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Impact of the lavender rhizosphere on the mercury uptake in field conditions

M.J. Sierra *, J. Rodríguez-Alonso, R. Millán

CIEMAT – Environmental Department, Avda. Complutense 40, E-28040 Madrid, Spain

HIGHLIGHTS

- ▶ Differences between rhizosphere and bulk soil: parameters of influence.
- ▶ Influence of edaphic parameters on mercury behavior in soil-lavender plant system.
- ▶ Distribution of mercury throughout the lavender plant.

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ABSTRACT

Lavender plants as well as their rhizosphere and bulk soil were sampled on a wide range of soils with different land use within the Almadén mercury mining district. The aim of this work is to evaluate the role of the rhizosphere on mercury behavior in soil-lavender plant system including chemometric analysis. The edaphic parameters that significantly differed between lavender rhizosphere and bulk soil were: total Hg; easily available Hg; electrical conductivity; organic matter; cation exchange capacity; soluble ions (Cl^-; SO_4^{2-}; PO_4^{3-}; NO_3^-; Al^+; Mn^{2+}; Ca^{2+} and Mg^{2+}). The most important variable in the differentiation is electrical conductivity. Furthermore, both organic matter and Mn^{2+} in rhizosphere soil seem to block Hg availability to plant. However, the presence of sulfates seems to favor it. Regarding other relationships, Hg seems to block Pb uptake by lavender plants and, on the other hand, the presence of Mn^{2+} seems to favor it.

Furthermore, Hg root uptake by lavender and its distribution throughout the plant have been studied. The more available Hg in rhizosphere soil, the more Hg is translocated to aerial part and less Hg is retained by root. In all cases, the Hg concentration in the root was higher than in the aerial part.

1. Introduction

The mining district of Almadén (Ciudad Real, Spain) has been the most important producer in the world of Hg from cinnabar for centuries (since the Celtic and Roman times). Cinnabar extraction ceased in 1999 and the final production of primary Hg occurred in 2004, when the mine closed down completely (Newman, 2002; Gray et al., 2004; Hernández Sobrino, 2007; Carrasco Milara, 2009). Because of this anthropogenic impact (open pits and underground mines, storage areas or metallurgical plants) besides lithological features of the area, the soil from this district has significant contents of Hg even in zones far from pollution sources (Higueras et al., 2003; Schmid et al., 2003; Millán et al., 2006, 2011).

Mercury is a heavy metal considered as a global contaminant and one of the most toxic pollutants that has left an important record in the history of humankind (Gochfeld, 2003). Mercury can cause adverse ecological and toxicological impacts through the mechanism of bioaccumulation and biomagnification. Therefore, the study of Hg transfer to biotic components is necessary, including its transfer to vegetation. The absorption of heavy metal by plants depends on factors such as plant species and soil properties.

Lavender (*Lavandula stoechas* L., botanical family: Labiateae) is a Mediterranean aromatic and perennial shrub which grows naturally in the study area. This plant shows drought resistance and heat tolerance, which are prerequisites for survival and good performance in areas with a Mediterranean-type climate. In addition, as Durán Zuazo et al. (2008) argued the aromatic herbs protect the soil against erosion and improve soil quality. Furthermore, since it could be an economic alternative due to their oil and fragrance producing biomass, previous studies were performed under controlled conditions to evaluate the possible cultivation of lavender as a safe alternative land use in Almadén district (Sierra et al., 2009). To confirm these results, field studies have been carried out in this work with wild plants of lavender from the study area.

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* Corresponding author. Tel.: +34 91 346 6157; fax: +34 91 346 6269.
E-mail addresses: mj.sierra@ciemat.es (M.J. Sierra), rocio.millan@ciemat.es (R. Millán).

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Mercury is predominantly in cinnabar form (HgS) in the Almadén area, but in spite of the low solubility of this mineral, it has been observed that Hg is mobilised and absorbed by plants (Adriano, 2001). The existence of a micro-ecosystem in plant rhizosphere different from bulk soil has been discussed by a number of authors although little is known about the role of rhizosphere in metal accumulation (Schöttelndreier and Falkengren-Grerup, 1999; Walter and Wenzel, 2002; Puschenreiter et al., 2001, 2003, 2005; Kidd et al., 2009; Martínez-Íñigo et al., 2009; Kim et al., 2010). In this case, the plant may modify rhizosphere conditions through processes such as production of metal-solubilising root exudates or alteration of pH (Adriano, 2001; Kabata-Pendias, 2001; Díez Lázaro et al., 2006). Therefore, the basic question is: How do the edaphic parameters differ in the rhizosphere soil from those in the bulk soil? In this paper, lavender plants from the Almadén district are analyzed to answer this question applying statistics. Selected soil parameters are compared between lavender rhizosphere and bulk soil. Furthermore, this work evaluated Hg absorption and Hg distribution through the wild lavender plants grown in different soils from the Almadén area. Previous data on mercury uptake by lavender in the district can be found in Molina et al. (2006).

2. Materials and methods

2.1. Study sites

The study area is located in the Almadén Hg mining district in Central Spain within the Province of Ciudad Real. The mining district extends over an area of about 100 km^2; and has a Mediterranean climate with wet and cool winters, and dry warm summers. Seven test plots with different land use and where *L. stoechas* grew wild were identified within the district. These plots are located in old abandoned mining sites surrounded by natural areas (Fig. 1):

- P1. "Fuente del Jardinillo"; Land use: Open Mediterranean forest; Altitude (Alt.): 438 m
- P2. "Dehesa de Castilseras"; Land use: Open Mediterranean forest; Alt.: 482 m
- P3. South facing slope of "Sierra de Cordoneros". Lower part; Land use: Open Mediterranean forest; Alt.: 516 m
- P4. South facing slope of "Sierra de Cordoneros". Upper part; Land use: Forest; Alt.: 546 m
- P5. "El Entredicho" Upper talus; Land use: Abandoned mine. Open pits; Alt.: 465 m
- P6. "El Entredicho" Lower talus; Land use: Abandoned mine. Open pits; Alt.: 452 m
- P7. "Las Cuevas"; Land use: Abandoned mine. Underground mining; Alt.: 536 m

2.2. Plant and soil sampling

The vegetation sampling was carried out in seven test plots coinciding with the end of flowering in 2007. At each sampling site, 3–4 lavender plants were sampled depending on their presence and abundance in each area so that the sampling was representative. Sampling consisted of taking the whole plant (aerial part and root) carefully, using a pick and hoe. The collected samples were further separated into flowers, leaves, stems and root. Thereafter, each plant organ was cleaned in distilled water using an ultrasonic bath (Ultrasons-H, Selecta) to remove external contamination (six cycles of 10 min) at room temperature. All samples were dried in individual beakers at room temperature until constant weight was reached and grinded to obtain homogeneous samples.

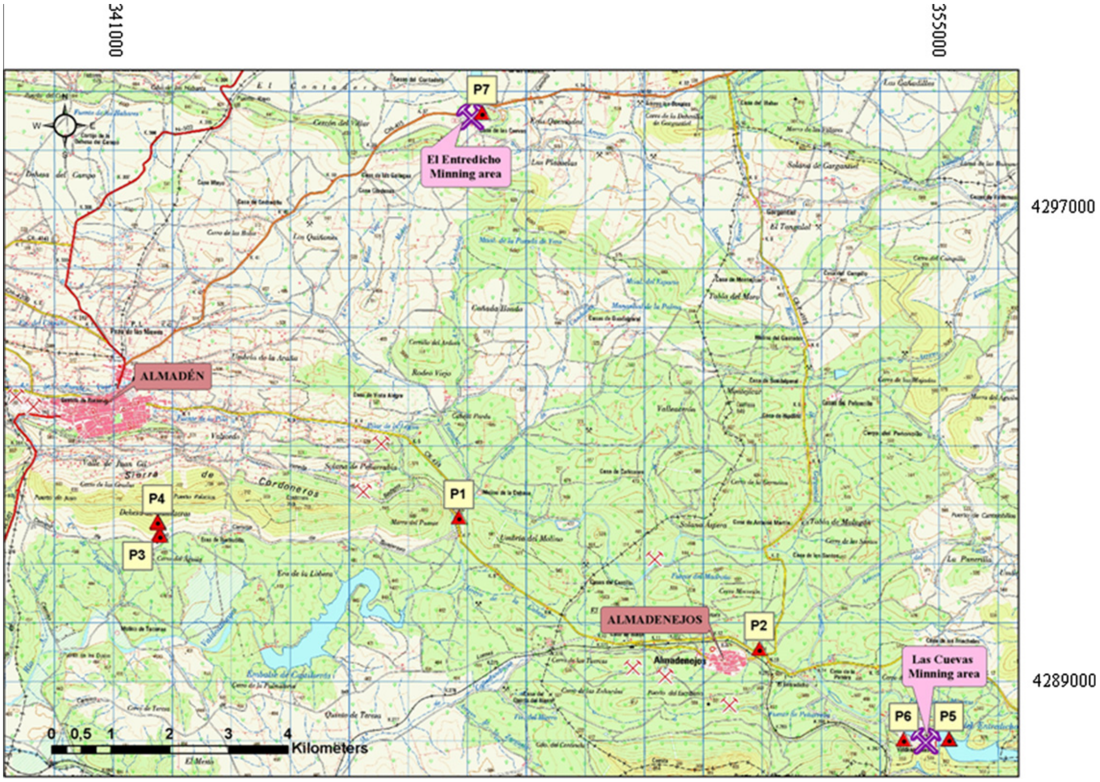

Fig. 1. The location of the test plots within the study area of Almadén. Source: Elaborated using topographic maps from the National Geographic Institute of Spain. Topographic map (2012) (http://www2.ign.es/iberpix/visoriberpix/visorign.html).

A soil profile 20 × 30 × 20 cm around each lavender sample was dug up. The roots were removed carefully by hand. All soil fractions free of roots were collected to give the bulk soil fraction and were air dried. The roots with the adhering soil aggregates <1 cm were also air dried. Then, the remaining dry root-soil fraction was shaken gently to separate some of the soil film covering the roots and was added to the soil fraction that detached easily from the surrounding roots after drying. This soil was collected as the rhizosphere fraction (Luster and Finlay, 2006). Both the bulk soil and the rhizosphere fraction were carefully homogenized.

2.3. Plant and soil analyses

The plant leaves were digested according to the acid digestion method (HNO_3, H_2O_2) under pressure given by Lozano-Rodríguez et al. (1995). Concentrations of Al, Ca, Cu, Fe, Mg, Mn, P and Zn were measured by atomic absorption spectroscopy (Perkin Elmer mod. 5000). Furthermore, K and Na were measured by flame emission (Perkin Elmer mod. 5000).

Different edaphic parameters were measured in the rhizosphere and bulk soil. These parameters included: Electrical conductivity (EC) and pH (1:2.5) that were determined according to the official methods given by Spanish Ministry (MAPA, 1994); cation exchange capacity (CEC) that was determined using the EPA 9081 method (Sodium acetate); organic matter (OM) that was measured following the Walkey–Black method; water soluble anions and easily available micro- and macro-elements for plants that were obtained using the AB-DTPA method (Soltanpour and Schwab, 1977). Furthermore, in the rhizosphere and bulk soil, easily available Hg for plants was obtained by the same AB-DTPA method. The use of this method is based on previous studies by Lucena et al. (1993) and Sierra et al. (2008a, 2009) in the Almadén area. They found high significant correlations between extracted Hg and plant Hg. Soluble anions (Cl^-, SO_4^{2-}, PO_4^{3-}, NO_3^-) were measured by ion chromatography, and micro- and macro-elements by atomic absorption and flame emission (Na and K).

The Hg concentration was determined in plant, soil and the soil extracts (rhizosphere and bulk soil) obtained by AB-DTPA method using an Advance Mercury Analyser (AMA-254, LECO Instruments). This equipment is based on an atomic absorption spectrophotometer specifically designed for Hg determination. Moreover, the solid or liquid samples are analyzed without a need of a chemical pretreatment. This equipment has a detection limit of 0.01 ng of Hg.

Certified reference materials (CRM, SRM and BCR) were used to determine the accuracy and precision of the Hg measurements and validate the applied methods. These reference materials are CRM 027 (soil from USA contaminated area, 3.80 ± 0.65 mg kg^{-1} of Hg), SRM 2709 (San Joaquin agricultural soil, 1.40 ± 0.08 mg kg^{-1} of Hg), SRM 1573a (tomato leaves, 0.034 ± 0.004 mg kg^{-1} of Hg) and BCR-CRM 150 (spiked skim milk powder, 0.0094 ± 0.0017 mg kg^{-1} of Hg). The mean values determined for ten measurements using the AMA-254 equipment were 4.13 ± 0.41 mg kg^{-1}, 1.47 ± 0.03 mg kg^{-1}, 0.032 ± 0.001 mg kg^{-1} and 0.0106 ± 0.0007 mg kg^{-1}, respectively. At a 95% confidence level, no significant differences were detected between the certified value and the experimental one, so this method was considered to be accurate for total Hg determination.

2.4. Statistical analysis

The software package used to perform the statistical analysis was SPSS for Windows (version 11.5). Standard descriptive analyses as well as the Kendalĺs tau-b correlation tests were applied. Correlation is significant at the 0.05 level. Furthermore, ANOVAs were carried out with the Turkey test when the Levene test assumed homogeneity of variance, or Welch's test with the Games-Howell test when the Levene test rejected homogeneity of variance. A probability of 0.05 or lower was considered as significant.

Multivariate statistical analyses were performed. Discriminant analysis was used to predict the variables that discriminate between groups: rhizosphere and bulk soil; and principal components analysis was performed separately on the bulk soil and on the rhizosphere. Furthermore, multiple regression analysis was carried out to define a regression equation that predicts Hg in the aerial part of the lavender plants. In this analysis, the available Hg and total Hg in the rhizosphere have been added together as independent variables.

3. Results and discussion

The results and discussion have been organized in two sections with their own sub-sections. The first section presents the differences between the bulk soil and the rhizosphere and the second section discusses the results related to soil-lavender system.

3.1. Soil

3.1.1. Edaphic parameters that make the rhizosphere different from bulk soil

Discriminant analysis is performed to assess how well the different measured edaphic parameters (Tables 1 and 2) separate the categories: bulk soil and rhizosphere in the classification. These measured parameters were chosen to discriminate between these two groups more significantly. They include: total Hg, easily available Hg, EC, OM, CEC, Cl^-, SO_4^{2-}, PO_4^{3-}, NO_3^-, Al^{3+}, Mn^{2+}, Ca^{2+} and Mg^{2+}. Using these variables, Wilks' Lambda presented a value of 0.011 that represents the total variability which is not due to differences between the two groups (rhizosphere and bulk soil). This low value indicated that these groups are well separated. Furthermore, there was a high canonical correlation (0.994) which confirmed that the chosen parameters are the best for discriminating between the rhizosphere and bulk soil. According to Standardized Canonical Discriminant Function Coefficients, the EC is the most important variable in the differentiation between the bulk soil and rhizosphere.

In the rhizosphere samples, Mn^{2+}, Mg^{2+}, Ca^{2+}, SO_4^{2-}, PO_4^{3-}, Cl^- and easily available Hg tend to be higher than those in the bulk soils. On the opposite, EC, CEC, NO_3^-, total Hg and OM in the rhizosphere tend to be lower than those in bulk soil samples.

Regarding the other measured edaphic parameters, there was a light pH increase (around 0.3 units) in the rhizosphere compared to the bulk soil, in most of the cases (Table 1). This effect was also observed by Puschenreiter et al. (2005) in the case of *Thlaspi goesingense* as well as by other authors, in the case of the hyperaccumulators *Alyssum murale*, *Thlaspi caerulescens*, and *T. goesingense* (Bernal et al., 1994; McGrath et al., 1997; Puschenreiter et al., 2001). However, this difference was not significant and, therefore, in this study this parameter does not make the rhizosphere different from the bulk soil.

An increase in pH may decrease metal bioavailability in general, whereas the increase of OM might trigger metal mobilization, as proposed previously by Salt et al. (2000) and Puschenreiter et al. (2003). In our case, in the rhizosphere there was a slight pH increase with respect to pH in the bulk soil, and a negative correlation between the available Hg and pH. It is linked with significant positive correlation between the available Hg and Al^{3+} and negative correlation between the soluble NO_3^- and the available Hg in the rhizosphere. Furthermore, the OM tended to be lower in the rhizosphere than in the bulk soil; thus, according to Salt et al. (2000) and Puschenreiter et al. (2003), the metal mobilization in the rhizosphere would be lower than in

Table 1
pH, EC, OM, CEC and soluble anions in the soils from different plots.

Soil		P1	P2	P3	P4	P5	P6	P7
pH	B.S.	5.4–5.6	6.2–6.4	6.0–6.1	5.8–6.1	7.8–7.9	7.1–7.2	4.5–4.7
	R.S.	5.9–6.3	5.2–5.3	6.3–6.4	5.5–5.6	8.0–8.2	7.4–7.6	5.0–5.1
EC (μS m^{-1})	B.S.	241 ± 2	121 ± 1	210 ± 1	112 ± 4	293 ± 2	531 ± 11	77 ± 1
	R.S.	86 ± 23	83 ± 2	205 ± 28	58 ± 4	190 ± 5	386 ± 16	48 ± 1
OM (%)	B.S.	2.4–2.5	2.3–2.4	2.4–2.9	5.5–7.1	2.4–2.5	6.6–7.7	4.0–4.9
	R.S.	3.9–4.0	4.5–4.7	5.3–8.2	3.0–4.2	1.1–1.3	3.6–3.8	1.9–2.0
CEC (cmol$_c$ kg^{-1})	B.S.	11.7 ± 0.0	21.0 ± 0.7	13.5 ± 1.4	14.6 ± 1.0	11.0 ± 0.1	20.8 ± 0.2	11.8 ± 0.4
	R.S.	10.2 ± 0.7	13.9 ± 0.5	13.8 ± 1.1	9.6 ± 0.4	7.5 ± 0.3	12.8 ± 1.3	9.8 ± 0.6
Soluble anions								
Cl$^-$ (mg kg^{-1})	B.S.	45.6 ± 0.1	13.0 ± 0.8	16.1 ± 4.2	3.8 ± 2.6	1.7 ± 1.4	9.8 ± 0.4	3.0 ± 1.4
	R.S.	34.7 ± 2.8	3.0 ± 0.7	58.6 ± 16.4	18.8 ± 20.6	6.0 ± 0.1	55.3 ± 0.1	52.5 ± 5.1
SO$_4^{2-}$ (mg kg^{-1})	B.S.	10.2 ± 9.6	9.7 ± 6.6	28.3 ± 13.4	13.2 ± 4.4	66.6 ± 0.1	194.1 ± 6.2	10.8 ± 0.7
	R.S.	15.3 ± 4.4	10.7 ± 1.9	25.8 ± 10.3	15.7 ± 6.0	62.0 ± 6.5	109.2 ± 3.1	40.5 ± 0.9
PO$_4^{3-}$ (mg kg^{-1})	B.S.	13.2 ± 0.1	15.7 ± 3.1	28.8 ± 18.7	14.0 ± 2.0	8.9 ± 1.7	21.2 ± 4.9	17.5 ± 1.4
	R.S.	16.0 ± 3.2	16.8 ± 1.2	49.6 ± 25.7	17.5 ± 1.4	13.2 ± 0.1	17.3 ± 0.1	ND
NO$_3^-$ (mg kg^{-1})	B.S.	104.9 ± 0.7	84.8 ± 0.2	60.2 ± 35.5	21.0 ± 5.6	23.3 ± 1.1	199.4 ± 16.8	5.0 ± 0.1
	R.S.	11.9 ± 7.0	8.4 ± 0.6	40.6 ± 3.0	2.4 ± 0.8	13.5 ± 0.1	39.5 ± 3.7	1.5 ± 0.1

The values are mean ± standard deviation ($n \geqslant 4$). BS: Bulk soil; RS: Rhizosphere soil. ND: No data.

Table 2
Easily available micro- and macro-elements for plants in the soils from different plots.

Soil		P1	P2	P3	P4	P5	P6	P7
Easily available micro–macro-elements								
Al$^+$ (mg kg^{-1})	B.S.	10.8 ± 1.7	9.6 ± 0.0	4.3 ± 3.2	1.2 ± 0.0	1.8 ± 0.0	3.7 ± 0.0	81.3 ± 2.1
	R.S.	2.7 ± 0.5	35.7 ± 1.8	2.3 ± 0.8	6.4 ± 1.7	2.8 ± 1.8	2.9 ± 0.5	108.0 ± 17.8
Cu^{2+} (mg kg^{-1})	B.S.	0.7 ± 0.1	1.0 ± 0.0	1.4 ± 0.3	1.2 ± 0.1	0.4 ± 0.0	3.7 ± 0.3	0.2 ± 0.0
	R.S.	3.9 ± 1.6	2.4 ± 0.0	1.4 ± 0.2	2.7 ± 1.4	1.5 ± 0.1	1.3 ± 0.2	2.4 ± 0.1
Fe^{2+} (mg kg^{-1})	B.S.	14.7 ± 0.8	27.6 ± 0.4	42.3 ± 4.7	4.1 ± 0.6	7.7 ± 0.4	73.4 ± 3.1	61.5 ± 2.3
	R.S.	48.8 ± 25.4	112.3 ± 0.4	29.3 ± 1.6	40.2 ± 11.4	46.0 ± 5.2	20.6 ± 0.8	66.3 ± 1.7
Mn^{2+} (mg kg^{-1})	B.S.	39.1 ± 0.6	103.0 ± 0.4	66.0 ± 6.9	8.6 ± 1.2	3.2 ± 0.3	20.3 ± 0.5	97.2 ± 7.6
	R.S.	33.4 ± 24.5	208.5 ± 10.7	123.9 ± 25.5	75.0 ± 2.0	10.9 ± 8.0	54.0 ± 2.8	45.6 ± 0.4
Pb^{2+} (mg kg^{-1})	B.S.	3.0 ± 0.2	2.0 ± 0.1	1.8 ± 0.9	1.2 ± 0.1	2.1 ± 0.1	2.7 ± 0.1	5.6 ± 0.2
	R.S.	57.4 ± 26.6	4.2 ± 0.2	2.5 ± 0.6	1.8 ± 0.5	4.1 ± 0.5	3.1 ± 0.1	6.5 ± 0.5
Zn$^+$ (mg kg^{-1})	B.S.	1.7 ± 0.0	1.2 ± 0.1	1.6 ± 0.3	1.2 ± 0.1	1.6 ± 0.1	5.2 ± 0.3	0.9 ± 0.1
	R.S.	4.3 ± 2.8	2.4 ± 0.2	3.4 ± 0.2	1.8 ± 0.4	1.6 ± 0.1	2.5 ± 0.1	2.4 ± 0.0
Ca^{2+} (mg kg^{-1})	B.S.	254.0 ± 0.0	406.3 ± 0.4	462.0 ± 53.9	423.5 ± 27.7	218.6 ± 0.4	494.0 ± 1.7	45.6 ± 0.4
	R.S.	444.8 ± 403.2	414.5 ± 1.3	459.3 ± 114.1	344.2 ± 21.1	355.3 ± 21.0	371.7 ± 15.6	413.0 ± 3.3
Mg^{2+} (mg kg^{-1})	B.S.	97.6 ± 4.7	48.1 ± 0.1	129.3 ± 10.2	63.8 ± 31.0	12.0 ± 0.1	189.3 ± 4.0	17.0 ± 1.5
	R.S.	118.9 ± 110.9	227.1 ± 2.4	240.1 ± 45.2	64.9 ± 19.2	120.5 ± 8.3	351.9 ± 0.1	92.3 ± 0.7
K$^+$ (mg kg^{-1})	B.S.	28.6 ± 3.1	52.6 ± 0.0	253.0 ± 28.4	141.4 ± 8.3	18.9 ± 0.3	115.0 ± 1.1	63.7 ± 0.5
	R.S.	116.8 ± 104.0	309.6 ± 2.2	722.3 ± 436.8	171.5 ± 66.2	247.4 ± 93.8	437.1 ± 0.1	200.9 ± 1.6
Na$^+$ (mg kg^{-1})	B.S.	9.8 ± 0.0	23.9 ± 0.0	10.2 ± 1.1	4.6 ± 0.7	1.1 ± 0.0	11.8 ± 0.1	3.8 ± 0.0
	R.S.	8.6 ± 7.7	13.7 ± 1.9	24.1 ± 6.3	5.9 ± 1.0	8.0 ± 0.9	10.6 ± 0.0	12.1 ± 0.4

The values are mean ± standard deviation ($n \geqslant 4$). BS: Bulk soil; RS: Rhizosphere soil.

the bulk soil. These results may be related to some Hg tolerance mechanism of *L. stoechas* L. since there were not these correlations in the bulk soil.

There was a higher percentage of the available Hg with respect to the total Hg in the rhizosphere than in the bulk soil (Table 3) except in the case of "Sierra de Cordoneros" (P3 and P4) in which both values were similar. Puschenreiter et al. (2005) observed the same tendency in the cases of soluble Ni, Cr and Zn in *Thlaspi goesingense* rhizosphere. A reasonable explanation for this fact may be that the Hg had been moved to the rhizosphere by mass flow (i.e. plant transpiration). However, despite the latter fact and according to the rest of the mentioned results the lavender plants seem to influence in decreasing the easily available Hg in their rhizosphere. It would appear to indicate that there would be some tolerance mechanism of *L. stoechas*.

There was a great variability in soluble anions both in the rhizosphere and the bulk soil from plot "Sierra de Cordoneros" (Table 1). The presence of livestock is typical in this area and this fact could

Table 3
Mercury in the soils from different plots.

Mercury in soil		P1	P2	P3	P4	P5	P6	P7
Total Hg (mg kg^{-1})	B.S.	2.6 ± 0.4	22.7 ± 3.8	3.3 ± 0.6	7.7 ± 0.3	13.5 ± 0.3	727.8 ± 1.2	56.0 ± 1.2
	R.S.	6.6 ± 0.4	5.1 ± 0.4	1.5 ± 0.1	5.6 ± 0.8	7.9 ± 0.8	54.4 ± 1.4	39.0 ± 0.3
Easily available Hg (μg kg^{-1})	B.S.	0.5 ± 0.1	2.6 ± 0.1	7.0 ± 1.7	22.0 ± 2.7	0.8 ± 0.0	62.6 ± 1.3	26.9 ± 2.1
	R.S.	6.9 ± 1.6	26.7 ± 6.0	2.2 ± 0.3	12.3 ± 1.1	3.1 ± 0.4	20.3 ± 1.3	40.9 ± 3.1
% of available Hg with respect to total Hg	B.S.	0.02	0.01	0.21	0.29	0.01	0.01	0.05
	R.S.	0.10	0.52	0.15	0.22	0.04	0.04	0.10

The values are mean ± standard deviation ($n \geqslant 4$). BS: Bulk soil; RS: Rhizosphere soil.

be associated with random organic residues from animals that could be causing this variability.

3.1.2. Mercury and edaphic parameters

3.1.2.1. Rhizosphere. Regarding the rhizosphere and according to test of Kendalĺs tau-b, there was significant negative correlation between the available Hg and pH linked to a significant positive correlation between the available Hg and Al^{3+} as mentioned above. Also, there were significant negative correlations between the soluble NO_3^- and the available Hg; and between EC and the available Hg. This former relationship would seem to suggest that the more salts (higher EC values), the more insoluble Hg precipitates like Hg_2SO_3 or Hg_2Cl_2 are formed.

Finally, it is also interesting to note that an increase of pH significantly increased the NO_3^- concentration; and an increase in the total Hg concentration significantly increased the available Hg concentration.

3.1.2.2. Bulk soil. Regarding the bulk soil and according to the test of Kendalĺs tau-b, there were other significant correlations between the available Hg and some edaphic parameters different from the ones found in the rhizosphere. For instance, it is interesting to point out the positive correlation between the available Hg and the OM. Only two significant correlations coincided in both soils. They were the significant positive correlation between the available Hg and the total Hg and the significant negative correlation between the available Hg and EC. It seems that the significant correlations found in the rhizosphere different from those found in the bulk soil could have been influenced by the plant.

3.1.3. Distribution and presence of mercury

According to Welch's test with the Games–Howell test (Levene test rejected homogeneity of variance), there was a significant positive correlation between the total Hg in the bulk soil and the total Hg in the rhizosphere and; between the total Hg in the bulk soil and the available Hg in the rhizosphere. It seems there was a resupply from fixed Hg fractions to the soil solution in the bulk soil and subsequently to the available pool in the rhizosphere.

Regarding the ratio of the percentage of the available Hg concentration with respect to the total Hg concentration in the bulk soil and in the rhizosphere, it remained below 1% in all cases (Table 3). This low ratio agrees with studies carried out by Lucena et al. (1993), Millán et al. (2006) and Millán et al. (2011) who reported that Hg availability for plants was normally low. It has to be taken into account that in all of these samples, Hg is mainly in cinnabar form, which is very stable.

Regarding the Hg concentration in the different plots (Table 3), variations among the different soils would be expected. The significantly highest values of the total and available Hg were found at the mine sites ("El Entredicho" (P6) and "Las Cuevas" (P7)) in the case of the bulk soil and rhizosphere. On the other hand, the significantly lowest values were found at "Fuente del Jardinillo" (P1) in the case of the bulk soil, whereas, the lowest values of the total and available Hg in the case of the rhizosphere were found in the pastures of "Sierra De Cordoneros" (P3). The total Hg concentrations for the soil in all plots were within the range found by Lindberg et al. (1979), Higueras et al. (2003), Millán et al. (2006), Molina et al. (2006) and Bueno et al. (2009) for the Almadén area.

3.2. Soil–plant system

3.2.1. Mercury in lavender plants

Total Hg concentrations were analyzed in the different plant organs of lavender plants (Fig. 2). The Hg concentration in the leaves varied from 0.18 to 3.05 mg kg^{-1}, whereas the Hg concentration measured in the stems ranged from 0.04 to 2.01 mg kg^{-1}. Finally, the Hg concentration in the root varied from 0.22 to 5.48 mg kg^{-1}. The roots of plants usually act as a barrier to Hg uptake (Patra and Sharma, 2000). However, Salt et al. (1998) observed that a relevant metal fraction is exported to the shoot and primarily takes place through the xylem.

The results indicate that there are marked differences between the distribution of this metal throughout the plants collected in mine plots ("El Entredicho" (P6) and "Las Cuevas" (P7)) and those collected in the rest of the plots (Fig. 2). In lavender plants from the mine areas, there were no significant differences between the Hg concentration in the roots and leaves and these concentrations were significantly higher than the flower and stem Hg concentration. On the other hand, in the rest of the lavender plants there were no significant differences between the different organs of the aerial part. The Hg concentration in the root is significantly higher which coincides with references by different authors (Du et al., 2005; Greger et al., 2005; Millán et al., 2006; Skinner et al., 2007; Sierra et al., 2008a,b, 2011).

Díez Lázaro et al. (2006) found that this plant species bioaccumulates different metals (Co, Cr, Cu, Mn, Ni, Pb and Zn) in its aerial parts. However, the metal distribution pattern of plants grown in different types of soils from Tras-os-Montes (Portugal) where the dominant lithology is serpentine, shows the influence of Ni. This is different from our case, since metal concentration in the leaves in those plants were significantly higher than in stems and roots where metals were accumulated in the same way. On the other hand, Hg distribution within our study of lavender plants in the natural environment is similar to that observed in a preliminary study performed under greenhouse conditions (Sierra et al., 2009).

Regarding the root uptake pathway, the distribution of this metal through the plants collected in mine plots coincides with the fact that the available and total Hg values in these soils were the highest compared to the rest of sample points. Nevertheless, the Hg concentrations measured in the leaves collected in the abandoned mine plots also coincide with the higher air Hg concentrations measured at these sites (Higueras et al., 2006; Llanos et al., 2010; Martínez-Coronado et al., 2011). This means that a fraction of the Hg measured in the leaves of the plants in this study (grown in the soils with high concentration of total and available Hg) could enter via the stomata pathway (Iverfeldt, 1991; Munthe et al., 1995).

The ability of different plants to absorb trace elements varies greatly (Kabata-Pendias, 2001; Molina et al., 2006). According tests of Kendall's tau-b in the study case, there was no correlation neither between the easily available Hg and total Hg in the bulk soil, and the lavender plant Hg concentration. However there were significant correlations between the rhizosphere Hg and the plant Hg. In the case of the root, there was a significant negative correlation. Metal uptake has been found to be associated with partial depletion of Hg from the rhizosphere. However, in the case of the plant aerial part, there was a significant positive correlation.

If the available and total Hg are taken together like independent variables in a multiple regression analysis where the dependent variable is leaf Hg, the analysis adjustment improves with respect to simple regression analysis where there was only one independent variable (available Hg or total Hg). The regression equation that was obtained is the following: Leaf Hg (forecast) = $-3.574 + 225.26 \times Hg_{\text{available in rhizosphere}} + 0.433 \times Hg_{\text{total in rhizosphere.}}$ The more Hg in the rhizosphere the more Hg is translocated to the aerial part and less Hg is retained by the root.

The soil used in the preliminary greenhouse study with lavenders, performed by Sierra et al. (2009) was from the area "Dehesa de Castilseras", the study plot P2. Furthermore, the chemical characteristics of the soils in both cases were similar with two exceptions. One of them was that the CEC was around 9 units lower in the soil used in the greenhouse pots and the other was that the

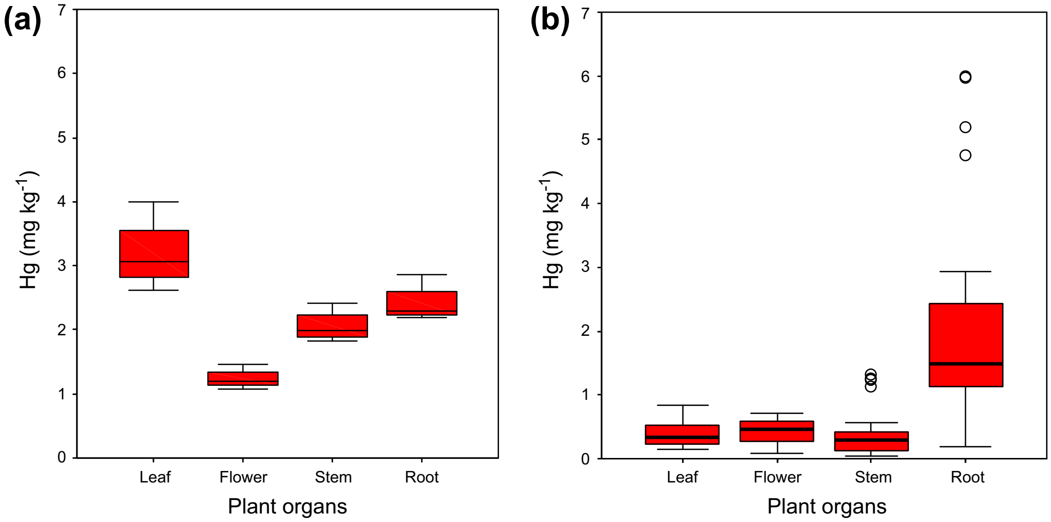

Fig. 2. (a) Distribution of mercury in different plant organs taking into account lavender samples collected in mine plots ("El Entredicho" (P6) and "Las Cuevas" (P7)) and (b) distribution of mercury in different plant organs taking into account lavender samples collected in the rest of the plots (P1, P2, P3, P4 and P5). Box and whisker plot; (a): *n* = 21; (b): *n* = 48. ○: Outliers (values that are between 1.5 and 3 times the interquartile range).

percentage of the available Hg with respect to the total Hg in field was around five times higher than in the soil used in the greenhouse pots. Moreover, the Hg concentration in the field (P2) and greenhouse plants was significantly different from each other. It is important to point out that the plant biomass of the field (P2) and greenhouse plants was similar. The aerial part of the plants collected in field (P2) had metal concentration around two times higher than the plants that grew under greenhouse conditions. Furthermore, the field roots (P2) had Hg concentration around three times lower than the plants that grew under greenhouse conditions. This coincides with the correlation between the easily available Hg and the plant Hg showed above.

Conesa et al. (2007) studied the response of *Lygeum spartum* plants to different metals and observed that the plants sampled in the field had metal concentrations of one order of a magnitude lower than the plants raised in conditions of a growth chamber. In that study, the field plant biomass was more than 47 times higher than the chamber plant biomass. Therefore, lower metal concentration in the field plants can be explained by phytodilution process as the authors commented.

The results obtained in this study show that shoot/root concentration ratio was less than 1 in all cases. Therefore, according to Baker (1981) these lavender plants present excluder behavior. These lavender plants would be metal-tolerant excluders, which would be able to grow on metalliferous soils without distinct bioaccumulation of the metals (Baker and Brooks, 1989). However, there are two exceptions, the case of "Las Cuevas" (P7) and "Dehesa de Castilseras" (P2) where the ratio was approximately 1. Therefore, according to Baker (1981), the plant-soil relationship could be of indicator type. The behavior of the same plant species (*L. stoechas*) with Hg in soil was not always the same and changes depending on soil properties as shown in the literature for other metals. Furthermore, our plant samples did not show visual symptoms of toxicity. It is interesting to point out that the values of the rhizosphere easily available Hg of these two cases were the highest. Furthermore, there was significant positive correlation between ratio of the Hg concentration in the aerial part and root and the easily available Hg concentration in the rhizosphere.

3.2.2. Plant-rhizosphere system. Relationships between Hg and different ions

According to the Principal Component analyses performed with the rhizosphere data, information on the following variables: Available Hg, leaf Hg, aerial part Hg, root Hg, rhizosphere Mn^{2+}, leaf Mn, leaf Pb, rhizosphere SO_4^{2-} and rhizosphere NO_3^-, can be represented by three components that explain 84.3% of the total variance in the variables. These components are as follows:

Component 1: Available Hg, Mn^{2+} from rhizosphere, leaf Mn, leaf Hg, leaf Pb.
Component 2: Available Hg, SO_4^{2-} from rhizosphere, leaf Hg, aerial part Hg, root Hg.
Component 3: SO_4^{2-} from rhizosphere and NO_3^- from rhizosphere.

Taking into account these results, correlation tests were applied for these variables in order to obtain more information.

Manganese is a micro-nutrient that plays a role as a structural component and co-factor for a number of key plant enzymes. It is absorbed by the plant as Mn^{2+} (Burnell, 1998; Shuman, 1991). The findings in the work presented here show that there is a strong positive significant correlation between this available rhizosphere Mn^{2+} and leaf Mn (Fig. 3a and Table 2). Furthermore, an antagonism effect of manganese against Hg is suggested. There was a negative significant correlation between the available rhizosphere Mn^{2+} and the aerial part and the root Hg (Fig. 3b and c). The more available Mn^{2+} in the rhizosphere, the less Hg in the aerial part and root. Other authors have studied antagonism effects of other micro-nutrients against other contaminant elements. He et al. (2004) suggested the antagonism of Zn and Se against Pb and Cd in vegetables. They showed that the enrichment of Zn and Se restrained the accumulation of Pb and Cd in the lettuce. Taking into account these types of antagonism effects against contaminant elements, the results in this study could suggest opportunities for future research. An example would be to study the effect of supplementing Mn in crops grown in Hg contaminated matrix.

According to Ghabbour et al. (2006) the Hg^{2+} binding affinity by soil humic acid is higher than Mn^{2+}. In that sense, it would appear that the Hg in soil could be moving the Mn from the exchange positions and favoring the manganese availability for the lavender plants, therefore, decreasing the Hg availability for the plants.

Regarding the relationships between Hg and the OM in the plant-rhizosphere system, there was a significant negative correlation between Hg in the aerial part and the OM in the rhizosphere. This would be explained by the great affinity of Hg by OM (Haitzer et al., 2002; Ravichandran, 2004; Han et al., 2006) since OM could

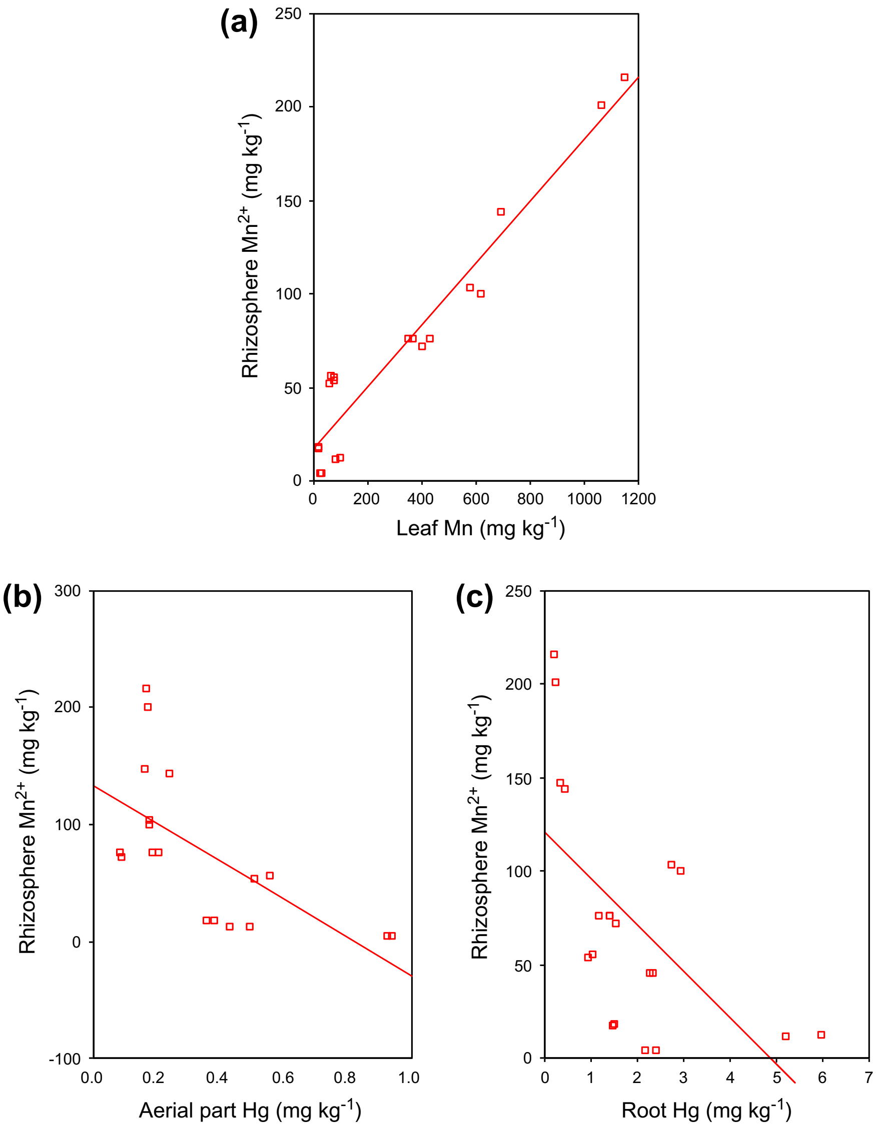

Fig. 3. (a) Relation between the available Mn^{2+} in the rhizosphere and the lavender leaf manganese concentration; (b) relation between the available Mn^{2+} in the rhizosphere and the aerial part mercury concentration; and (c) relation between the available Mn^{2+} in the rhizosphere and the root mercury concentration.

block the Hg availability for the lavender in our study case. Ravichandran (2004) observed the formation of extremely strong ionic binding between Hg and reduced sulfur sites in soil OM, which supports the results presented in this study.

On the other hand, our findings would seem to show that available rhizosphere Mn^{2+} favor Pb translocation to the aerial part of the lavender plant. The authors hypothesize a synergic effect of Mn and Pb in lavender plants. There was a positive significant correlation between the available rhizosphere Mn^{2+} and the Pb in lavender leaves (Fig. 4a). Furthermore, there was a positive significant correlation between the leaf Mn and leaf Pb in contrast to the negative significant correlations between leaf Mn and leaf Hg (Fig. 4b and c) as well as between leaf Pb and leaf Hg (Fig. 4d). The Pb could be entering cells by means of the same uptake processes as Mn. These results are similar to those reported by Zornoza et al. (2010) focused on the effects of the interaction between Mn and Cd on the growth of *Lupinus albus*. According to Zornoza et al. (2010), when the Mn supply was adequate, Cd uptake and accumulation increased. It seems that manganese could have synergic or antagonistic effects depending on the contaminating element.

Furthermore, it is interesting to note that the presence of sulfates seems to favor the Hg uptake by the plant. There was a positive significant correlation between the SO_4^{2-} in the rhizosphere and the Hg within the aerial and root parts of the lavender plant. Toxic metal ions enter cells by means of the same uptake processes that move essential micronutrient metal ions. Hg seems to bind preferentially with sulfur, nitrogen-rich ligands and the less electronegative halides (Nieboer and Richardson, 1980; Stumm and Morgan, 1995). Because of high affinity of Hg by –S bonds, this metal could be entering in the same way as the sulfates that are the main source of inorganic S in soils for plants. The sulfates enter with a high affinity transport system when there are low concentrations in soil and by diffusion system when there is a high concentration in soil. The sulfates are absorbed by root and mainly transported by xylem to leaves (Azcón-Bieto and Talón, 2000).

Finally, regarding NO_3^-, an increase in this ion in the rhizosphere significantly decreased the available Hg concentration in the rhizosphere, however, there were positive significant correlations between the NO_3^- concentrations in the rhizosphere and bioaccumulation factors (BAF = Hg in tissue/Hg available in soil)

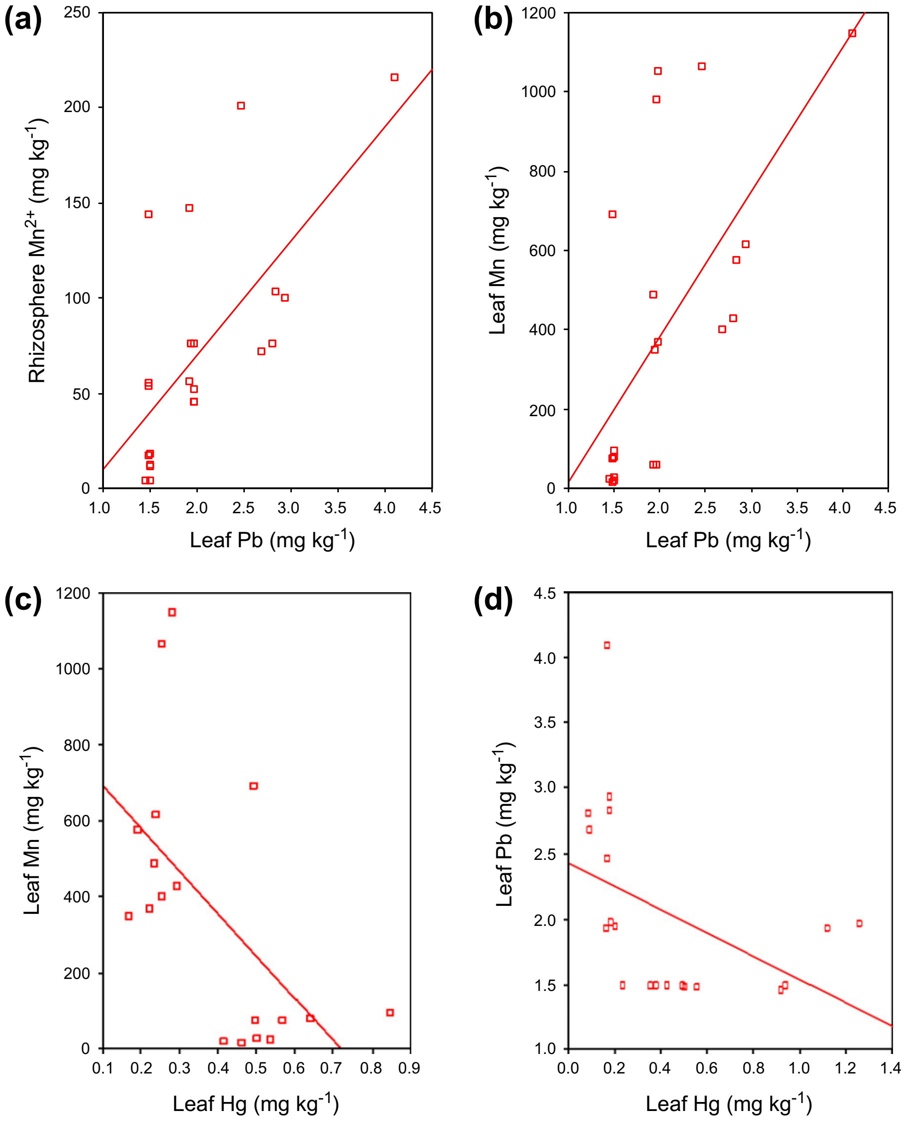

Fig. 4. (a) Relation between the available Mn^{2+} in the rhizosphere and the lavender leaf lead concentration; (b) relation between the lavender leaf manganese and lavender leaf lead concentration; (c) relation between the lavender leaf manganese and lavender leaf mercury concentration; (d) relation between the lavender leaf lead and lavender leaf mercury concentration.

in the root and aerial part of lavender plants that could mean an increase the uptake of Hg from the rhizosphere. This could be related to the significant negative correlation between the available Hg and the soluble NO_3^- mentioned above. The high NO_3^- concentrations in soil may increase the foliar development and the evapotranspiration. Therefore, the plant nutrient demand would be greater and as a result the Hg uptake could also be greater. This agrees with Carrasco-Gil et al. (2012) who observed that the supply of NO_3^- prevented oxidative stress in roots and may improve root metabolism and development, but this was followed by an increase in Hg uptake from the soil by *Medicago sativa* L. plants.

4. Conclusions

The main edaphic properties and parameters that differ between the lavender rhizosphere and the bulk soil are: total Hg; easily available Hg; EC; OM; CEC; Cl^-; SO_4^{2-}; PO_4^{3-}; NO_3^-; Al^{3+}; Mn^{2+}; Ca^{2+} and Mg^{2+}. In this case, the EC is the most important variable that shows the greatest difference.

The highest values of the *L. stoechas* Hg concentration corresponded to the plants sampled in the old mining areas (regarding the aerial part of the plants). The lowest values correspond to the forest area of the high part of “Sierra de Cordoneros”. This trend coincides with the results obtained for the rhizosphere and total Hg concentration of the bulk soil. Hg concentrations in lavender depends on Hg concentrations in soils. In lavender plants from the mine areas, there were no significant differences between the Hg concentration in the roots and leaves and these concentrations were significantly higher than the flower and stem Hg concentration. In the rest of the lavender plants, the root Hg concentration was higher than the concentration found in the aerial part. Regarding the Hg distribution in the aerial part, there were no significant differences between the Hg concentrations in the different organs.

Furthermore, the obtained results indicate that there are significant correlations between the mercury concentrations in the rhizosphere and in the plant. Lavender plants uptake available Hg from the rhizosphere. The more easily Hg is available in the rhizosphere, the greater is the translocation of Hg to the aerial part and less Hg is retained by the root.

The lavender plants included in this study are tolerant to Hg toxicity. It has been found that lavender plants are Hg tolerant excluders. The exceptions are plants from soils with the highest Hg concentration, in which the soil–plant relationship shows indicator behavior.

Both OM and rhizosphere Mn^{2+} seem to block Hg uptake by the lavender roots and its translocation to the aerial part of the plant. However, the presence of sulfates seems to favor it. Furthermore, Hg seems to block Pb uptake by lavender plants, whereas the presence of Mn^{2+} seems to favor the Pb uptake by these plants.

An increase in NO_3^- concentration in the rhizosphere significantly decreased the rhizosphere available Hg concentration. However, a NO_3^- increase would seem to increase the uptake of Hg from the rhizosphere.

The results from this study point towards the idea that rhizosphere interactions are considered to play a key role in controlling bioavailability to lavender plants. This study indicates complex interrelationships between the different elements.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2012.06.017.

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