample. Eight samples of a reference material (Eurovector E11037, certified value for C=4.401%) were also analyzed, with recovery
percentages of 99-114%.

2.5 Statistical analysis

Statistical analysis was performed using SPSS Statistics 17.0. The relation between the variables was evaluated by Factor Analysis, considering the correlation matrix. Factors were extracted by Principal Components Method, followed by Varimax rotation. Retained factors presented eigenvalues greater than 1; this observation was confirmed by Scree Plot analysis.

3 Results

3.1 Total mercury and soil characteristics

Results obtained for the determination of total mercury in the fourteen samples are shown in Table 2. Total mercury concentration ranges between 1.0 and 91 mg kg\(^{-1}\) for Estarreja samples and 1.1 and 98 mg kg\(^{-1}\) for soils of Caveira. The soil properties are also shown in Table 3.

Soil pH in Caveira varied between 3.6 and 5.3. Although all soils analysed were acidic, an unusually low pH value was observed in sample 11 (pH 3.6). The Caveira area is known to be affected by acid mine drainage (Cardoso Fonseca and Ferreira da Silva, 2000) which may explain the low pH. Acid mine drainage is formed when pyrite (FeS\(_2\)) and other metal sulphides are exposed to oxygen and water and subjected to oxidising conditions resulting in the production of sulfuric acid (low pH), sulphates and dissolved metal ions (Ziemkiewicz et al., 1997).

Total carbon % values varied between 1.6 and 5.1% while organic carbon % varied in the range of 1.6-4.3%. A considerable fraction of the total carbon content is in the form of organic carbon, in the entire dataset.

Variable soil textures were obtained for these soils: loamy sand, sandy loam, loam and silt loam with clay percentages between 3.2 and 16.9%. In general, soils from Caveira showed higher clay percentages than soils from Estarreja.

The “active” forms of aluminium and iron (which occur as amorphous hydroxides and are bond to organic matter) were extracted as oxalates from soil samples and measured in an ammonium oxalate-oxalic acid extract. A large variability between soil samples was
observed with respect to amorphous aluminium oxides and amorphous iron oxides (which varied in the range of 0.057 – 0.21% and 0.068 – 0.16%, respectively). In general, aluminium oxides were present in relatively higher concentrations in samples from Estarreja while the highest contents of iron oxides were found in samples from Caveira. The iron amorphous oxides contents of these soils, particularly at the Caveira area, are relatively higher than those from a study of Portuguese agricultural acid soils which reported a iron oxides range of 0.0073 – 0.46% and a median of 0.096% (Horta and Torrent, 2007). The contents of aluminium oxides observed in Estarreja were also higher than those observed by Horta and Torrent (2007).

Manganese concentrations and sulfur % were higher in Caveira soils than in those from Estarreja.

These soil samples cover a wide range of mercury contamination and allow testing of the Kingston method both in soils with very different mercury concentrations and in soils with different origins and characteristics.

3.2 Fractionation of mercury

The fractionation (Figure 2) revealed that in all samples mercury was mainly present in the semi-mobile phase (between 63 and 97%). The mobile fraction represented a much lower contribution to the total mercury content in both Caveira samples (between 0.29 and 2.0%), and Estarreja samples (median 1.3%). Two exceptions were observed, with samples 9 and 12 presenting an anomalous high percentage of mobile mercury (6.2% and 4.8%, respectively). The higher percentage of mobile mercury in these samples may be explained by the fact that these soils are used for agricultural purposes and are consequently subjected to human influence, including oxidation and the application of fertilizers. Also, characteristics of the soils may partially explain this occurrence, as will be clarified later.

Non-mobile mercury species were the second most abundant fraction present in Caveira soils, with percentages ranging between 1.3% and 35%. For Estarreja soils, however, mercury seems to be present in low contents both in mobile and non-mobile phase (less than 2% for both cases). These data could not be compared to others since there is no existing data about speciation of mercury in these areas.

Results from the fractionation of mercury in the samples can be seen in Table 2.

Recovery, defined as the sum of extracted mercury fractions divided by the
independently determined total mercury concentration, ranged between 78 and 101 % and was considered satisfactory (Table 2). Recoveries higher than 100% can be explained by the heterogeneity associated with soils. Because mercury is not homogenously present in soil, it is likely than the aliquot taken for total mercury analysis does not have exactly the same mercury content as the one taken for mercury fractionation, despite the fact that each sample was thoroughly homogenised prior to analysis. Recoveries lower than 100% can be also justified by the lack of homogeneity of the sample, but can also result from losses of volatile mercury during the process. The same problem was observed by Kocman et al. (2004). Better recoveries were obtained for industrial soil samples, probably because of soil characteristics. Estarreja’s soils are richer in sand particles and poorer in clay particles than Caveira’s soil, which means that the extraction solutions can more easily access mercury in the first case.

As total mercury concentration of the fourteen samples ranged between 1.0 and 98 mg kg\(^{-1}\), this method of fractionation proved to give good results both for high and low total mercury concentrations.

### 3.3 Factor Analysis

According to the criteria explained in the statistical analysis section, factor analysis was performed for each Hg fraction. Table 4 presents the loadings for all factors extracted, the respective communalities, and the variance explained by each factor as well as the cumulative variance. All communalities are elevated, demonstrating that the factors retained are fit to describe the correlational structure of the variables. The distribution of the samples according to the factor plots was examined for each fraction (Figure 3).

For mobile fraction, factor 1 explains 37% and factor 2 explains 34% of total variance. The mobile fraction has its highest loading on factor 2; the same factor also has high loadings for aluminium and manganese (positive) and organic carbon and sulfur content (negative). Samples 5 and 9 are separated by factor 2, due to their high concentration of manganese, aluminium and particularly low concentration of organic carbon (Figure 3a). As shown in Figure 3a, Factor 2 which includes the mobile fraction of mercury did not separate samples by their different geographic origin. By contrary, Factor 1 differentiates Caveira samples for their high content in manganese, iron and clay.

For semi-mobile fraction, three factors were identified that, in total, explain 81% of
variance (Table 4). The semi-mobile fraction has its highest loading on Factor 3, as well as aluminium, indicating that the distribution of this variable is related with this particular fraction. As shown in Figure 3b, samples 1, 6, 8, and 10 have the highest percentage of semi-mobile mercury and also of aluminium. This factor did not allow distinguishing Estarreja from Caveira samples (Figure 3b). Both Factor 1 (highest loadings of organic carbon, sulfur, and clay) and Factor 2 (highest manganese and iron loadings) allowed to separate specific Caveira samples from the dataset (Factor 1: highest scores for samples 3, 11, 13; Factor 2: highest scores for samples 5 and 9) (Figure 3b).

Finally, the factor analysis considering the non-mobile fraction allowed identifying three factors, with factor 3 exhibiting a 0.88 loading for the non-mobile fraction (Table 4). Aluminium has a strong, negative correlation with factor 3 (loading = -0.78). pH also had a negative loading in factor 3 (Table 4). Although with low loading values, a positive correlation between organic carbon and sulfur content and Factor 3 was observed (Table 4). Sample 7 has a high score in Factor 3 and is clearly distinguishable from the rest (Figure 3c) which relates to the presence of non-mobile species and a combination of relatively low pH and aluminium contents and medium organic carbon and sulfur levels.

4 Discussion

Although the mercury fraction in the mobile phase generally did not exceed 2% of total mercury, given the high contamination of some samples this fraction may still represent significant amounts of bioavailable mercury. The importance of this fraction should not be underestimated, since it includes among others the alkyl species (Han et al., 2003). These mercury species are more mobile, more toxic and more readily bioaccumulated than any other mercury species (Han et al., 2003). In the mobile fraction are also present soluble inorganic mercury species. These species, such as mercury chloride (HgCl₂) are more easily transported by natural processes than other inorganic mercury species and can also serve as substrates for mercury methylation (Bloom et al., 1999; Han et al., 2003).

Combined, these extractable organomercury species and extractable soluble inorganic species contribute to the major portion of mercury potential toxicity in soils. Considering that the majority of these soils are predominantly used for agricultural and livestock
purposes (Reis et al., 2009), the presence of mobile and toxic mercury species, even in low concentrations, may be of concern.

Although the mobile mercury fraction (measured by HCl and ethanol extraction) is not entirely identical to in-situ soil pore water concentrations, it can be used as a first indicator for potential groundwater pollution or risk of metal leaching from soils. The Portuguese legislation defines a maximum admissible concentration of 0.0010 mg L$^{-1}$ for mercury in groundwater to be used for drinking water supply (Decreto-Lei n.º 236, 1998). Thirteen of the fourteen samples analysed exhibited mobile mercury concentrations above this legal limit. The highest metal concentration observed in the liquid extracts reached 0.21 mg L$^{-1}$ in Estarreja, and 0.087 mg L$^{-1}$ in Caveira. The exceedance of the maximum admissible concentration in groundwater by mobile mercury contents may be an indication of environmental risk, confirming the need for a comprehensive assessment of the impacts of soil mercury contamination at these sites.

Despite the different characteristics of the soils from Estarreja and from Caveira, when the mobile mercury fraction of both sets of samples was compared by means of the Mann-Whitney test, it proved that there was no difference between the two ($U=6.0; p=0.100$). This may be related to the fact that soil characteristics that were found to play most influence in the mobile fraction are similar for soils from both sampling sites.

The mercury species that fall into the semi-mobile category, such as elemental mercury, are less toxic than easily extractable mercury species (Han et al., 2003). Such species include Hg$^0$ or amalgams of mercury with another metal, Hg$^{2+}$ complexes, which can be also present in the mobile phase, and Hg$_2$Cl$_2$ to a small extent (Table 1). Therefore, although this fraction is not immediately available, its species can be easily converted into more readily available ones. The soils from Estarreja and Caveira presented different distribution of mercury in the semi-mobile phase (Mann-Whitney $p=0.003$), with soils of Estarreja showing higher concentration of semi-mobile mercury species. Considering that these soils are used for agricultural purposes, the presence of semi-mobile mercury species in significant concentrations can pose a risk upon exposure.

The non-mobile fraction includes the less available and less toxic species of mercury, such as HgS, HgSe or Hg$_2$Cl$_2$ (Han et al., 2003). The percentage of mercury in the non-mobile and residual fractions was different for mine and industrial soils, as confirmed by the Mann-Whitney test ($p=0.018$ for non-mobile fraction and $p=0.018$ for residual...
fraction), with mine soils exhibiting higher concentrations and higher variability in concentrations in both fractions.

In all samples, mercury was found within the residual fraction, despite the harsh extraction conditions already applied to liberate the non-mobile phase. This means that species present here are hardly available. Caveira soils have higher percentage of residual mercury species (median 2.6%) compared to industrial soils (median 0.29%). Considering that the percentage of non-mobile mercury is also higher in the first case, mine soils have elements that retain mercury tightly, so that it becomes less available, and, therefore, less dangerous.

4.1 Influence of soil properties on mercury fractions

Factor analysis suggested that specific soil properties play a relevant role in determining mercury mobility at both sampling areas. In general, aluminium and manganese contents have a positive influence on mercury mobility. The concentration of aluminium is particularly associated with the mercury semi-mobile fractions. By contrary, organic matter and sulfur contents contribute to mercury retention in the soil matrix and inhibit mercury mobility.

Several authors have regarded crystalline and amorphous aluminium as efficiently adsorbents for mercury in soils (He et al., 2007; Kim et al., 2004). As extractions were performed at low pH, the increasing acidity of the medium mobilized the aluminium ions and consequently mercury. This could explain the positive relation between mercury in mobile and semi-mobile fractions and the aluminium content.

The association of mercury mobility with the distribution of manganese can be explained by the fact that the presence of manganese oxides is known to significantly promote the solubility of HgS in an HCl solution (Fernández-Martínez and Rucandio, 2005). The influence of manganese on the mobility of mercury is evident, particularly in sample 9, which has one of the highest percentages of extracted mobile mercury and the highest content of manganese.

Organic carbon was one of the factors controlling mercury retention in soils. This was expected given the well know strong affinity of mercury to soil organic matter (Bloom and Preus, 2003).

Similarly, sulfur contributes to the retention of the metal in the non-mobile solid-phase.
Cardoso Fonseca and Ferreira da Silva (2000) and Ferreira da Silva et al. (2005) reported the abundance of sulfides at the surface around the mine, explaining the occurrence of stable forms of mercury (such as cinnabar and other mercury sulfides) in the area of the Caveira mine.

Factor Analysis did not clearly separate samples from Caveira and Estarreja, but did group some samples, according to their characteristics. Samples 5 and 9 (Caveira) are characterized by their high content in manganese and aluminium and low organic carbon, which in turn favours mercury mobility. Samples 1, 6, 8, and 10 (Estarreja) were characterised by higher semi-mobile mercury contents in association with higher aluminium levels. And finally, sample 7 (Caveira) was separated from the remaining samples due to conditions for higher retention of mercury in the solid-phase.

5 Conclusion

This study focussed on the determination of the extractability of mercury in soils with different contamination sources and on the evaluation of the influence of specific soil properties on the behaviour of the contaminant. Results revealed that mercury was mainly present in the semi-mobile phase of soils from both locations. Analysis has also shown that the metal was more mobile in soils from the industrial sampling site than the mine area. The study conducted to evaluate the influence of soil properties in the distribution of mercury demonstrated that the presence of mercury in the mobile phase could be related to manganese and aluminium soil contents. A positive relation between mercury in the semi-mobile fraction and the aluminium content was also observed. By contrary, organic matter and sulfur contents contributed to mercury retention in the soil matrix reducing the mobility of the metal.

Despite known limitations of sequential extraction procedures, the methodology applied here for the fractionation of mercury in contaminated soil samples provided relevant information on mercury’s relative mobility and it may be useful in the implementation of risk assessment methodologies in contaminated sites. In relation to future assessments of risks to human health, crop quality and the environment it could be more useful to define a simple and robust approach that could give information on the distribution of mercury, considering not only its mobility, but also its reactivity and availability to plants and organisms.
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