

Comparison of extractants used for the assessment of mercury availability in a soil from the Almadén mining district (Spain)

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Abstract Single extraction methods have been extensively used to assess the availability of metals in polluted soils. This work focused on checking the feasibility of several chemicals, i.e. CaCl₂, EDTA, diethylenetriaminepentaacetic acid (DTPA) and a low-molecular-weight organic acid mixture (rhizosphere-based method), to be used as extractants for mercury (Hg) in a soil from the Almadén mining district (Spain). Moreover, the effect of several experimental parameters, i.e. extraction time (0.5, 1, 2, 5, 16 and 24 h), concentration of extractant (0.01, 0.05, 0.1 and 1 M) and soil/extractant ratio (1:2, 1:5 and 1:10), on the amount of Hg extracted was investigated. The Hg extraction ability followed the descending order EDTA > rhizosphere-based method > DTPA ≈ CaCl₂. This ranking was attributed to the higher complexation power of EDTA and organic acids. It was also found that extraction times between 2 and 5 h are required to avoid underestimation of mobile Hg and re-adsorption of the Hg previously extracted. Although some exceptions were found, Hg extraction efficiency was generally enhanced with higher extractant concentrations. Finally, the amount of Hg extracted by the four extractants increased with decreasing soil/extractant ratios.

Keywords Mercury · Metal availability · EDTA · DTPA · Rhizosphere-based method · Calcium chloride · Single extraction

Introduction

Pollution of soils by metals and other potentially toxic elements is a global environmental concern that is receiving increasing attention due to the hazards for ecosystems and human health. The environmental risk of metal pollution depends not only on the total soil concentrations but also on the geochemical and biochemical properties of a given metal and soil (Gupta and Sinha 2006). It is generally accepted that the evaluation of the potential risk and toxicity of metals in soils requires an assessment of the proportion of the mobile and available forms of the total metal (Feng et al. 2005a; Monteiro et al. 2016). Availability determines the fate of metals in the soil and their capacity to be leached to groundwater or taken up by plants. A complex variety of abiotic and biotic processes affects metal availability; these include adsorption onto and desorption from mineral surfaces, precipitation, complexation by organic matter and interactions with plants and microorganisms (Wang et al. 2004; Evangelou et al. 2007; Menzies et al. 2007).

Several chemical methods have been developed in an attempt to predict availability of metals, which have largely been applied to agricultural, industrial and mining soils. They can be divided into single and sequential extraction methods. In general, sequential extraction methods are time-consuming and their complexity limits the procedural robustness; in fact, they require an elevated technical skill to ensure the quality of the results. On the contrary, the single or one-step extraction methods are fast and cost-effective and require low technical skill (Reis et al. 2016). Single extraction

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methods are preferred when the objective is to assess the metal fraction available for plant absorption, which is named as phytoavailable (Menzies et al. 2007). It may include water-soluble, exchangeable, carbonate-associated, Fe and Mn oxide-bound and, in some cases, organic matter-bound fractions; moreover, these are the most toxic metal species in polluted soils. Single extraction methods have been carried out using different chemical extractants: (i) mineral acids at various concentrations, e.g. HCl and HNO₃ (Menzies et al. 2007; Reis et al. 2015; Monteiro et al. 2016); (ii) chelating agents, e.g. EDTA, DTPA (Wang et al. 2004; Feng et al. 2005a, b; Gupta and Sinha 2007); (iii) buffered salts, e.g. NH₄OAc (Reis et al. 2015); (iv) neutral salts, e.g. CaCl₂, NaNO₃ (Wang et al. 2004; Feng et al. 2005a, b; Gupta and Sinha 2007) and (v) a low-molecular-weight organic acid mixture, composed of acetic, lactic, citric, malic and formic acids (rhizosphere-based extraction method) (Feng et al. 2005a, b).

Mercury is considered one of the most toxic pollutants because of its harmful effects on the environment and public health. Mercury pollution in soils may be caused by direct sources, i.e. industrial discharges, dumping of wastes and deposition of mine tailings directly on the soil, or by indirect sources, such as the deposition of atmospheric mercury emissions (Leterme et al. 2014). Some of the environmental hazards regarding mercury pollution are due to its toxicity and its ability to biomagnify while it passes through the ecosystems and the food chain (Boening 2000). Despite the considerable progress in Hg fractionation and speciation in soils and their relationship with phytoavailability, there is not a generally accepted approach to determine this parameter in the risk assessment studies (Monteiro et al. 2016; Reis et al. 2016). The use of single extraction methods is often the most useful option in order to estimate Hg transfer into the food chain, although the validity of a soil test for the evaluation of Hg availability has not still been developed (Monteiro et al. 2016). Reis et al. (2016) have recently reported an extensive overview of the Hg fractionation and speciation procedures used in soils. According to it, single extraction of Hg has been conducted using water (for the assessment of the water-soluble Hg), ammonium acetate (for the exchangeable Hg) and diluted hydrochloric acid (for the carbonate-bound fraction of Hg). In addition, the organic matter-bound fraction of mercury can be determined using oxidizing reagents or reagents known for their ability to extract humic substances, such as NaOH, diluted HNO₃ and Na₂P₂O₇ (Issaro et al. 2009; Monteiro et al. 2016). Na thiosulphate has been reported to be an effective reagent for soil-Hg single extractions, especially when the metal is strongly bound to specific sites of organic matter (Issaro et al. 2010). Nevertheless, together with the chemicals used as extractants, other operational conditions, such as extraction time, soil to extractant ratio and concentration of the extractant, need to be studied in order to harmonize mercury single extraction procedures (Reis et al. 2015).

The objective of this work was to check the feasibility of several chemicals that have been widely used for single extraction methods, i.e. CaCl₂, EDTA, diethylenetriaminepentaacetic acid (DTPA) and a low-molecular-weight organic acid (LMWOA) mixture, to be used as extractants for mercury in a soil from the historical mining district of Almadén (Spain). Additionally, this study was focused on establishing optimal procedural conditions for the extraction of the available mercury, considering extraction time, concentration of extractant and soil/extractant ratio.

Materials and methods

Soil characterization

The soil used in the experiment was randomly collected from a farming area next to the metallurgical plant of Almadenejos, in the Almadén district (Ciudad Real, Spain). It is a mercury-mining area located in central Spain, 300 km southwest of Madrid, with a long history of mining activities (more than 2000 years). This fact, together with natural emissions, has resulted in high levels of mercury in soils, water and air of the surrounding areas (Higuera et al. 2006). Surficial samples (0–20 cm) of cultivated soil were collected and mixed. The soil was air-dried, disaggregated and screened through a 2-mm sieve. The <2-mm fraction was used for the experiments and for the measurement of the soil physical-chemical properties.

Soil pH and electrical conductivity (EC) were measured in the supernatant of a 1:5 soil/water mixture (w/v), organic carbon was determined using a total organic carbon analyser (Shimadzu TOC-VCSH, Columbia, USA), particle size distribution (clay, silt and sand content) was determined using laser diffractometry (Beckman Coulter LS, Fullerton, USA) and cation exchange capacity (CEC) was measured by the ammonium acetate saturation method (MAPA 1994). The EPA 3051A method was used to determine the total concentration of Hg in soil. The procedure was as follows: a 0.5 g sample (fraction <2 mm) was ground into a fine powder by a ball mill (Retsch MM200, Haan, Germany), and then it was digested in a microwave oven (CEM MARS 5, Matthews, USA) using a mixture of 9 mL of concentrated HNO₃ (Panreac, ACS reagent grade, 69% w/w) and 3 mL of concentrated HCl (Panreac, ACS reagent grade, 37% w/w). The Hg concentration of the obtained extracts was analysed as described in the “Mercury analysis” section, using three replicates of the soil sample.

Extraction experiments

The first experimental series of soil extractions was carried out using the extraction procedures reported in Table 1. The

Table 1 Single extraction procedures used in the first experimental series of this study

Extraction method	Procedure	Refs.
CaCl ₂	2.0 g soil + 20 mL 0.01 M CaCl ₂ , shaking for 3 h	Novozamsky et al. 1993
EDTA	2.0 g soil + 20 mL 0.05 M EDTA adjusted to pH 7.0, shaking for 1 h	Quevauviller et al. 1996
DTPA	10.0 g soil + 20 mL 0.005 M DTPA + 0.01 M CaCl ₂ + 0.01 M TEA adjusted to pH 7.3, shaking for 2 h	Lindsay and Norvell 1978
RHIZO	2.0 g soil + 20 mL LMWOAs; total concentration of acetic, lactic, citric, malic and formic acids was 0.01 M; their molar ratio was 4:2:1:1:1 (c/c), shaking for 16 h	Feng et al. 2005a

TEA triethanolamine, RHIZO rhizosphere-based method, LMWOAs low-molecular-weight organic acids

procedure employing the mixture of low-molecular-weight organic acids is called the ‘RHIZO method’ from here on. EDTA solution was prepared using ethylenediaminetetraacetic acid dipotassium salt dehydrate (Panreac, 98% purity); pH of the EDTA solution was adjusted to 7.0 by adding 0.1 M ammonia solution. DTPA and LMWOA solutions were prepared from DTPA (Panreac, 99% purity), citric (Panreac, ACS reagent grade, 99.5% purity), malic (Panreac, 99.5% purity), formic (Panreac, ACS reagent grade, 98% purity), lactic (Panreac, 92% purity) and acetic (Panreac, ACS reagent grade, 99.7%) acids. The extractions were carried out in 100-mL glass containers using an orbital end-over-end shaker at a constant rate of 80 rpm and at temperature of 30 °C. The resulting extracts were centrifuged at 3000 rpm for 15 min. The pH of the supernatant was measured and then acidified with 5% nitric acid and stored at 4 °C before mercury analysis. All the extraction experiments were carried out in triplicate.

The influence of the extraction time was studied in the second experimental series. Single extractions were performed in the same way described for the first experimental series, using extraction times of 0.5, 1, 2, 5, 16 and 24 h. A third experimental series was conducted to study the influence of the concentration of the extractant solution; four concentrations were used, i.e. 0.01, 0.05, 0.1 and 1 M, while the extraction times adopted were those which had shown the highest efficiency in the second experimental series. At last, the influence of the soil/extractant ratio was studied (fourth experimental series) using three different values for this parameter, i.e. 1:2, 1:5 and 1:10 g of soil per millilitre of extractant solution. Contact time and concentration of extractant were defined according to the optimized values found in the previous experimental series.

Mercury analysis

The Hg content of the soil extracts (coming from digestion and single extraction experiments) was measured using an atomic absorption spectrophotometer Varian SpectraAA 240FS (Varian Inc., California, USA) equipped with a hydride

generator VGA-77. The cold vapour atomization was conducted using an acid solution of stannous chloride (SnCl₂ 25% w/v in HCl 20% v/v; SnCl₂ was ACS reagent grade, 98% purity, from Panreac) as reductant agent for the samples. Three different measurements of each extract were carried out to determine average Hg concentration (a typical variation of less than 5% was found among different replicates). The 2711 Standard Reference Material with a certified Hg concentration of $6.25 \pm 0.19 \mu\text{g g}^{-1}$ (Montana Soil, from LGC Promochem) was used to assess the accuracy of the analytical method, obtaining a typical agreement of 95–103% between the certified value and the concentrations found ($n = 3$).

Statistical analyses

IBM SPSS Statistics program version 22.0 was used for all the statistical analyses. The effect of the different studied parameters on mercury extraction from the soil, i.e. type of extractant, concentration of extractant and soil/extractant ratio, was checked using one-way ANOVA. Data normality was previously assessed by using the Kolmogorov-Smirnov test.

Results and discussion

Effect of the type of extractant

The main basic physicochemical properties of the soil are listed in Table 2. The soil was classified as sandy loam with a total Hg concentration of $41.1 \mu\text{g g}^{-1}$. As expected, the amount of mercury extracted varied greatly with the different extraction methods and reagents (Table 3). The extracted Hg followed the descending order 0.05 M EDTA > RHIZO method > 0.005 M DTPA \approx 0.01 M CaCl₂. It must be pointed out that the highest amount of mercury extracted from the soil, corresponding to the EDTA method, accounts for only 0.06% of the total soil Hg. It indicates the low availability of mercury in the used soil and is in agreement with other previous studies on agricultural soils belonging to the Almadén district where

Table 2 Basic physicochemical properties of the soil used in the extraction experiments

Soil properties	Values
pH	5.3
OM (%)	3.1
CEC (cmol _c kg ⁻¹)	20.1
EC (μS cm ⁻¹)	267.0
% sand	56.2
% silt	28.8
% clay	15.0
Total Hg (μg g ⁻¹)	41.1

OM organic matter content, CEC cation exchange capacity, EC electrical conductivity

mercury was poorly available and mainly associated to cinnabar particles (Sánchez et al. 2005; Millán et al. 2006; Rodríguez et al. 2007; Zomoza et al. 2010).

The observed trend of Hg extractability may be explained as follows. EDTA is one of the best chelating agents and forms highly stable soluble complexes with many metal pollutants; it is able to remove carbonate-bound metals, organically bound metals (in soils with low carbonate content) (Sahuquillo et al. 2003; Gupta and Sinha 2007; Ruiz et al. 2009) and metals occluded in oxides and secondary clay minerals (due to its capacity to release trace metals out of the non-silicate-bound phases) (Feng et al. 2005a; Žemberyová et al. 2006). For the RHIZO method, the relatively high extractability of mercury is mainly due to the complexation of Hg by organic acids (Gabriel and Williamson 2004; Quin et al. 2004; Feng et al. 2005b), although the observed decrease of the soil pH (Table 3) could also contribute to some extent, e.g. by means of the dissolution of carbonates (Reis et al. 2016). Metals extracted by the RHIZO method correspond to the potentially available metal fractions in soil; in fact, this method was shown to predict the bioavailability of metals to plants (Feng et al. 2005a, b). The DTPA method was initially developed for calcareous soils since the solution used is buffered at pH 7.3, thus preventing the dissolution of carbonates (Novozamsky et al. 1993); its extracting power is mainly based on the ability of DTPA to chelate different metals, e.g. Fe, Cu, Mn and Zn,

Table 3 Effect of the type of extractant on Hg extraction from soil: solution pH and amount of Hg extracted (ng Hg per g of soil)

Extractant	pH	Hg (ng g ⁻¹)
CaCl ₂	4.41 ± 0.27b	3.80 ± 0.89a
EDTA	7.48 ± 0.04c	26.57 ± 3.17c
DTPA	6.95 ± 0.05c	4.06 ± 0.35a
RHIZO	2.35 ± 0.07a	10.45 ± 2.44b

Values are given as mean ± standard deviation ($n = 3$). Different letters indicate significant differences ($p < 0.05$, Tukey's test) between extractants

together with the ability of CaCl₂ (which is one of the chemicals included in the extraction solution, Table 1) to exchange rapidly with bivalent cations (Hammer and Keller 2002). In our study, mercury was poorly extracted by DTPA, with similar results to those of CaCl₂ (Table 3). This result is in agreement with that reported by Jing et al. (2008) for the Hg extraction from an acid loamy soil. Despite that DTPA is a chelating agent slightly stronger than EDTA (Hong et al. 2002), there are numerous studies showing that its ability for extracting metals from soils is significantly lower than that of EDTA (Feng et al. 2005b; Van Engelen et al. 2007; Gupta and Sinha 2006, 2007). EDTA is assumed to extract both carbonate and organically bound metal fractions (Gupta and Sinha 2007), while DTPA would only be capable of extracting the metals associated to Fe, Al and Mn oxides (Feng et al. 2005b; Rózański et al. 2016). The low Hg availability in the studied soil together with the low concentration of DTPA in the extracting solution could be suggested as additional reasons for the low efficiency shown by this method in the present study. Zhu et al. (2015) used the DTPA extraction method to predict the accumulation of methylmercury (MeHg) in rice grains. They found that the ability of DTPA to extract MeHg was similar to that of CaCl₂, concluding that DTPA, containing no strong binding sites for MeHg, was a weak complexing agent for MeHg; moreover, extraction with DTPA was not a good method to assess MeHg phytoavailability. At last, the concentration of Hg extracted by CaCl₂ was lower since this salt is only able to extract the easily exchangeable Hg fraction, e.g. Hg adsorbed onto mineral surfaces (Feng et al. 2005a; Jing et al. 2008; Issaro et al. 2009). The effectiveness of CaCl₂ extraction to predict plant availability is not clear from the results reported in the literature. Zhu et al. (2015) found that MeHg levels in rice grains were not correlated with CaCl₂-extracted MeHg, while Jing et al. (2008) reported good correlations between Hg extracted by CaCl₂ and its concentration in rice seeds and radish roots.

Effect of the extraction time

Figure 1 shows the amount of Hg extracted per unit of weight of soil (ng g⁻¹) as a function of the extraction time (h) for the four chemicals used. In spite of the different extractant power of the different reagents and, therefore, the dissimilarity of the extracted Hg concentrations, some common trends can be observed. All the mercury extraction profiles showed two different stages: a first stage where the concentration of extracted Hg sharply increases with the extraction time and a second stage where the extracted Hg concentration decreases as the extraction time increases. Specifically, the highest concentration values of extracted Hg were reached at 2 h for EDTA and CaCl₂, 5 h for DTPA and 16 h for the RHIZO method. Moreover, the equilibrium was not apparently reached for any extractant after 24 h of contact. pH of the extractant

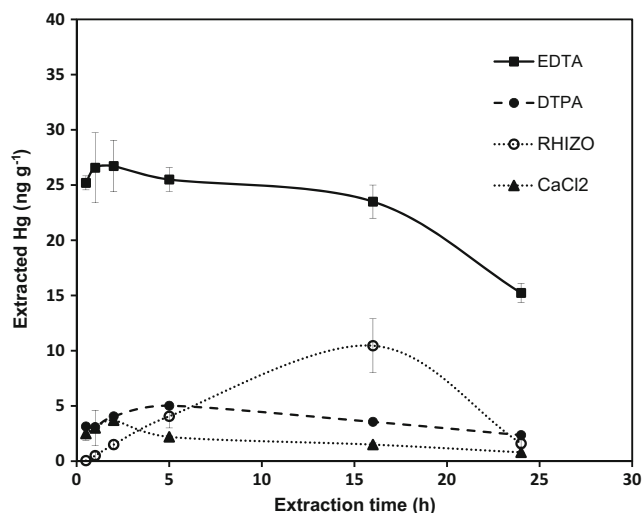


Fig. 1 Effect of extraction time on Hg extracted from soil (ng of Hg per g soil DW) for the different extractants. Experimental conditions: **a** EDTA concentration 0.05 M, soil/extractant ratio 1:10; **b** DTPA concentration 0.005 M, soil/extractant ratio 1:2; **c** RHIZO, LMWOA concentration 0.01 M, soil/extractant ratio 1:10; **d** CaCl₂ concentration 0.01, soil/extractant ratio 1:10. Error bars represent the STD of three replicates

solutions after the experiments was measured. The pH values obtained were in the ranges 6.9–7.5, 6.8–7.2, 4.5–5.0 and 2.0–2.3 for EDTA, DTPA, CaCl₂ and the RHIZO method, respectively; they did not change significantly for the different contact times and no evident trend was found.

The initial rise in the extracted Hg in the graphs of Fig. 1 suggests that a significant portion of the available Hg can be readily extracted using relatively short extraction times, i.e. 1–2 h. It is in agreement with the results previously reported by Reis et al. (2015) for the extraction of Hg using NH₄OAc and diluted HCl as extractants. Those authors showed that the mercury extraction profile consists of two different stages; in the first extraction stage (*t* < 10 h), Hg was released at a faster rate than afterward, most likely because the latter mercury species were more strongly associated with the soil matrix. Moreover, the fact that the soil used in our study was a sandy loam type would contribute to a faster release of mercury (the lower the clay content is, the higher the diffusion rate) (Reis et al. 2014). From our results, it can be concluded that extraction times of at least 2 h could be necessary to avoid underestimation of the Hg that can migrate from the soil to other environmental compartments, e.g. plants or groundwater (Reis et al. 2016). On the other hand, the use of too long extraction times led to a re-adsorption of the Hg previously released to some extent (Fig. 1). This phenomenon has been previously reported for Pb and Zn extractions using the chelating agent EDDS ([S,S]-stereoisomer of ethylenediaminesuccinic acid) (Yan et al. 2010) and in the Hg extraction with ammonium acetate (Reis et al. 2015). According to this, all Hg species extracted from the soil, as Hg²⁺ free ions and/or as dissolved complexes with EDTA, DTPA or LMWOAs (RHIZO method), could be re-adsorbed onto the soil surfaces (Yan et al. 2010).

For longer contact times, the shape of the extraction profiles is the result of a balance between the relative rates of mercury extraction and re-adsorption processes (Fig. 1). The fact that the peak of extraction for the RHIZO method was reached after 16 h of contact instead of the considerably shorter times observed for the other extractants could be explained taking into account that Hg adsorption on soil particles generally decreases with decreasing pH, due to the competition between H⁺ ions and metal ions on soil surfaces (Gabriel and Williamson 2004). Therefore, our results demonstrate that it is extremely important to consider the pH of the extractant solutions when chemical extractions are used to assess the mercury availability in soils. Together with pH, other soil physicochemical properties such as organic matter content; iron, manganese and sulphur contents; texture and redox and humidity conditions have been shown to control mercury retention or release on/from soil matrix (Reis et al. 2016). Finally, it is worth pointing out that other authors have also found that the equilibrium in the Hg extraction takes place between 24 h and 1 week; this fact is relevant because it may contribute to the increase in the environmental risk deriving from a continuous release of Hg to the environment in polluted areas (Reis et al. 2015; Reis et al. 2016).

Effect of the concentration of the extractant

A third experimental series was carried out to study the effect of increasing extractant concentrations on Hg extraction from the soil. The conditions for the other fixed experimental parameters were the following: (i) EDTA, contact time 2 h, soil/extractant ratio 1:10; (ii) DTPA, contact time 5 h, soil/extractant ratio 1:2; (iii) RHIZO method, contact time 16 h, soil/extractant ratio 1:10 and (iv) CaCl₂, contact time 2 h, soil/extractant ratio 1:10. The results of the extractions are shown in Fig. 2.

Two different trends can be observed when studying the relationship between the extractant concentration and the efficiency of mercury extraction (Fig. 2). For EDTA and CaCl₂, the amount of extracted Hg increased with the concentration of the extractant. However, that increase was not proportional, especially for the highest extractant concentrations: e.g. the concentration of the Hg extracted by EDTA 0.1 M was 77.1 ng per gram of soil, while by increasing EDTA concentration ten times (1 M) only an increase by approximately 25% of the extracted Hg was found (Fig. 2). Differently, Hg removal decreased with respect to that of 0.01 M concentration for higher LMOWA concentrations. At last, for DTPA extraction, the highest concentration of extracted Hg was reached with the 0.05 M concentration; similar values were found for 0.01 and 0.1 M DTPA concentrations, and the lowest extracted Hg concentration was obtained with the 1.0 M concentration (Fig. 2).

The pH of the extractant solutions was measured after the experiments, and the results are shown in Table 4. As

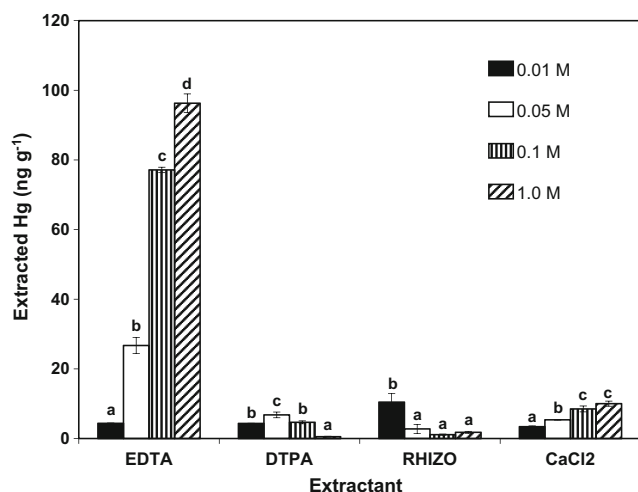


Fig. 2 Effect of extractant concentration on Hg extracted from soil (ng of Hg per g soil DW) for the different extractants. Experimental conditions: **a** EDTA, contact time 2 h, soil/extractant ratio 1:10; **b** DTPA, contact time 5 h, soil/extractant ratio 1:2; **c** RHIZO, contact time 16 h, soil/extractant ratio 1:10; **d** CaCl₂, contact time 2 h, soil/extractant ratio 1:10. Error bars represent the SD of three replicates. Different letters indicate significant differences ($p < 0.05$, Tukey's test) between extractant concentrations

expected, pH did not practically change for the different concentrations of CaCl₂, while it increased for increasing concentrations of EDTA. Regarding the RHIZO method, the pH of the extractant solutions decreased significantly as the LMWOA concentration was increased, reaching a pH value of 1.24 for the highest concentration. In the DTPA extraction, the concentration of DTPA was varied without varying the amounts of the other reagents; as a result, the pH of the extractant solution remained approximately between 6 and 7 for the 0.01, 0.05 and 0.1 M concentrations but it decreased to 2.13 for the 1.0 M concentration.

The results found for the RHIZO method and for DTPA point out the importance of pH in influencing the adsorption/desorption behaviour of Hg in soils. From our results, it seems that Hg release from the soil decreased for very low pH values (around 2 and below). This observation is surprising since there is a general consensus to consider that metal mobility and availability increase as pH decreases (Wang et al. 2004; Clemente et al. 2005); this trend has been also reported for Hg (Reis et al. 2016). Two reasons may be suggested to explain

Table 4 Effect of the extractant concentration on Hg extraction from soil: solution pH after extraction

Concentration	0.01 M	0.05 M	0.1 M	1 M
CaCl ₂	5.12 ± 0.02	5.23 ± 0.08	5.18 ± 0.06	5.24 ± 0.03
EDTA	4.68 ± 0.04	6.67 ± 0.05	7.33 ± 0.08	8.83 ± 0.04
DTPA	7.21 ± 0.08	5.93 ± 0.06	6.11 ± 0.09	2.13 ± 0.03
RHIZO	2.35 ± 0.07	1.69 ± 0.07	1.66 ± 0.04	1.24 ± 0.03

Values are given as mean ± standard deviation ($n = 3$)

the observed trend. On the one hand, several authors have reported that mercury can form insoluble Hg and humic acid-bound Hg at low pH values (Winfrey 1990; Biester and Zimmer 1998). On the other hand, DTPA and LMOWAs are non-specific extractants and, therefore, other metals present in the mineral surfaces in larger concentrations than Hg, such as Fe or Ca, could be released largely from the soil at this low pH and compete with Hg for binding to the complexation agents (Subirés-Muñoz et al. 2011). Gabriel and Williamson (2004) also pointed out the increase in Hg adsorption to humic matter in soil at low pH, due to the abovementioned competition with other ions that are released to a higher extent than Hg. However, in the case of EDTA and CaCl₂ extractants, for which pH was between slight acid to alkaline values, a higher concentration of extractant led to an increase in the Hg release from the soil. In agreement with our results, Subirés-Muñoz et al. (2011) reported a higher Hg extraction with increasing EDTA concentrations for a soil coming from the same mining area (Almadén, Spain). Zou et al. (2009) also showed that the removal of several metals (Pb, Zn, Cd, Cu) using EDTA increased progressively with increasing doses until the value corresponding to the soil requirement (i.e. the stoichiometric dose) was reached; after that, the removal was slightly increased for higher doses. This agrees to what was observed in our study, where the 0.1 M EDTA concentration seemed to correspond to the soil requirement.

Effect of the soil to extractant ratio

A fourth experimental series was carried out to study the effect of the soil to extractant ration on Hg extraction from the soil. The conditions fixed for the other experimental parameters were the following: (a) EDTA concentration 1 M, contact time 2 h; (b) DTPA concentration 0.05 M, contact time 5 h; (c) RHIZO method, LMWOA concentration 0.01 M, contact time 16 h and (d) CaCl₂ concentration 1 M, contact time 2 h. The results of the extraction are shown in Fig. 3.

The variation of Hg extraction with the soil/extractant ratio (1:2, 1:5 and 1:10) for the different chemicals is shown in Fig. 3. The amounts of Hg extracted by the four extractants generally increased with decreasing soil/extractant ratio; however, in most cases the increases were not statistically significant, i.e. for the three ratio values of CaCl₂, for the two highest values of EDTA and for the two lowest ratio values of DTPA and the RHIZO method. This trend agrees with those reported in previous studies (Jing et al. 2008; Reis et al. 2015). However, it seems that this parameter is not as important as the extraction concentration in affecting Hg extraction. The pH of the extractant solutions after the experiments was similar for the different soil/extractant ratios employed for each extractant (data not shown); so, the differences when studying the effect of extractant concentration related to the pH changes in the extraction solution were not observed in this case. Reis

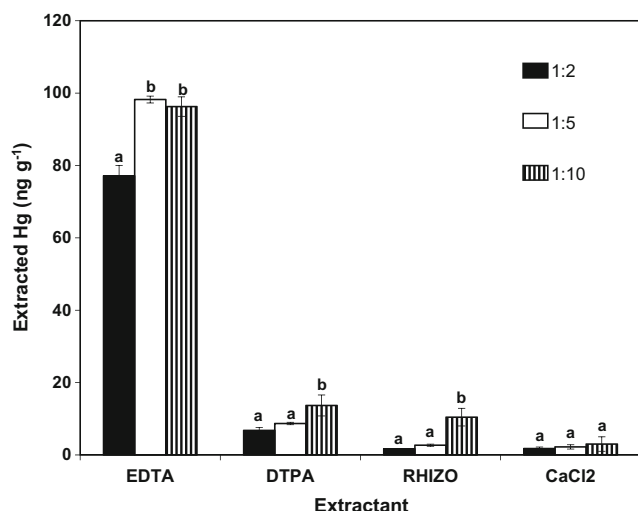


Fig. 3 Effect of the soil to extractant ratio on Hg extracted from soil (ng of Hg per g soil DW) for the different extractants. Experimental conditions: **a** EDTA concentration 1 M, contact time 2 h; **b** DTPA concentration 0.05 M, contact time 5 h; **c** RHIZO, LMWOA concentration 0.01 M, contact time 16 h; **d** CaCl₂ concentration 1 M, contact time 2 h. Error bars represent the SD of three replicates. Different letters indicate significant differences ($p < 0.05$, Tukey’s test) between soil/extractant ratios

et al. (2015) reported similar results as shown here, and they concluded that when choosing the best solid-to-liquid ratio other parameters must be considered, i.e. the soil sample should be homogeneous and representative of the bulk soil, and it is convenient to use soil/extractant ratios high enough to avoid any potential problems with detection limits of the methods and instruments used for mercury analysis. From our results, a 1:10 soil/extractant ratio is recommended for the Hg extraction regardless of the extractant used.

Conclusions

Single extraction methods are a useful and easy tool to assess the availability of metals in polluted soils, as demonstrated in this study for Hg in an agricultural soil collected nearby the Almadén mining district (Spain). However, both the chemical properties of the extractant and the operational parameters employed, i.e. contact time, concentration of the extractant solution and soil/extractant ratio, have a great influence on the amount of metal extracted and, therefore, in the assessment of the hazards derived from the metal pollution.

In this study, we found that, among extractants, EDTA and LMWOAs (RHIZO method) were the most effective reagents to extract mercury from soil due to the strong complexation capacity of EDTA and organic acids. The contact time had an important role on mercury extraction as well. Extraction times of at least 2 h appear to be necessary to avoid underestimation of the mobile Hg. Moreover, higher contact times (more than 5 h) led to a significant re-adsorption of the Hg previously

extracted as free ion or as Hg complex. The increase in the extractant concentration had a different effect on the Hg extraction depending on the chemical and its concentration. Hg removal generally increased by increasing the concentration of the extractant. However, it was noticed that the pH of the extracting solutions had a significant influence on Hg extraction; this effect was observed for high concentrations of extractants used in DTPA and RHIZO methods where pH values around 1.5–2 were reached. Finally, the amount of Hg extracted by the four extractants generally increased with decreasing soil/extractant ratios, although these differences were not statistically significant in all the cases; so, this parameter seems to be less important in influencing Hg extraction compared to the other assessed parameters, including the pH of the extraction solution. To summarize, the experimental conditions that allowed the highest Hg extraction were the following:

- EDTA: contact time 2 h, extractant concentration 1 M and soil/extractant ratio 1:5
- DTPA: contact time 5 h, extractant concentration 0.05 M and soil/extractant ratio 1:10
- CaCl₂: contact time 2 h, extractant concentration 1 M and soil/extractant ratio 1:10
- RHIZO method: contact time 16 h, extractant concentration 0.01 M and soil/extractant ratio 1:10

These findings could contribute to a better estimation of the available fractions of mercury in soils and, therefore, to the development of an established method to assess the environmental risk derived from mercury-polluted soils.

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References

Biester H, Zimmer H (1998) Solubility and changes of mercury binding forms in contaminated soils after immobilization treatment. *Environ Sci Technol* 32:2755–2762

Boening DW (2000) Ecological effects, transport, and fate of mercury: a general review. *Chemosphere* 40:1335–1351

Clemente R, Walker DJ, Bernal MP (2005) Uptake of heavy metals and As by *Brassica juncea* grown in a contaminated soil in Aznalcóllar (Spain): the effect of soil amendments. *Environ Pollut* 138:46–58

Evangelou MWH, Ebel M, Schaeffer A (2007) Chelate assisted phytoextraction of heavy metals from soil. Effect, mechanism, toxicity, and fate of chelating agents. *Chemosphere* 68:989–1003

Feng MH, Shan XQ, Zhang SZ, Wen B (2005a) A comparison of the rhizosphere-based method with DTPA, EDTA, CaCl₂ and NaNO₃ extraction methods for prediction of bioavailability of metals in soil to barley. *Environ Pollut* 137:231–240

Feng MH, Shan XQ, Zhang SZ, Wen B (2005b) Comparison of a rhizosphere-based method with other single extraction methods for

- assessing the bioavailability of soil metals to wheat. *Chemosphere* 59:939–949
- Gabriel MC, Williamson DG (2004) Principal biogeochemical factors affecting the speciation and transport of mercury through the terrestrial environment. *Environ Geochem Hlth* 26:421–434
- Gupta AK, Sinha S (2006) Chemical fractionation and heavy metal accumulation in the plant of *Sesamum indicum* (L.) var. T55 grown on soils amended with tannery sludge: selection of single extractants. *Chemosphere* 64:161–173
- Gupta AK, Sinha S (2007) Assessment of single extraction methods for the prediction of bioavailability of metals to *Brassica juncea* L. Czern. (var. Vaibhav) grown on tannery waste contaminated soil. *J Hazards Mater* 149:144–150
- Hammer D, Keller C (2002) Changes in the rhizosphere of metal-accumulating plants evidenced by chemical extractants. *J Environ Qual* 31:1561–1569
- Higuera P, Oyarzun R, Lillo J, Sánchez-Hernández JC, Molina JA, Esbrí JM, Lorenzo S (2006) The Almadén district (Spain): anatomy of one of the world's largest Hg-contaminated sites. *Sci Total Environ* 356:112–124
- Hong PK, Li C, Banerji SK, Wang Y (2002) Feasibility of metal recovery from soil using DTPA and its biostability. *J Hazard Mater* 94(3):253–272
- Issaro N, Abi-Ghanem C, Bermond A (2009) Fractionation studies of mercury in soils and sediments: a review of the chemical reagents used for mercury extraction. *Anal Chim Acta* 631:1–12
- Issaro N, Besancon S, Bermond A (2010) Thermodynamic and kinetic study of the single extraction of mercury from soil using sodium-thiosulfate. *Talanta* 82:1659–1667
- Jing YD, He ZL, Yang XE, Sun CY (2008) Evaluation of soil tests for plant-available mercury in a soil-crop rotation system. *Commun Soil Sci Plant* 39:3032–3046
- Leterme B, Blanc P, Jacques D (2014) A reactive transport model for mercury fate in soil—application to different anthropogenic pollution sources. *Environ Sci Pollut Res* 21:12279–12293
- Lindsay WL, Norvell WA (1978) Development of DTPA soil test for zinc, iron, manganese, and copper. *Soil Sci Soc Am J* 42:421–428
- MAPA (Ministerio de Agricultura, Pesca y Alimentación de España) (1994) Métodos oficiales de análisis de suelos y aguas. Madrid (Spain).
- Menzies NW, Donn MJ, Kopitke PM (2007) Evaluation of extractants for estimation of the phytoavailable trace metals in soils. *Environ Pollut* 145:121–130
- Millán R, Gamarra R, Schmid T, Sierra MJ, Quejido AJ, Sánchez DM, Cardona AI, Fernández M, Vera R (2006) Mercury content in vegetation and soils of the Almadén mining area (Spain). *Sci Total Environ* 368:79–87
- Monteiro RJR, Rodríguez SM, Cruz N, Henriques B, Duarte AC, Römken PFAM, Pereira E (2016) Advantages and limitations of chemical extraction tests to predict mercury soil-plant transfer in soil risk evaluations. *Environ Sci Pollut Res*. doi:10.1007/s11356-016-6564-x
- Novozamsky U, Lexmond TM, Houba VJG (1993) A single extraction procedure of soil for evaluation of uptake of some heavy metals by plants. *Int J Environ An Ch* 51:47–58
- Quevauviller P, Lachica M, Barahona E, Rauret G, Ure A, Gomez A, Muntau H (1996) Interlaboratory comparison of EDTA and DTPA procedures prior to certification of extractable trace elements in calcareous soil. *Sci Total Environ* 178:127–132
- Quin F, Shan XQ, Wen B (2004) Effects of low-molecular-weight organic acids and residence time on desorption of Cu, Cd, and Pb from soils. *Chemosphere* 57:253–263
- Reis AT, Davidson CM, Vale C, Pereira E (2016) Overview and challenges of mercury fractionation and speciation in soils. *Trends Anal Chem* 82:109–117
- Reis AT, Lopes CB, Davidson CM, Duarte AC, Pereira E (2014) Extraction of mercury water-soluble fraction from soils: an optimization study. *Geoderma* 213:255–260
- Reis AT, Lopes CB, Davidson CM, Duarte AC, Pereira E (2015) Extraction of available and labile fractions of mercury from contaminated soils: the role of operational parameters. *Geoderma* 259:260:213–223
- Rodríguez L, Rincón J, Asencio I, Rodríguez-Castellanos L (2007) Capability of selected crop plants for shoot mercury accumulation from polluted soils: phytoremediation perspectives. *Int J Phytoremediat* 9:1–13
- Rózański, S. L., Castejón, J. M. P., & Fernández, G. G. (2016). Bioavailability and mobility of mercury in selected soil profiles. *Environmental Earth Sciences*, 75(13) doi:10.1007/s12665-016-5863-3
- Ruiz E, Rodríguez L, Alonso-Azcárate J, Rincón J (2009) Phytoextraction of metal polluted soils around a Pb-Zn mine by crop plants. *Int J Phytoremediat* 11:360–384
- Sahuquillo A, Rigol A, Rauret G (2003) Overview of the use of leaching/extraction tests for risk assessment of trace metals in contaminated soils and sediments. *Trends in Anal Chem* 22:152–159
- Sánchez DM, Quejido AJ, Fernández M, Hernández C, Schmid T, Millán R, González M, Aldea M, Martín R, Morante R (2005) Mercury and trace element fractionation in Almadén soils by application of different sequential extraction procedures. *Anal Bioanal Chem* 281:1507–1513
- Subirés-Muñoz JD, García-Rubio A, Vereda-Alonso C, Gómez-Lahoz C, Rodríguez-Maroto JM, García-Herruzo F, Paz-García JM (2011) Feasibility study of the use of different extractant agents in the remediation of a mercury contaminated soil from Almadén. *Sep Purif Technol* 79:151–156
- Van Engelen DL, Sharpe-Pedler RC, Moorhead KK (2007) Effect of chelating agents and solubility of cadmium complexes on uptake from soil by *Brassica juncea*. *Chemosphere* 68:401–408
- Wang X, Shan X, Zhang S, Wen B (2004) A model for evaluation of the phytoavailability of trace elements to vegetables under the field conditions. *Chemosphere* 55:811–822
- Winfrey MR (1990) Environmental factors affecting the formation of methylmercury in low pH lakes. *Environ Toxicol Chem* 9:853–869
- Yan DYS, Yip TCM, Yui MMT, Tsang DCW, Lo IMC (2010) Influence of EDDS-to-metal molar ratio, solution pH, and soil-to-solution ratio on metal extraction under EDDS deficiency. *J Hazard Mater* 178:890–894
- Žemberyová M, Barteková J, Hagarová I (2006) The utilization of modified BCR three-step sequential extraction procedure for the fractionation of Cd, Cr, Cu, Ni, Pb and Zn in soil reference materials of different origins. *Talanta* 70:973–978
- Zhu DW, Zhong H, Zeng QL, Yin Y (2015) Prediction of methylmercury accumulation in rice grains by chemical extraction methods. *Environ Pollut* 199:1–9
- Zornoza P, Millán R, Sierra MJ, Seco A, Esteban E (2010) Efficiency of white lupine in the removal of mercury from contaminated soils: soil and hydroponic experiments. *J Environ Sci* 22:421–427
- Zou Z, Qiu R, Zhang W, Dong H, Zhao Z, Zhang T, Wei X, Cai X (2009) The study of operating variables in soil washing with EDTA. *Environ Pollut* 157:229–236