

Davidson, C.M. Extractability and mobility of mercury from agricultural soils surrounding industrial and mining contaminated areas. Chemosphere . ISSN 0045-6535

<http://strathprints.strath.ac.uk/27622/>

This is an author produced version of a paper published in Chemosphere .
ISSN 0045-6535. This version has been peer-reviewed but does not include the final publisher proof corrections, published layout or pagination.

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

3
4 **Authors:** Ana Teresa Reis¹, Sónia Morais Rodrigues¹, Christine M. Davidson², Eduarda
5 Pereira¹; Armando C. Duarte¹

6
7 **Address:** ¹CESAM and Chemistry Department, University of Aveiro, Campus de
8 Santiago, 3810-193, Aveiro (Portugal)

9 ² WestCHEM, Department of Pure and Applied Chemistry, University of Strathclyde, 295
10 Cathedral Street, Glasgow G1 1XL, UK

11
12 **Corresponding Author:** Ana Teresa Reis (teresareis@ua.pt)

13 Phone: +351 234 370 737

14 Fax: +351 234 370 084

15
16
17
18 **Abstract**

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

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

29 Results of factor analysis indicate that the presence of mercury in the mobile phase
30 could be related to manganese and aluminum soil contents. A positive relation between
31 mercury in the semi-mobile fraction and the aluminium content was also observed. By

32 contrary, organic matter and sulfur contents contributed to mercury retention in the soil
33 matrix reducing the mobility of the metal.

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

37

38

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

40

41

42 **1 Introduction**

43

44 Soil mercury contamination is a problem found at many industrial and mining sites
45 both active and inactive (Biester and Scholz, 1997; Fernández-Martínez *et al.*, 2005; Inácio
46 *et al.*, 1998). Soil concentrations of a few to several thousand mg of mercury per kg of soil
47 were found in the vicinity of industrial facilities where mercury and its compounds are or
48 were used (Biester and Scholz, 1997; Reis *et al.*, 2009). At mining sites, the weathering of
49 waste materials and mankind's action can redistribute mercury, if present, in additional
50 chemical forms, facilitate dispersion in watersheds or through atmospheric emissions, and
51 increase its bioavailability for organisms (Brown *et al.*, 1999).

52 Soils play an important role in the biological cycle of mercury acting both as a sink and
53 a source of this metal to biota, atmosphere and hydrological compartments (Oliveira *et al.*,
54 2007). However, the speciation and the way the different species interact with the soil
55 matrix cause changes in solubility, toxicity and bioavailability of the metal (Biester *et al.*,
56 2002). Han *et al.* (2003) highlighted the high mobility and toxicity of alkyl species, such as
57 methylmercury (MeHg^+) and ethylmercury(II) (EtHg^+), and the high solubility and
58 mobility of soluble inorganic species, such as mercuric chloride (HgCl_2), in comparison
59 with other inorganic mercury species. As observed by the same authors, alkyl and
60 inorganic soluble mercury species contribute to the major portion of potential mercury
61 toxicity in soil (Han *et al.*, 2003). On the other hand, chemically stable species, such as
62 mercuric sulfide (HgS) are considerably less mobile, and, therefore, less toxic (Han *et al.*,
63 2003).

64 As different species exhibit different behavior, mobility and availability, measurements

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

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

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

97 in that order. The operationally-defined mercury fractions are summarized in Table 1.

98 In order to assess the dynamics of mercury within the soil system it is of paramount
99 importance better to understand the relationships between mercury species and soil
100 properties. The fractionation of mercury in soils can be affected by clay minerals, metal
101 oxides, organic matter and pH (Sánchez *et al.*, 2005). Complexes formed by divalent
102 mercury with soluble organic matter, chlorides and hydroxides may contribute to its
103 mobility (Millán *et al.*, 2006; Sánchez *et al.*, 2005). Organic matter presence in soils can
104 also lead to the formation of mercury complexes and inhibit mercury biomethylation
105 processes (Bloom and Preus, 2003). The pH affects the speciation of mercury in the soil
106 solution and influences the mobility and availability of mercury in soil (Yin *et al.*, 1996).
107 According to Jing *et al.* (2007) and Miretzky *et al.* (2005) desorption of inorganic divalent
108 mercury species from soil components increases with decreasing pH since, in general, trace
109 element cations become more soluble and therefore more mobile as pH decreases (Chopin
110 and Alloway, 2007). The presence of sulfur is very important in the chemistry of mercury,
111 as in the presence of sulfides mercury becomes tightly bound to them, forming the
112 insoluble HgS (Boszke *et al.*, 2003). Because HgS is not very reactive or mobile, the
113 formation of this compound allows mercury to be retained; it therefore becomes less
114 available for methylation and potentially less harmful to the environment.

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

123

124 **2 Material and methods**

125 **2.1 Study site description**

126

127 The first set of samples was collected in the vicinity of the industrial complex of
128 Estarreja, North-Western coast of Portugal (**Figure 1**). This complex dates back to 1950
129 (Inácio *et al.*, 1998) and is home to a large chlor-alkali plant which used to produced
130 chlorine and caustic soda by the mercury cell process, where liquid elemental mercury is
131 utilized as a cathode in the electrolysis of a saturated brine solution (Ullrich *et al.*, 2007).
132 As other studies show (Lacerda and Salomons, 1998; Ullrich *et al.*, 2007) mercury-cell
133 chlor-alkali plants have been identified as major sources of mercury to the environment.
134 Although the plant started to change the production process in 1994 and completely ceased
135 the use of mercury in 2002 (Ospar Commission, 2006), mercury emitted from the existing
136 plant still remains significant in the surrounding environment. Until 1975 the liquid
137 effluents from this plant, containing many different types of contaminants (Batista *et al.*,
138 2002), including mercury, were discharged directly into man-made effluent streams.
139 Consequently, the pollutants were transported for several kilometres through the
140 agricultural fields surrounding the chlor-alkali plant (Costa and Jesus-Rydin, 2001).
141 Although after 1975 impermeable pipes were constructed, and the streams are no longer
142 used for effluent transport, these are still present in fields.

143 A second set of samples was collected in the Caveira sulfide mine, which is located in
144 Grândola, in the North-West region of the Iberian Pyrite Belt (IPB, Figure 1). The IPB is a
145 well known mining district of worldwide significance, due to its unusual concentration of
146 large and medium sized mineral deposits, including ores of copper, iron, lead, sulfur and
147 zinc. Antimony, arsenic, cadmium, cobalt, gold, mercury, selenium and silver can also be
148 found in soils from the IPB (Barriga, 1990). Past mining activities at the Caveira mine
149 included pyrite (FeS_2) and Cu extraction. From 1936 until the 1970's Caveira massive
150 sulfides were exploited for sulfur. Although the mine is now closed, soil metal
151 contamination and acid mine drainage still pose severe environmental problems at the site.
152 Large volumes of waste were produced by the mining activities and various types of
153 tailings deposited in the area (the amount of waste stored on the site is estimated to be
154 higher than 2 Mt) (Cardoso Fonseca e Ferreira da Silva, unpublished results). Rainwater
155 circulates and percolates easily over and through these tailing materials causing significant
156 erosion and transport of tailings debris to areas nearby and downstream.

157

158 2.2 Sampling

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

168 Sampling at both sites was performed using a plastic spatula and samples were placed
169 in plastic bags during transport to the laboratory, where they were pre-treated within one
170 hour. The soil sampling depth was 0–15 cm. Once in the laboratory, soil samples were air
171 dried at room temperature to constant weight. Stones were removed and soil aggregates
172 were crushed and homogenised, during the drying stage. The dried samples were sieved to
173 < 2 mm using a nylon sieve. The air-dry soil, < 2 mm fraction, was used both for general
174 characterization of these soils and for mercury extraction.

175

176 2.3 Soil samples characterisation

177 The soil pH (CaCl_2) was determined using a WTW pH meter-538, according to the ISO
178 10390:1994 method. Total carbon (TotC) percentages were measured on an Elemental
179 Analysis instrument (LECO CNH-2000), according to ISO 10694:1995. For the
180 determination of organic carbon content (OrgC), an excess of solution of 4 M of
181 hydrochloric acid (HCl) was added to a crucible containing a weighed quantity of soil. The
182 crucibles were left to stand for 4 h and then were dried for 16 h at 60-70 °C. The analysis
183 of carbon content after the removal of carbonates (organic carbon) was performed using
184 the same procedure of total carbon determinations.

185 The particle size distribution and clay contents of the soil samples were determined
186 using a Coulter LS230 laser diffraction particle size analyzer. The classification of soils
187 followed the USDA Texture Classes: sand fraction ($0.050 < \% < 2\text{mm}$), silt fraction
188 ($0.002 < \% < 0.050\text{mm}$), and clay fraction ($\% < 0.002\text{mm}$). Classification of samples was
189 achieved by using the *Talwin 42*[®] classification software program.

190 The pseudo-total contents of aluminium (Al), iron (Fe), manganese (Mn) and sulfur (S)
191 were extracted by *aqua regia* (according to ISO 11466:1995) and analysed by ICP-MS
192 (ICP-MS THERMO X Series, Peltier Nebulizing Camera, Burgener Nebulizer; CETAC
193 AS510 auto-sampler; the CeO⁺/Ce⁺ ratio was optimized at < 2%; Internal standard: In).
194 The instrument was tuned using a 10 µg kg⁻¹ multi-element tuning solution. The
195 operational conditions used are summarized as follow: RF power: 1400 W; plasma gas
196 flow (argon): 13 L min⁻¹; auxiliary gas (argon): 0.90 L min⁻¹; nebulizer flow (argon): 0.95
197 min⁻¹.

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

205

206

207 **2.4 Sequential extraction procedure**

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

210

211 **2.4.1 Extraction of the mobile fraction (M)**

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

214 A sample (1.0–2.0 g) was weighed and added to a 10 mL centrifuge tube with 2.5 mL
215 of the extract solution. The sample and the extract solution were mixed well by vigorous
216 shaking for 2 min. The pH was checked and, when necessary, concentrated HCl was added
217 drop-wise until the pH of the mixture was between 1.5 and 3. The sample was then
218 sonicated at room temperature (not at 60±2 °C, as referred in [Han *et al.* 2003](#)) for 7 min,
219 and centrifuged (3200 rpm, 5 min) to separate the supernatant from the soil matrix. The
220 supernatants were collected using a Pasteur pipette and transferred to a vial. This extraction

221 was repeated three more times. The residue was then rinsed by adding 2.5 mL of DDI
222 water, shaken for 2 min and centrifuged. All the extraction supernatants and the water
223 rinse were combined. This final solution was kept at 4 °C and analysed within 48 hours.
224

225 2.4.2 Extraction of the semi-mobile fraction (SM)

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

234 For the extraction of semi-mobile species, a solution of 1:2 (v/v) HNO₃:DDI water is
235 required. A 5 mL aliquot of this solution was added to the residue and mixed by shaking it
236 vigorously. The mixture was heated to 95±2 °C for 20 min in a sand bath. To avoid losses
237 of volatile mercury species, cap tubes were replaced by glass spheres during the heating
238 step, providing both sufficient cover and reflux. After cooling to room temperature,
239 samples were centrifuged (3200 rpm, 5 min), the supernatant was collected, and the
240 extraction was repeated. The remaining soil residue was washed with 5 mL DDI water.
241 The rinse water was combined with both supernatants and the solution stored at 4 °C until
242 analysis.
243

244 2.4.3 Extraction of the non-mobile fraction (NM)

245 The procedure for the extraction of the non-mobile phase was similar to the one used
246 for the semi-mobile phase except that the extraction solution was 1:6:7 (v/v/v)
247 HCl:HNO₃:DDI water. The remaining residue (RES) was dried at 40 °C and analysed for
248 mercury content.
249

250 2.4.4 Mercury analysis

251 Total mercury contents in all samples, extracted solutions and residual matrix were

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

261

262 2.4.5 Quality control and quality assurance

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

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

272 The sequential extraction method was also tested by applying the procedure to CRM-
273 021 Dry soil No. 3 (sandy loam) from RTC (USA). Although this reference material is not
274 certified for the mercury fractions targeted by the Kingston method, the sum of the three
275 fractions was compared to the certified value for total Hg (4.7 mg kg⁻¹). The mean results
276 found for the 8 replicate samples analyzed were 0.0199 mg kg⁻¹ and 4.5 mg kg⁻¹, for mobile
277 and semi-mobile fractions, respectively. Mercury levels for the non-mobile and residual
278 fractions were below the detection limit (0.05 ng). The mean sum (4.5 mg kg⁻¹) was within
279 the confidence interval (4.5 – 5.1 mg kg⁻¹) and, as a recovery of 96% was obtained, the
280 extraction efficiency was found acceptable.

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

284 percentages of 99-114%.

285

286 **2.5 Statistical analysis**

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

292 **3 Results**

293 **3.1 Total mercury and soil characteristics**

294 Results obtained for the determination of total mercury in the fourteen samples are
295 shown in Table 2. Total mercury concentration ranges between 1.0 and 91 mg kg⁻¹ for
296 Estarreja samples and 1.1 and 98 mg kg⁻¹ for soils of Caveira. The soil properties are also
297 shown in Table 3.

298 Soil pH in Caveira varied between 3.6 and 5.3. Although all soils analysed were acidic,
299 an unusually low pH value was observed in sample 11 (pH 3.6). The Caveira area is known
300 to be affected by acid mine drainage (Cardoso Fonseca and Ferreira da Silva, 2000) which
301 may explain the low pH. Acid mine drainage is formed when pyrite (FeS₂) and other metal
302 sulphides are exposed to oxygen and water and subjected to oxidising conditions resulting
303 in the production of sulfuric acid (low pH), sulphates and dissolved metal ions
304 (Ziemkiewicz et al., 1997).

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

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

311 The “active” forms of aluminium and iron (which occur as amorphous hydroxides and
312 are bond to organic matter) were extracted as oxalates from soil samples and measured in
313 an ammonium oxalate-oxalic acid extract. A large variability between soil samples was

314 observed with respect to amorphous aluminium oxides and amorphous iron oxides (which
315 varied in the range of 0.057 – 0.21% and 0.068 – 0.16%, respectively). In general,
316 aluminium oxides were present in relatively higher concentrations in samples from
317 Estarreja while the highest contents of iron oxides were found in samples from Caveira.
318 The iron amorphous oxides contents of these soils, particularly at the Caveira area, are
319 relatively higher than those from a study of Portuguese agricultural acid soils which
320 reported a iron oxides range of 0.0073 – 0.46% and a median of 0.096% (Horta and
321 Torrent, 2007). The contents of aluminium oxides observed in Estarreja were also higher
322 than those observed by Horta and Torrent (2007).

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

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

328

329 **3.2 Fractionation of mercury**

330 The fractionation (Figure 2) revealed that in all samples mercury was mainly present in
331 the semi-mobile phase (between 63 and 97%). The mobile fraction represented a much
332 lower contribution to the total mercury content in both Caveira samples (between 0.29 and
333 2.0%), and Estarreja samples (median 1.3%). Two exceptions were observed, with samples
334 9 and 12 presenting an anomalous high percentage of mobile mercury (6.2% and 4.8%,
335 respectively). The higher percentage of mobile mercury in these samples may be explained
336 by the fact that these soils are used for agricultural purposes and are consequently
337 subjected to human influence, including oxidation and the application of fertilizers. Also,
338 characteristics of the soils may partially explain this occurrence, as will be clarified later.

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

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

345 Recovery, defined as the sum of extracted mercury fractions divided by the

346 independently determined total mercury concentration, ranged between 78 and 101 % and
347 was considered satisfactory (Table 2). Recoveries higher than 100% can be explained by
348 the heterogeneity associated with soils. Because mercury is not homogeneously present in
349 soil, it is likely than the aliquot taken for total mercury analysis does not have exactly the
350 same mercury content as the one taken for mercury fractionation, despite the fact that each
351 sample was thoroughly homogenised prior to analysis. Recoveries lower than 100% can be
352 also justified by the lack of homogeneity of the sample, but can also result from losses of
353 volatile mercury during the process. The same problem was observed by *Kocman et al.*
354 (2004). Better recoveries were obtained for industrial soil samples, probably because of
355 soil characteristics. Estarreja's soils are richer in sand particles and poorer in clay particles
356 than Caveira's soil, which means that the extraction solutions can more easily access
357 mercury in the first case.

358 As total mercury concentration of the fourteen samples ranged between 1.0 and 98
359 mg kg⁻¹, this method of fractionation proved to give good results both for high and low
360 total mercury concentrations.

361

362 **3.3 Factor Analysis**

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

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

377 For semi-mobile fraction, three factors were identified that, in total, explain 81% of

378 variance (Table 4). The semi-mobile fraction has its highest loading on Factor 3, as well as
379 aluminium, indicating that the distribution of this variable is related with this particular
380 fraction. As shown in Figure 3b, samples 1, 6, 8, and 10 have the highest percentage of
381 semi-mobile mercury and also of aluminium. This factor did not allow distinguishing
382 Estarreja from Caveira samples (Figure 3b). Both Factor 1 (highest loadings of organic
383 carbon, sulfur, and clay) and Factor 2 (highest manganese and iron loadings) allowed to
384 separate specific Caveira samples from the dataset (Factor 1: highest scores for samples 3,
385 11, 13; Factor 2: highest scores for samples 5 and 9) (Figure 3b).

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

394

395 **4 Discussion**

396 Although the mercury fraction in the mobile phase generally did not exceed 2% of total
397 mercury, given the high contamination of some samples this fraction may still represent
398 significant amounts of bioavailable mercury. The importance of this fraction should not be
399 underestimated, since it includes among others the alkyl species (Han *et al.*, 2003). These
400 mercury species are more mobile, more toxic and more readily bioaccumulated than any
401 other mercury species (Han *et al.*, 2003). In the mobile fraction are also present soluble
402 inorganic mercury species. These species, such as mercury chloride (HgCl₂) are more
403 easily transported by natural processes than other inorganic mercury species and can also
404 serve as substrates for mercury methylation (Bloom *et al.*, 1999; Han *et al.*, 2003).
405 Combined, these extractable organomercury species and extractable soluble inorganic
406 species contribute to the major portion of mercury potential toxicity in soils. Considering
407 that the majority of these soils are predominantly used for agricultural and livestock

408 purposes (Reis et al., 2009), the presence of mobile and toxic mercury species, even in low
409 concentrations, may be of concern.

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

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

426 The mercury species that fall into the semi-mobile category, such as elemental
427 mercury, are less toxic than easily extractable mercury species (Han et al., 2003). Such
428 species include Hg^0 or amalgams of mercury with another metal, Hg^{2+} complexes, which
429 can be also present in the mobile phase, and Hg_2Cl_2 to a small extent (Table 1). Therefore,
430 although this fraction is not immediately available, its species can be easily converted into
431 more readily available ones. The soils from Estarreja and Caveira presented different
432 distribution of mercury in the semi-mobile phase (Mann-Whitney $p=0.003$), with soils of
433 Estarreja showing higher concentration of semi-mobile mercury species. Considering that
434 these soils are used for agricultural purposes, the presence of semi-mobile mercury species
435 in significant concentrations can pose a risk upon exposure.

436 The non-mobile fraction includes the less available and less toxic species of mercury,
437 such as HgS , HgSe or Hg_2Cl_2 (Han et al., 2003). The percentage of mercury in the non-
438 mobile and residual fractions was different for mine and industrial soils, as confirmed by
439 the Mann-Whitney test ($p=0.018$ for non-mobile fraction and $p=0.018$ for residual

440 fraction), with mine soils exhibiting higher concentrations and higher variability in
441 concentrations in both fractions.

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

449

450 **4.1 Influence of soil properties on mercury fractions**

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

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

462 The association of mercury mobility with the distribution of manganese can be
463 explained by the fact that the presence of manganese oxides is known to significantly
464 promote the solubility of HgS in an HCl solution (Fernández-Martínez and Rucandio,
465 2005). The influence of manganese on the mobility of mercury is evident, particularly in
466 sample 9, which has one of the highest percentages of extracted mobile mercury and the
467 highest content of manganese.

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

471 Similarly, sulfur contributes to the retention of the metal in the non-mobile solid-phase.

472 Cardoso Fonseca and Ferreira da Silva (2000) and Ferreira da Silva *et al.* (2005) reported
473 the abundance of sulfides at the surface around the mine, explaining the occurrence of
474 stable forms of mercury (such as cinnabar and other mercury sulfides) in the area of the
475 Caveira mine.

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

483 **5 Conclusion**

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

495 Despite known limitations of sequential extraction procedures, the methodology
496 applied here for the fractionation of mercury in contaminated soil samples provided
497 relevant information on mercury's relative mobility and it may be useful in the
498 implementation of risk assessment methodologies in contaminated sites.

499 In relation to future assessments of risks to human health, crop quality and the
500 environment it could be more useful to define a simple and robust approach that could give
501 information on the distribution of mercury, considering not only its mobility, but also its
502 reactivity and availability to plants and organisms.

503

504

505 **Acknowledgements**

506 This study was supported by the Portuguese Foundation for Science and Technology via a
507 PhD grant (SFRH/BD/43681/2008; A.T.Reis) and Project SEPMERCURY - Sequential
508 extraction methods for assessing the origin, mobility and toxicity of mercury from
509 contaminated solid matrices and preparation of new sediment and soil reference materials
510 (PTDC/AAC-AMB/105157/2008).

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