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Spatial variation of biological and pedological properties in an area affected by a metallurgical mercury plant: Almadenejos (Spain)

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ABSTRACT

Almadén, Spain, has the most important known cinnabar deposits, which have provided a third of the entire world production of Hg, and has been the scene for numerous studies on Hg. This mining district includes several mines where cinnabar has been extracted in the past two millennia as well as facilities related to the production of primary Hg. The aim of the work is to evaluate the spatial distribution of Hg in the soil–plant system within an area where intense activity occurred over a long period. An abandoned metallurgical plant from the 17th–18th centuries was chosen as the study area, situated in Almadenejos a distance of 12 km from Almadén. Nowadays, this plot is covered with cinnabar mine tailings and it is used by village inhabitants for livestock grazing. The area has elevated Hg concentrations of natural origin and from human activities. Soil parameters are similar within the study area; however, data reveal high variability in total and available Hg concentrations in soils, making it difficult to establish a trend. *Marrubium vulgare* L. has been studied due to its abundance in the plot, and there is no evidence of phenological toxicity. In spite of elevated Hg concentrations, high biological activity is found in the sampled soils. All these characteristics, spatial variation, high Hg concentration, good biological activity, make this a particularly good area for studies involving Hg.

1. Introduction

The Almadén area in Central Spain was the mining area with the greatest exploitation of Hg, until mining activities ceased at the beginning of this decade. The Almadén Hg mining district included cinnabar extraction sites (open pits and underground mines), storage areas and metallurgical plants, including mineral furnaces. Cinnabar extraction ceased in 1999 and the final production of primary Hg occurred in 2004, when the mine closed down completely. In two millennia of activity, this area produced a third of the Hg used in human history (Hernández et al., 1999; www.mayasa.es).

Studies of Hg distribution in the environment of Almadén has been carried out by the US Environmental Protection Agency (Hildebrand et al., 1980). A detailed study on Hg in soils showed that total Hg concentrations in the surface soils ranged from 0.5 to 260 mg kg^{-1}, where maximum values were obtained near the Almadén mine (Lindberg et al., 1979). Furthermore, significant Hg concentrations in soils were found at distances of up to 14 km in the direction of the prevailing wind. In the nearby village of Almadenejos, high Hg concentrations in the range of 6–8889 mg kg^{-1} (Higueras et al., 2003) and values of up to 550 mg kg^{-1} (Millán et al., 2006) were obtained in soils within an abandoned metallurgical plant. Studies on soils East and NE of the site, at a distance of 1 km and in the direction of the prevailing wind, have shown Hg contents of 17 and 22 mg kg^{-1} in the top soil and maximum contents of 40 and 45 mg kg^{-1} at depths of 29 and 43 cm, respectively (Schmid et al., 2003).

The study area for this work is focused on the abandoned metallurgical site in Almadenejos due to the intense Hg activity that was carried out in the village. The site dates from the 18th century and is located 12 km from Almadén. Close to this site, there are two former underground Hg mines called “La Concepción Vieja” and “La Concepción Nueva” (17th and 19th centuries, respectively) and the open pit mine named “El Entredicho”. This latter mine was active until the end of the 20th century and is now an artificial lagoon. The studied area is approximately 30,000 m^2 surrounded by a wall built between 1756 and 1759, and where six pairs of abandoned roasting furnaces are found in ruins. These furnaces were used to obtain primary Hg from cinnabar.

The climate of the region is considered semi-arid with a mean annual temperature of 16 °C and a mean annual precipitation of 587 mm, with maximum values during spring and autumn. The lithology is comprised of metamorphosed detrital rocks and the experimental plot is 508 m above sea level (a.s.l.). Presently,

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the abandoned site is covered with cinnabar mine tailings and village inhabitants use it as pasture land for livestock, mainly for pig farming, but also for sheep and horses. The dominant vegetation includes *M. vulgare* L. and *Asparagus acutifolius* L. plants, as well as the nitrophilous species *Cynoglossum cheirifolium*. Species of limited extent are *Urtica urens* and *Diplotaxis virgata* and together with *A. acutifolius* form the nitrophilous assemblage (Millán et al., 2006). Identification of flora growing on mine soils enriched with Hg is of particular interest, because of its potential use in phytotechnologies and as important bioindicators of contamination (Millán et al., 2006).

The main aim of this study was to evaluate the spatial distribution of Hg in the soil–plant system within an area where human activity was dominant over a long period. This includes: (1) determination of total and available Hg concentration in the soil; (2) identification of plant species growing in the field plot; (3) selection of a plant species that copes with high concentrations of Hg; and (4) study of biological soil parameters.

2. Material and methods

The overall experimental design for the work included a careful selection of sampling points within the study area that were located with GPS. Soil and plant samples were obtained in three field campaigns between 2006 and 2008 during the autumn. After each campaign, laboratory analyses were carried out to determine physical, chemical and biological soil properties as well as total and available Hg concentrations for both plant and soil samples.

2.1. Sampling strategy

Soil samples were taken from seven points at regular 10 m spacing initiating from the highest point at 501 m a.s.l. and moving down the slope to the lowest point at 486 m a.s.l. In this way, six samples were obtained upslope, nine samples along side and six samples downslope from the furnaces corresponding to areas A1–A3. At each sampling point of this transect, composite samples were collected from a 1 m^2 by obtaining 10 cm of the surface soil at five points (Fig. 1). At each sampling point, *M. vulgare* L. plants were also collected. All the collected plants had the same age and biomass in order to compare the results. This plant species was chosen due to its dominance within the area of the abandoned metallurgic plant.

2.2. Sample preparation

Soil samples for standard physical and chemical analyses were air dried and sieved to obtain the fraction <2 mm.The methodology for preparing plant samples included a controlled removal of deposited Hg from the surface of the plant washing them and using an ultrasonic bath treatment (Ultrasons-H, Selecta; 3 cycles of 10 min at room temperature) to remove all the particles deposited on the plant material. Plants were divided into two fractions: the aerial part of the plant (stem and leaves) and the root system. All samples were dried at room temperature to constant weight. In addition, soil and plant samples were specifically prepared for Hg analysis. In this case, all samples were homogenized and ground with an agate mortar and sieved to obtain a particle size of less than 420 μm.

2.3. Soil analysis

Laboratory analyses were carried out according to standard methods that included: Electrical conductivity (EC), pH in saturated paste in water and pH in KCl, 0.1 M, was determined according to the MAPA methods (MAPA, 1994). Cation Exchange Capacity (CEC) was determined using the EPA 9081 Method. Texture was obtained by the Bouyoucos densimeter method and soil color was established using the International Munsell Soil Color Charts. Organic matter (OM) was measured following the Walkey-Black method. Nitrogen and C content was determined with a LECO Elemental Analyser. Phosphorous was determined spectrophotometrically by means of the Olsen method (MAPA, 1994). Available Hg in the soil was obtained using the AB-DTPA method (Soltanpour

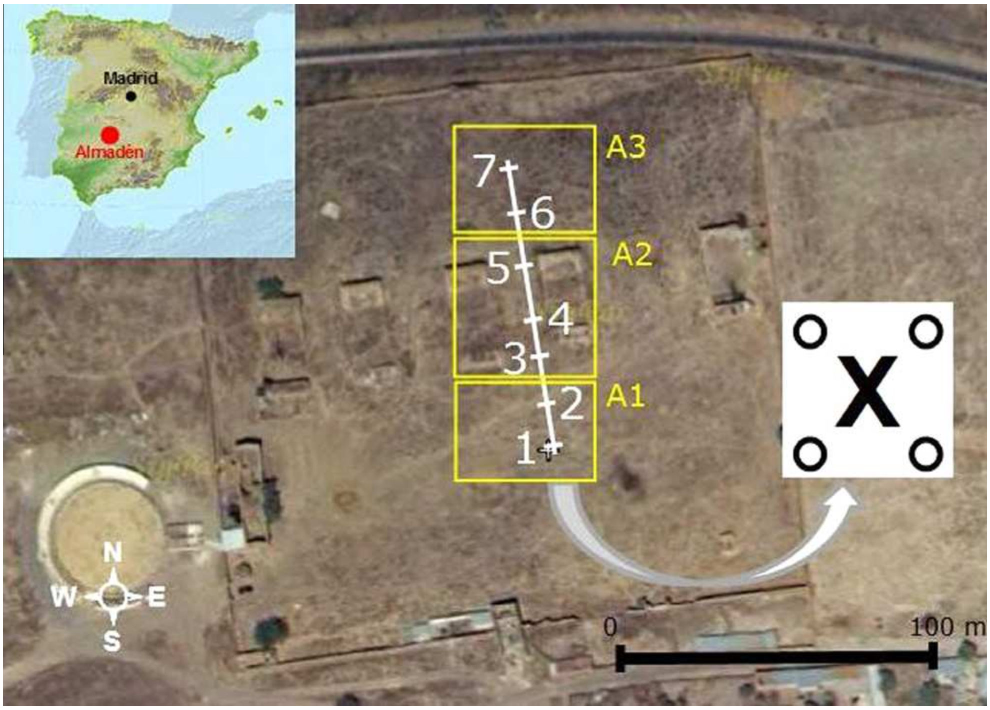

Fig. 1. Experimental field plot in Almadenejos (Spain) and location of the sampling areas (O = soil and X = soil + plant).

and Schwab, 1977). The use of this method was based on previous studies by Lucena et al. (1993) and Sierra et al. (2008, 2009) in the Almadén area. Furthermore, a specific 6-step sequential extraction procedure developed by Sánchez et al. (2005) was applied to study the distribution of Hg in soil samples. The certified reference material CRM 051 was used as a control for the sequential extraction procedure, and gave an average recovery percentage of 96%.

2.4. Mercury analysis

Mercury concentration in all the samples of soil, soil extracts and vegetation was measured using an atomic absorption spectrophotometer specifically designed for Hg determination (Advanced Mercury Analyser – AMA254 – LECO Company). Both the solid and liquid samples were analyzed without any chemical pre-treatment and with a detection limit of 0.01 ng of Hg. Certified reference materials (CRM) were used to determine the accuracy and precision of the measurements and validate the applied methods. These reference materials are SRM 1573a (tomato leaves, 0.034 ± 0.004 mg kg^{-1}), BCR-CRM 151 (spiked skim mild powder, 0.101 ± 0.010 mg kg^{-1} of Hg), CRM 027 (soil from USA contaminated area, 3.80 ± 0.65 mg kg^{-1}), SRM 2711 (Montana soil, 6.25 ± 0.19 mg kg^{-1}) and CRM 051 (soil from USA contaminated area, 29.90 ± 5.96 mg kg^{-1}). The mean values determined for 10 measurements using the AMA-254 equipment were 0.032 ± 0.002 mg kg^{-1}; 0.094 ± 0.007 mg kg^{-1}; 3.33 ± 0.40 mg kg^{-1}; 6.32 ± 0.45 mg kg^{-1} and 30.04 ± 1.76 mg kg^{-1}, respectively. At a 95% confidence level, no significant differences were detected between the certified values and the experimental ones, so this method was considered to be accurate for total Hg determination.

When samples recorded a high Hg content, out of the equipment range limit (>600 ng), they were subjected to an acidic digestion following the EPA 3052 Method (US EPA, 1996). The process was performed in a microwave ETHOS 1 (Milestone SRL). The recovery percentage for certified reference material (CRM 051) was 99%.

2.5. Biological parameters

Biological parameters were obtained for soil samples collected during the autumn and spring season of 2006 and 2007, respectively. Freshly sieved sample (<2 mm) was kept at 4 °C until all biological analyses were performed. The respirometric assays, either for Substrate Induced Respiration (SIR) or basal respirometry, were performed with the Micro-Oxymax respirometer (Columbus Instruments) that simultaneously used up to 10 reaction chambers. The air sample was dried previously to measure O_2 with a paramagnetic sensor (0–21% O_2) and CO_2 with an infrared sensor (0–1.5% CO_2).

SIR was estimated as described by Anderson and Domsch (1978) and modified as follows: After analysing their Water Holding Capacity (WHC), soil samples were remoistened and incubated overnight at 22 °C. The pre-incubated samples were remoistened with a solution containing glucose at several concentrations (0, 3, 6 and 9 g glucose kg^{-1} dry soil) and a respirometry assay was performed to determine the glucose concentration that optimizes the soil respiration. Thereafter, a second respirometry assay was prepared with the optimum glucose concentration, as previously described, where the respiration rate was measured hourly for 7 h. Ten grams of soil were used and each assay was done in triplicate. The soil microbial biomass C was calculated from the equation: SIR-C_{mic} (mg kg^{-1} soil) = 40.04 MIRR + 3.75, where MIRR is the maximum induced respiration rate of the soil (ml CO_2 kg^{-1} soil h^{-1}).

In order to measure the Basal Respirometry, soil samples were remoistened to between 55% and 60% of their WHC and were incubated at 25 °C for 24 h. Thereafter, the bottles containing soil samples were connected to the respirometer and the respiration rate was measured hourly. Data considered for respiration rates were produced between 30 and 40 h after the first incubation, when the rates show more stable values (Margesin et al., 2000). The Basal respirometry results were employed in obtaining the metabolic quotient (qCO_2).

Soil microbial biomass C was also determined by the Fumigation Extraction (FE) method (Vance et al., 1987). Two portions of moist soil (7.5 g oven-dry soil) were weighed, the first one (non-fumigated) was extracted with 30 mL of 0.5 M K_2SO_4 for 60 min in a shaker at 250 rpm and centrifuged for 20 min at 5000 rpm. The supernatants were filtered through (0.45 + 0.22) µm Sartorius nylon syringe filters into separate vials for organic C analysis. The second portion was fumigated for 24 h at 25 °C with ethanol-free $CHCl_3$ and then extracted as described above. The organic C in the extracts of the control and fumigated soils were determined with a TOC-V CSH analyzer (SHIMADZU, Shimadzu Scientific Instruments). Microbial biomass C was calculated as follows: Microbial Biomass C = EC/k_{EC}, where EC is the net flush of C, obtained from the difference between the extracted C in fumigated soil samples minus the extracted C in control soil samples. k_{EC} = 0.45 is a constant to compensate for the fraction of C extracted by 0.5 M K_2SO_4.

2.6. Statistical analysis

Standard descriptive analysis as well as the Pearson and Kendalĺs Taub correlation tests was applied. In the latter tests, a probability of 0.01 and 0.05 or lower was considered as significant, respectively. The analyses were carried out with the statistical package SPSS 11.5.

3. Results and discussion

3.1. Chemical and physical characteristics of soil

Standard soil parameters indicate slight variations along the studied transect. Soil color (7.5YR5/4–7.5YR5/3); texture (sandy-clay-loam); pH values (slightly acidic to neutral); OM content (4–9%) and C/N ratio (close to 10) parameters imply a good conditions for pasture land, which is the actual land use at this site. EC values indicate that there is no salinity risk. Finally, the CEC value seems to be related to the clay fraction as it is lower than expected for a predominantly organic soil horizon (Table 1). Furthermore, the presence of $CaCO_3$ was observed at this site. This is related to the use of quicklime to whitewash the walls and the furnace sides.

Total Hg concentration in A1 ranges from 5 to 1430 mg kg^{-1}; in A2 it ranges from 10 to 7560 mg kg^{-1} and in A3 from 59 to 40,000 mg kg^{-1} (Fig. 2). Available Hg concentration in A1 ranges from 0.04 to 0.37 mg kg^{-1}, in A2 from 0.11 to 0.78 mg kg^{-1} and in A3 from 0.7 to 15.8 mg kg^{-1} (Fig. 3). In all cases, minimum values were obtained in samples collected upslope from the furnace

Table 1
Maximum and minimum values of soil parameters (A1 n = 6; A2 n = 9; A3 n = 6).

	A1	A2	A3
pH	6.7–7.4	6.4–7.3	5.8–7.2
EC 1:5 (µS cm^{-1})	213–295	157–235	180–186
OM (%)	5.0–8.2	4.7–8.6	4.3–8.2
C (%)	4.2–5.1	3.4–4.3	3.1–7.6
N (%)	0.4–0.5	0.4–0.4	0.3–0.8
C/N	10.2–11.7	9.4–10.2	8.8–10.0
CaCO3 (%)	2.2–3.8	1.5–2.6	2.7–2.8
CEC (cmol$_c$ kg^{-1})	24.0–27.4	18.9–22.1	16.5–26.2

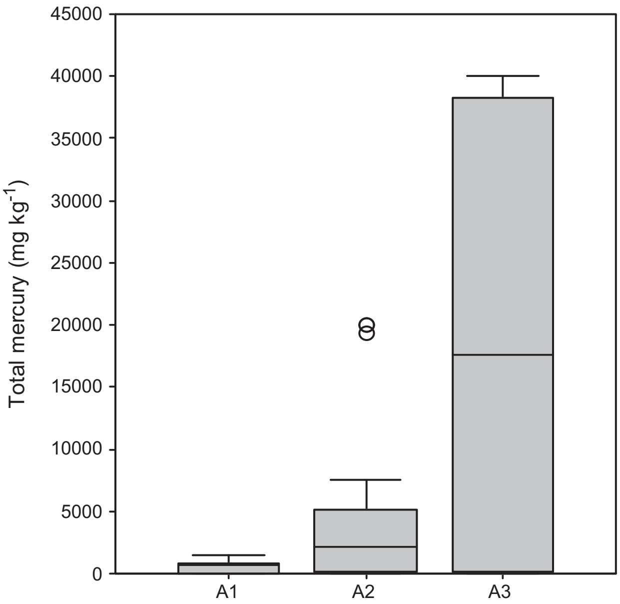

Fig. 2. Total Hg concentration in soil for the three study areas (A1, A2 and A3).

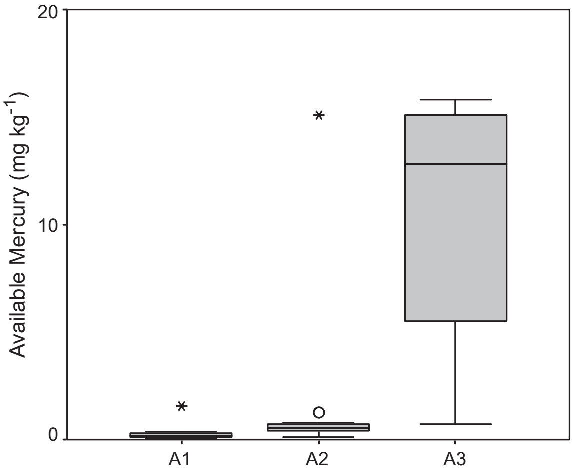

Fig. 3. Available Hg concentration in soil for the three study areas (A1, A2 and A3).

area, given maximum values in the area located downslope from the furnaces, cinnabar was stockpiled and, in some cases, slag and waste produced in the roasting processes was deposited there. Moreover, the lower part of the transect acts as the reception and accumulation area for the sediments that come from the upper part of the site (A1 and A2).

The analyses show great heterogeneity of soil Hg concentrations. This is consistent with ranges obtained by Gray et al. (2004) where values of mine wastes from Almadén are in the range 160–34,000 mg kg^{-1}. The values further coincide with results obtained by Bernaus et al. (2006) for analyses of calcine samples from the top of two of the old furnaces from Almadenejos that vary from 6700 ± 500 to 35,000 ± 6000 mg kg^{-1}, and with results obtained by Bernaus (2006), who studied Hg concentrations in different Almadén soil samples and ore samples also showing great variation ranging from 400 to 2000 mg kg^{-1} and from 30,000 to 40,000 mg kg^{-1}, respectively.

In this study, no significant correlation was obtained between any soil parameter measured and soil Hg concentration or between the different soil parameters. However, there was a significant correlation between total Hg concentration in soil and available Hg concentration in soil ($r = 0.658$, $\alpha = 0.01$).

Regarding the ratio of the percentage of available Hg concentration in soil with respect to total Hg concentration in soil, it remained below 3% in the three areas. This low ratio is in agreement with studies carried out by Lucena et al. (1993) and Millán et al. (2006) who reported that the Hg availability for plants was normally low. It has to be taken into account that in these samples, Hg is mainly in cinnabar, which is very stable.

3.2. Mercury in plant samples

Mercury concentrations in leaves of *M. vulgare* varied from 6 to 323 mg kg^{-1}, whereas Hg concentration measured in stems gave lower values, from 1.5 to 77 mg kg^{-1}, showing two extreme values, 150 and 176 mg kg^{-1}. Mercury concentrations in root varied from 8 to 651 mg kg^{-1} (Fig. 4). Previous studies carried out on *M. vulgare* plants in the same area reported levels of total Hg in shoots of 23.0 ± 2.6 mg kg^{-1} and in root of 67.2 ± 10.1 mg kg^{-1} (Millán et al., 2006) and total Hg in shoot up to 156 mg kg^{-1} (Higueras et al., 2003). In both cases, values are within the ranges found in this work.

The roots of plants usually act as a barrier to Hg uptake (Patra and Sharma, 2000). However, Salt et al. (1998) observed that a particular metal fraction is exported to the shoot and this primarily takes place through the xylem. In this study, the Hg content of the shoot is between 2.8 and 323 mg kg^{-1}. Regarding Hg accumulation in plants, McGrath and Zhao (2003) reported that plants with a shoot/root ratio greater than 1 are considered accumulators. The results obtained in this study show a shoot/root ratio in the range of 0.11–3 in A1; 0.18–0.91 in A2 and 0.03–0.07 in A3. The plant samples did not show visual symptoms of toxicity indicating that an effective defence mechanism was exerting its homeostatic response. Therefore *M. vulgare* is regarded as a Hg accumulator.

In the three studied areas, the Hg content in the stems were similar and in all cases lower than in leaves and roots. The area

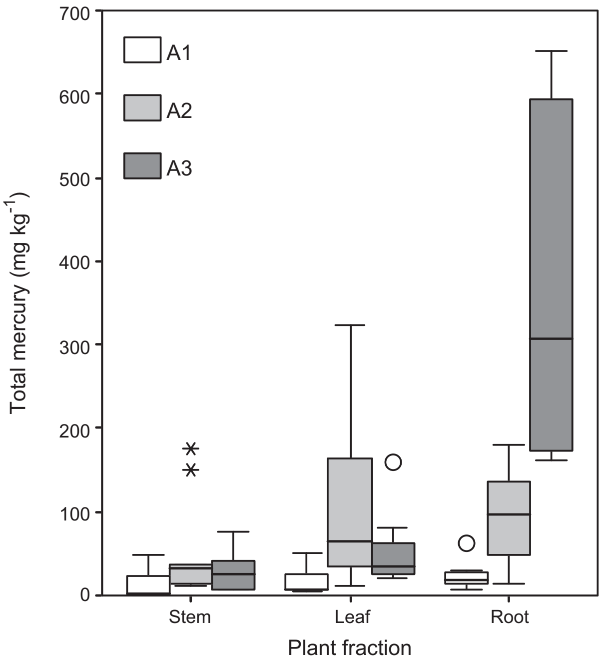

Fig. 4. Distribution of Hg in the different plant fraction for the three study areas (A1, A2 and A3).

located upslope from the roasting furnaces (A1), gave similar values in all plant fractions and were lower than the other sampling sites. In the area along the furnaces (A2), Hg in plant increased in roots and leaves. In the downslope area (A3), a maximum Hg concentration was reached and this coincides with the maximum total and available Hg content in soil (Figs. 2–4).

There is a significant correlation between root Hg concentration and available Hg concentration in soil ($r = 0.450$, $\alpha = 0.05$), but no correlation between root Hg concentration and total Hg concentration in soil was observed. The other plant fractions did not show a correlation either with available Hg concentration in soil or with total Hg in soil.

3.3. Soil chemical analysis by sequential extraction

A sequential extraction was performed on soil samples taken in autumn 2008 in order to study the affinity of Hg for different soil components. The study of Hg distribution in these soil samples has been performed by applying the 6-step CIEMAT sequential extraction procedure developed by Sánchez et al. (2005).

The results indicate that Hg distribution between different fractions in the three areas is similar (Table 2). Mercury is mainly found in the fraction assigned to the final insoluble residues (31–70%) that correspond to resistant Hg sulfides. The next major fraction of Hg, 22–59%, is released with 6 M HCl that indicated Hg mainly associated with crystalline Fe–Mn oxyhydroxides. The Hg associated with the oxidizable fraction, that can be assigned to the association with organic matter, and traces of elemental Hg is in the range of 5–20%. Finally, Hg associated with water-soluble phases, the exchangeable fraction, carbonates and amorphous oxyhydroxides is less than 0.2%.

Furthermore, using the same samples from autumn 2008, comparison of the sum of soluble and exchangeable fractions obtained from sequential extraction (0–0.13%) and the Hg extracted by the Soltanpur method (0–0.14%) showed similar values, in percentage terms.

The experimental area was an old cinnabar roasting site (18th and 19th century). The mineral roasting process in the 18th and 19th century was incomplete and, accordingly, less efficient than more modern processes. This accounts for the fact that most Hg was associated with resistant Hg sulfides and that an elevated quantity of Hg in the oxidizable fraction that could be associated with both high organic matter (Table 1) and traces of elemental Hg. In the Hg mine of Idrija (Slovenia), Biester et al. (1999) observed that the predominant Hg species in older tailings was cinnabar due to incomplete roasting techniques. In tailings of the 20th century the amount of cinnabar decreased due to a higher efficiency in the roasting process. In the case of the abandoned metallurgy site in Almadenejos, at the time of activity lower cinnabar roasting temperatures were used. This led to lower Hg recovery (Bernaus 2006) and as a consequence higher soil Hg concentrations are found at the site.

Sánchez et al. (2005) and Sierra et al. (2009) carried out the 6-step sequential extraction procedure on Almadén soils from an area whose traditional use has been agricultural and pastureland, and obtained different results. In these soils, the major part of the Hg was associated with crystalline Fe–Mn oxyhydroxides (70–100%). The Hg remaining in the final insoluble residue was in the range of 8–20% and the amount of Hg associated with the oxidizable fraction was lower.

Bernaus et al. (2006) carried out 6-step sequential extraction procedures on calcine samples from the top of two of the old furnaces from Almadenejos. Their results differ to the ones obtained in this work, in that Hg extracted with 6 M HCl was highest compared with other steps (from 44% to 51%), with the second major amount of Hg being associated with the final insoluble residue (from 16% to 42%). In this work, the percentages for these phases are the second highest and the highest, respectively. On the other hand, the values of Hg associated with oxidizable fractions were one of the lowest, which could be due to the low organic matter content in the calcines. This latter result is the most different with respect to this work where values of Hg associated with oxidizable fractions are the third highest. These differences are related to the type of sample used, where Bernaus et al. (2006) used samples of calcination residues while in this work the study is focused on soil samples.

Furthermore, data obtained by Higueras et al. (2003) using Hg-thermodesorption and TEM–EDX analysis for other soils from different Almadén mining sites are similar to the ones found in this work. They found Hg only in the forms of cinnabar particles and bound to organic matter, which must be related to deposition of Hg^0 that would have been generated from the furnaces.

3.4. Analyses of biological parameters

In the framework of the study of soils in the Almadén mining district, several assays related to soil microbial activity were performed. Microbial activity and biomass may indicate the incidence of environmental changes and reveal situations of stress and disruption in soil systems. To avoid the seasonal influence on the magnitude of these parameters, they were determined for samples obtained in autumn and spring of 2006 and 2007, respectively. This is when the biological activity has reached a maximum in this area. Parameters obtained in this study are: Soil microbial biomass carbon; metabolic quotient (qCO_2); C_{mic}/C_{org} and Maximum respiratory rate. All these parameters are appropriate as soil quality indicators; however, the lack of standard values is somewhat inconvenient for this purpose (Sparling, 1997). Soil samples from specific plots, such as the abandoned Almadenejos metallurgical plant form part of a large study carried out within the Almadén mining district. Reference values for these microbial activity

Table 2
Values of total Hg percentage in the different soil fractions and initial Hg concentration in each soil sample.

	A1		A2			A3	
	1.1	1.2	2.1	2.2	2.3	3.1	3.2
% Total Hg in different soil fractions							
Water-soluble (%)	0.03	0	0	0.01	0.01	0	0
Exchangeable (%)	0.01	0	0.01	0.04	0.04	0.02	0.01
Carbonates (%)	0.02	0	0.01	0.24	0.03	0.02	0.01
Easily reducible (%)	0.06	0	0.01	0.10	0.02	0.02	0.01
6 M HCl-soluble (%)	34.1	24.3	23.0	36.4	58.7	23.2	22.0
Oxidizable (%)	20.1	17.2	10.8	5.6	9.4	9.0	8.3
Residue (%)	45.8	58.6	66.2	56.6	31.7	67.8	69.7
Initial total mercury (mg kg^{-1})	498 ± 13	678 ± 42	3358 ± 417	7365 ± 423	1281 ± 168	7862 ± 1591	6219 ± 179

Table 3
Descriptive statistics of soil microbial activity parameters in the Almadén Mining District.

Parameter (units)	*n*	Min	Max	Median	Mean
Microbial biomass C					
SIR (mg C_{mic} kg soil^{-1})	42	117.50	2104.47	515.39	656.26
FE (mg C_{mic} kg soil^{-1})	15	222.79	1283.24	394.43	500.58
Metabolic quotient (qCO_2) (mg CO_2 g C_{mic}^{-1} h^{-1})	42	2.08	29.74	7.78	9.46
Ratio C_{mic} to C_{org} (%)	42	0.67	5.04	2.27	2.10
Maximum respiratory rate (mg CO_2 100 g dry soil^{-1} h^{-1})	42	1.00	16.70	3.33	4.47

indicators may be obtained from the whole study area, after selecting plots covering different land uses. The descriptive statistics for these parameters, in the Almadén mining district, are shown in Table 3.

Soil microbial biomass carbon was obtained by SIR for samples obtained during autumn 2006 and the following spring, while FE soil microbial biomass was obtained for samples taken in autumn 2006. SIR Soil Microbial Biomass C values (Fig. 5) range between 484 and 1352 mg C_{mic} kg soil^{-1}, the values are included in the range of values obtained in the SIR method calibration, from 150 to 2400 mg C_{mic} kg soil^{-1}. Only one of the values of soil microbial biomass C in Almadenejos is under the median value obtained for Almadén. Sometimes microbial biomass estimation by different methods shows a low correlation between pairs of methods (Wardle and Parkinson, 1990), so some authors have suggested the use of more than one method to estimate soil microbial biomass C (West et al., 1986). Moreover, other authors (Sparling and West, 1990; Theenhaus et al., 1997) have expressed some concern about SIR microbial biomass C overestimation in soils with high pH, due to carbonate solubilization and abiotic CO_2 production. In order to remove any doubt concerning soil microbial biomass C in this plot, the FE method was applied. This method is not influenced by abiotic CO_2, and soil microbial biomass C values between 730 and 1283 mg C_{mic} kg soil^{-1} were obtained. These values from the Almadenejos plot are the highest determined for the Almadén area by the FE method (Table 3).

Metabolic quotient (qCO_2) is the ratio between basal respirometry and soil microbial biomass, given the specific respiration rate. The units used are: mg CO_2 emitted/g of microbial biomass hourly. Highest values may be associated with soil stress and disruption situations, when microbial populations are using chemical energy in establishing resistance mechanisms, i.e. to potentially harmful metals, thus diverting C and energy source from growing. Expected values for the abandoned metallurgy area of Almadenejos should be high with respect to the potentially harmful metal concentration in these samples. However, values obtained in this plot range from 2.3 to 5.2 mg CO_2 g C_{mic}^{-1} h^{-1} (Fig. 6). These values are clearly under the median value obtained for the Almadén area, suggesting that these samples are not subject to any stress or disruption situation. Nevertheless, some authors doubt the capability of this parameter in assessing soil stress or disruption while studying on a temporal basis (Wardle and Ghani, 1995). They suggest that contradictory data is obtained unless different soils are compared, because the metabolic quotient values are quite similar. This

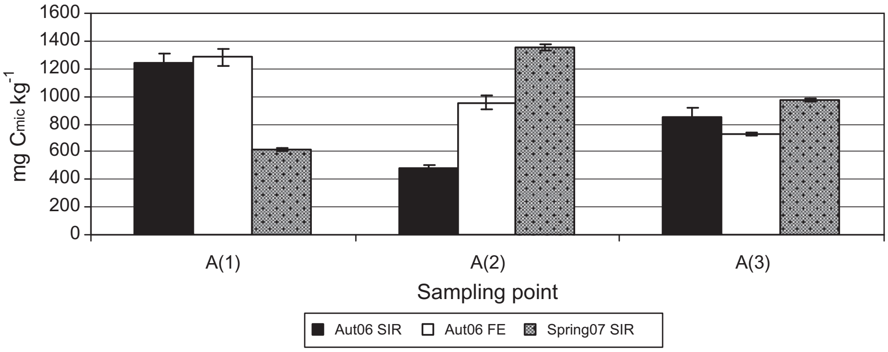

Fig. 5. Soil Microbial Biomass C obtained from the three sampling areas (A1, A2 and A3).

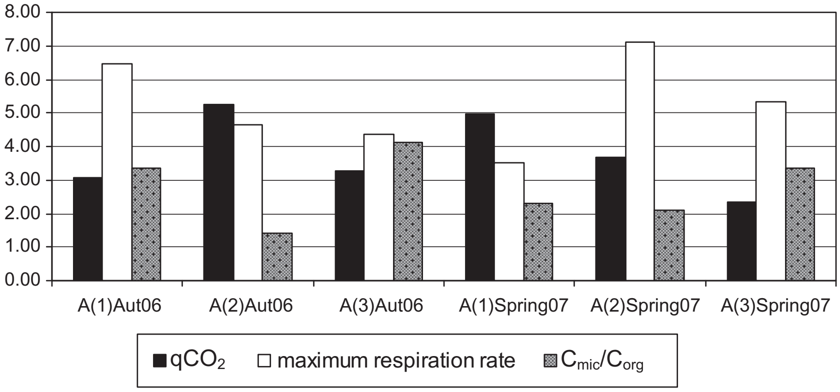

Fig. 6. Microbial activity parameters obtained in the three areas (A1, A2 and A3).

parameter does not give enough information about soil microbial activity and therefore, other authors (Anderson, 2003) relate this parameter with the C_{mic}/C_{org} ratio to obtain more complete information. In the Almadenejos samples, soil microbial biomass data support low metabolic quotient values.

The C_{mic}/C_{org} ratio is considered to have a constant value in each soil according to Anderson and Domsch (1989) who studied agricultural soils. However, this ratio may change with agricultural practices, i.e. when organic matter is added to monocultured agricultural soils. The C_{mic}/C_{org} ratio also follows a definite trend from early to successive stages. This is the case, when more soil organic C is included in the microbial structures leading to advanced successive stages as the soil organic matter evolves and increases the proportion of humified organic C. Values obtained for Almadenejos range from 1.4% to 4.1%. Only the lowest value of Almadenejos is under the median value of for the Almadén area (Fig. 6).

The maximum respiratory rate is a parameter proposed as an OECD test for assessing the activity of soils (Dott, 1995). The formal name of the parameter is “inhibition of microbial respiration” and assesses if soil contamination has any effect on soil microbial activity. It is suggested that soils with CO_2 production rates under $0.5\ mg\ CO_2\ 100\ g^{-1}$ dry soil h^{-1} may have been exposed to chemical pollutants. As all samples from Almadén have maximum respiratory rates that are higher than the reference value, a comparison between the obtained values was made with Almadenejos which are in the range from 3.5 to $7.1\ mg\ CO_2\ 100\ g^{-1}$ dry soil h^{-1}. All these values are higher than the Almadén median value (Fig. 6).

The results show that the Almadenejos site constitutes an important area that belongs to the cultural heritage of Almadén mining district. This implies that any study developed in this site has to take into account factors like current and past activities and land use, including anthropogenic impacts on soils as well as natural characteristics of the area.

4. Conclusions

Soil parameters are similar in all the samples from the study area. There is no correlation between them and the Hg concentration in soil (total and available). High total Hg concentrations are found within the soil, as it was expected in a mining area. There is correlation between total and available Hg in soil samples. However, it is important to point out the low percentage of available Hg despite the significantly high amounts of Hg in the soil.

Mercury is mainly found in a form associated with resistant Hg sulfides, the 6 M HCl-soluble fraction, mainly crystalline Fe–Mn oxyhydroxides, and in organic matter, in addition there are traces of elemental Hg. The lowest Hg concentrations were obtained for Hg associated with water-soluble phases, the exchangeable fraction, carbonates and amorphous oxyhydroxides.

M. vulgare could be a good candidate for phytoextraction techniques, due to its capacity to accumulate high Hg in its shoots, and the absence of phenological toxicological effects. Further studies are being undertaken with the rhizosphere soil–plant system in order to study the rizhosphere influence on the processes affecting Hg uptake and translocation.

Not one of the analysed soil microbial activity parameters indicate any type of stress or disruption in superficial soil samples from Almadenejos either when comparing the values obtained for all the analysed soil samples in Almadén or when comparing to external standards. This may be explained by a low microbial availability of Hg and to some extent the physical and chemical characteristics of the soils in the Almadenejos plot that show optimum values related with critical parameters such as organic matter.

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