

Article

Use of Genus *Cistus* in Phytotechnologies: Application in a Closed Mercury Mine

Araceli Pérez-Sanz ¹, Rocío Millán ^{2,*}, María José Sierra ², Thomas Schmid ² and Gregorio García ^{3,*}

¹ Department of Agricultural Chemistry and Food Science, Autonomous University of Madrid (UAM), 28049 Madrid, Spain; araceli.perezs@uam.es

² CIEMAT, Avenida Complutense 40, 28040 Madrid, Spain; mj.sierra@ciemat.es (M.J.S.); thomas.schmid@ciemat.es (T.S.)

³ Agronomical Engineering Department, Technical University of Cartagena (UPCT), 30203 Cartagena, Spain

* Correspondence: rocio.millan@ciemat.es (R.M.); gregorio.garcia@upct.es (G.G.); Tel.: +34-968-32-5755 (G.G.)

Abstract: The Almadén mining district is known to be one of the richest mercury areas in the world. Despite the high concentrations of this metal, this territory has well-established vegetation that provides a wide range of mercury-tolerant plants that can be used as ecosystem services. This is the case of some species of *Cistus* that grow wild and spontaneously as part of the natural flora of Almadén. The objective of this study was to evaluate if there were differences between the absorption and distribution of Hg of five species of the genus *Cistus* in spontaneous growth and to evaluate their potential application in phytotechnologies. The work has been carried out with plant samples collected under field conditions in the “Fuente del Jardinillo” located in the old mining area of Almadén (Ciudad Real). The experimental plot was divided into three previously characterised subplots to ensure that all the sampled plants had grown in similar soil conditions (pH, organic matter content, EC, CEC, total Hg and available Hg). Additionally, the experiment was carried out in triplicate. The results showed that despite the homogeneity of the soil, the absorption of Hg in the aerial part of the plants showed significant differences related to *Cistus* species. The values in the bioaccumulation of mercury in the aerial part were also different. Based on the uptake of mercury by the plants sampled in this study, its potential use in phytotechnologies was established, classifying them as phytoextractors (*Cistus albidus*, *C. ladanifer* and *C. monspeliensis*) and phytostabilisers (*C. crispus* and *C. salvifolius*).

Keywords: phytoremediation; soil pollution; ecosystem services; Almadén; mercury; intragenus; *Cistus*



Citation: Pérez-Sanz, A.; Millán, R.; Sierra, M.J.; Schmid, T.; García, G. Use of Genus *Cistus* in Phytotechnologies: Application in a Closed Mercury Mine. *Land* **2023**, *12*, 1533. <https://doi.org/10.3390/land12081533>

Academic Editor: Evangelia Golia

Received: 13 July 2023

Revised: 30 July 2023

Accepted: 31 July 2023

Published: 2 August 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Almadén, located in the province of Ciudad Real (central Spain) is the largest mercury-mining district in the world (Figure 1). It occupies an area of approximately 100 km². Almadén production accounted for more than 30% (285,000 tonnes) of the total global mercury production [1,2]. The district has several mineral mercury deposits, predominantly in the form of cinnabar (HgS), but elemental mercury (Hg⁰) and poorly soluble cations (Hg²⁺) are also found [3,4]. Part of the current contamination of the area comes from its atmospheric deposition, which is common in mining areas and a consequence of mining and processing activity over centuries [5]. Mercury mining has been carried out for over 2000 years, ceasing in May 2002. Metallurgy and distillation activities were suspended in Almadén in February 2004 [6].

This region can be regarded as one of the most mercury-affected in the globe because of the extensive mining district. Mercury can be transported over long distances after being emitted from the primary source because of atmospheric emissions, its dispersion and transport by rivers [7–10]. At present, the closure of the Almadén mines raises the need for a change of land use to maintain the region’s economic activity. Almadén also shows a

particular landscape and an environment rich in natural resources of great environmental importance that can be used to restore and guarantee the habitability of this area. In addition, this knowledge will allow us to select the most appropriate techniques to restore other contaminated areas where mercury is the main source of contamination.

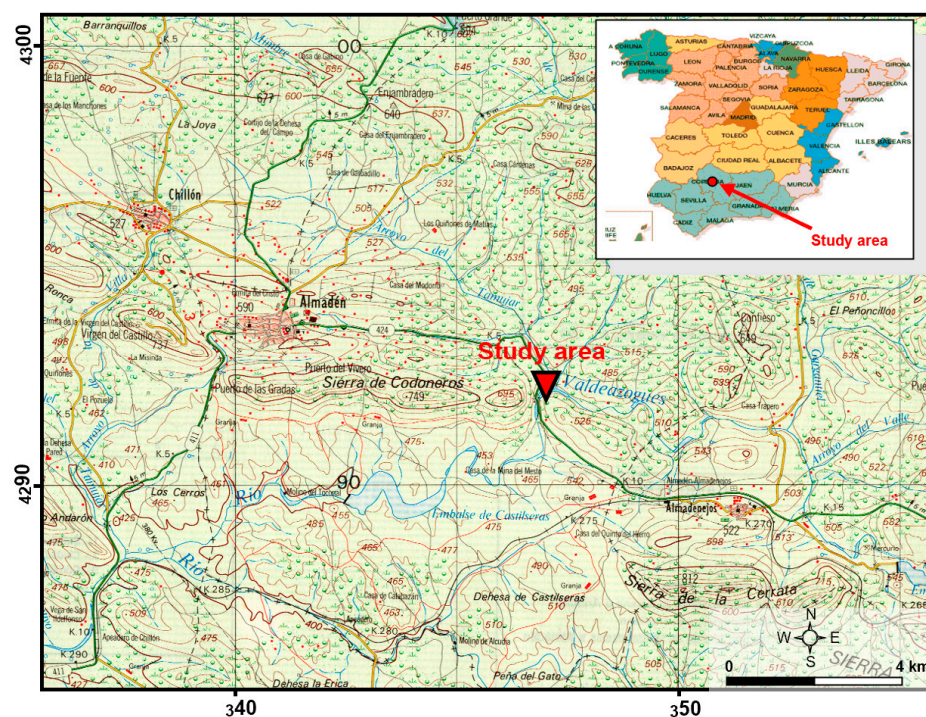


Figure 1. Location of the “Fuente de Jardinillo” (P2) in the “Sierra de Cordoneros” (Almadén, Spain).

In the Almadén area, mine stockpiles and tailings represent a source of mercury to nearby soils. Furthermore, they are relevant areas where mining and industrial activities was carried out related to the extraction (i.e., open-pit mines) or treatment of the mineral (furnaces and storage areas), some of them centuries old and representing the technology of the time [11]. Mercury occurs naturally and massively in the soil as HgS in the form of cinnabar and as an appreciable Hg^{2+} because of atmospheric deposition, the weathering of the lithological substrate, and, to a lesser extent, the decomposition of vegetation [9,12,13]. Once deposited in soil, mercury retention is controlled together with pH and other soil physicochemical properties, such as organic matter concentration, iron and manganese oxides, sulphur contents, redox and humidity, and clay minerals [14–16].

Generally, mercury is strongly bound to soil constituents because of its affinity for sulphur-containing functional groups present in the organic molecules of organic matter-rich topsoil layers [17]. Since Hg is poorly available and mobile in soil due to the strong bonds, only small amounts of Hg are typically present in soil solution, and uncharged complexes constitute the predominant form of Hg (II) in solution. Due to their high specific surface area and reactivity, clay minerals play a very important role in mercury immobilisation, especially in soils that are neutral and/or poor in organic matter.

However, under certain conditions, the mobilisation of mercury can occur, e.g., increased pH and chloride concentrations in the soil solution can improve the solubility of this element through complexation [18,19]. In acid soils, the formation of mercury complexes with soluble organic matter is also a major contributor to increased Hg solubility. On the other hand, in neutral soils or soils with low organic matter concentration, solubility would be dominated by Fe oxides and clay minerals, and Hg mobility would increase if pH decreased. Only the fraction of Hg in solution (soluble fraction) is taken up by plants, although this is in equilibrium with the fraction of Hg retained in soil constituents at a lower strength [20].

The natural geochemical anomalies that give rise to large mining areas, together with the presence of large amounts of heavy metals in paragenetic association often support characteristic plant species, which thrive in these heavy-metal-enriched environments through adaptive strategies [21]. These may include the accumulation of these metals in unusually high concentrations, at levels exceeding soil concentration levels. This particularity of heavy-metal-tolerant and -accumulating plant species can be exploited for the remediation of anthropogenically heavy metal contaminated soils, provided that suitable plant species can be identified if they produce enough biomass.

Previous work carried out on the soil of the Almadén region [22] revealed mercury concentrations in a range of 0.5–260 mg/kg. More recent studies [11,23–25] show a concentration of mercury between 5 and 40,000 mg/kg. The concentrations depended on their location with respect to the mining focus, and also, to the areas of mining and processing of ore and mercury. The study area corresponds to an old mine which is part of an old mining district composed of numerous mining exploitations.

The concentration of mercury measured in some plant species in the area [26] turned out to be greater than 100 mg/kg and 1 mg/kg at 0.5 and 20 km from the mining area, respectively. Subsequently, tests conducted at experimental sites in the Almadén mines revealed mercury contents in plants ranging from below the detection limits of the technique used to more than 100 mg/kg [23,24,26–28].

In view of the results, [11] focused their studies on knowing the behaviour of the soil-plant system and, thus, determining the possible application of phytotechnologies in the recovery of other contaminated ecosystems [11,28–31]. Phytotechnologies, considered biological soil treatment technologies, comprise various strategies such as phytostabilisation, phytoextraction, rhizodegradation or phytostimulation, rhizofiltration, phytodegradation or phytotransformation, phytovolatilisation and hydraulic control [32,33]. Furthermore, phytotechnologies could be improved by adding soil amendments or could be combined with other remediation techniques [34,35].

In this work, different physicochemical parameters have been evaluated to characterise the soil from “Fuente del Jardinillo” (Almadén) and to determine its total mercury content. At the same time, the potential of the five plant species of the genus *Cistus* (*C. albidus*, *C. crispus*, *C. ladanifer*, *C. monspeliensis* and *C. salviifolius*) present in the study area for their use in phytoextraction or phytostabilisation phytotechnologies has been studied and compared.

This work delves into phytoextraction, which involves the use of plants to extract contaminants from the soil and translocate them to the aerial parts, thus eliminating them from the soil, and phytostabilisation, a technique in which plants are used to avoid the bioavailability of toxic metals in the soil, reducing their mobility. The optimisation of these techniques involves the correct selection of the plant species with the greatest phytoremediation potential.

2. Materials and Methods

2.1. Plot Description

Previous work has thoroughly described the botanical categorisation of the vegetation in the Almadén mining area [11], taking into account the different degrees of impact of mining activities and evaluating the composition of the flora and soil of the mining area. The study area in this work corresponds to the “Fuente del Jardinillo”. The area (38°45′35″ N, 4°45′51″ W) is located on the southern side (25–30% slope) of the “Sierra de Cordoneros” (Figure 1) where there are remains of a silicicolous holm oak forest (*Pyro bourgaeanae*–*Quercetum rotundifoliae*) [36]. There are also formations of scrubland belonging to the *Genisto hirsutae*–*Cistetum ladaniferi* community composed of a dense rose bush and other shrubs and bushes typical of the holm oak formation in a Mediterranean climate.

This area is relatively small, about 1 Ha, with a homogeneous vegetable density where five species of genus *Cistus* coexist: *C. albidus*, *C. crispus*, *C. ladanifer*, *C. monspeliensis* and *C. salviifolius*. The coexistence of so many different species in such a small area makes it

possible to compare intragenus homogeneity (*Cistus*) on the individual behaviour of each species against mercury contamination as a tool to recover mercury-contaminated soils.

For the sampling to be as homogeneous as possible, the plot was delimited in such a way that all the plant species were subjected to the same abiotic variables, as well as perfectly mixed without following specific distribution patterns. In this way, a subplot of 200×60 m was selected, discarding the area of the slope most affected by erosion that could cause biases in the sampling. Subsequently, this plot was divided into three subareas (50×60 m), separated by 2-metre-wide safety channels (Figure 2).

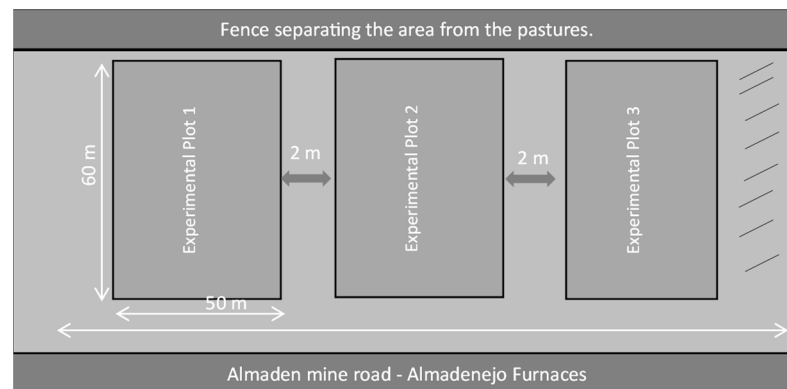


Figure 2. Experimental design to sample the “Fuente del Jardinillo” during the field experiment.

The sampling protocol and analysis are detailed and described by Millán et al., 2006 [11]. In brief, plant species were collected in triplicate with one specimen for each subplot. Similarly, in each subplot, 5 soil samples (2 kg each) were collected by introducing a cylinder with dimensions of 25 cm depth and 20 cm diameter into the soil area below each plant species. A total of 5 points per subplot were sampled, corresponding to a total of 15 samples in the field experiment.

All plant samples correspond to the same season and had been exposed to mercury during the same period to compare mercury uptake and distribution. The plant sampling was performed considering that some of them are biannual and others are pluriannual, and it was necessary to cut the part of the aerial part of the plant that corresponds to the same exposition period. The conceptual map of the analysed samples, both soil and plant samples, is shown in Table 1.

Table 1. Conceptual map of the soil and plant samples to be analysed in this experiment.

Species	Plot	Soil Sample	Plant Sample
<i>C. albidus</i>	1	S_CA1	P_CA1
	2	S_CA2	P_CA2
	3	S_CA3	P_CA3
<i>C. crispus</i>	1	S_CC1	P_CC1
	2	S_CC2	P_CC2
	3	S_CC3	P_CC3
<i>C. ladanifer</i>	1	S_CL1	P_CL1
	2	S_CL2	P_CL2
	3	S_CL3	P_CL3
<i>C. monspeliensis</i>	1	S_CM1	P_CM1
	2	S_CM2	P_CM2
	3	S_CM3	P_CM3
<i>C. salvifolius</i>	1	S_CS1	P_CS1
	2	S_CS2	P_CS2
	3	S_CS3	P_CS3

Once in the laboratory, plant samples were washed with Millipore water in an ultrasonic bath (Ultrasons-H SELECTA), subjecting the shoots and leaves to 4 cycles of 10 min to remove any dust particles or debris that might be adhered to the plant surface [37]. The samples were then left to air dry in paper envelopes and finally crushed.

The soil samples were air-dried, disaggregated, and sieved (<2 mm). In this fraction, pH, cation exchange capacity (CEC) and electrical conductivity (EC) were measured according to [38–40]. The fine soil fraction was oven dried (35 °C) for 48 h and ground with an agate mortar to measure soil organic matter using the Walkley–Black method and mercury concentration.

2.2. Measurement of Total Mercury Concentration in Soil and Plant

The total mercury content in soil and in the root and aerial part of plant species was measured by atomic absorption spectrophotometry, with an Advanced Mercury Analyser 254 LECO analyser (LECO Company, Plzeň, Czech Republic) according to [41]. This equipment has a detection limit of 0.01 ng of Hg. This is an atomic absorption spectrometer designed for the determination of mercury in solid and liquid samples without the need for chemical pre-treatment or pre-concentration of the sample. The operation of the equipment is based on the combustion of the sample in a furnace so that the mercury generated in the gas phase is amalgamated in a gold trap. Additionally, sequential extraction was determined. The sum of these fractions would correspond to what is readily available to plants. Certified reference materials (CRM) were used to determine the accuracy and precision of the measurements and validate the applied methods. These reference materials are BCR-CRM 62 (olive leaves, $0.28 \pm 0.02 \text{ mg kg}^{-1}$ of Hg) and BCR-CRM 281 (ryegrass, $0.0205 \pm 0.0019 \text{ mg kg}^{-1}$ of Hg). The recovery percentage for the ten measurements was $102 \pm 2\%$ and $97.6 \pm 0.5\%$, respectively.

2.3. Measurement of Soluble and Exchangeable Mercury Concentrations

Soil samples were subjected to the first two stages of the sequential extraction procedure developed by CIEMAT specifically to study the distribution of mercury in Almadén soils [42–44]. In this case, since the target is the soil–plant system, the fractions concerned are the soluble and exchangeable fractions that correspond to what is potentially available to the plant. The first stage is the extraction of the mercury soluble in water. For this purpose, 0.5 g of sieved soil sample was mixed in plastic screw-capped centrifuge tubes with 25 mL of Milli Q H₂O. After one hour of orbital shaking at 180 rpm and at room temperature, they were centrifuged for 15 min at 10,000 rpm. The supernatant was then filtered through 0.45 µm pore-size filters. Finally, it was acidified with 0.1 mL of HNO₃ Suprapur[®] stored and refrigerated.

In the second step of the sequential extraction, the exchangeable mercury is determined from the residue obtained in step 1, which was mixed with 20 mL of 1 M ammonium chloride at pH 7.0 for 1 h at room temperature. After 15 min of centrifugation at 10,000 rpm, the supernatant was filtered through 0.45 µm pore-size filters in 25 mL volumetric flasks. The residue was washed with 3 mL of ultrapure H₂O and centrifuged for 10 min at 10,000 rpm. The supernatant was filtered through 0.45 µm pore-size filters and combined with the previous filtrate. It was then acidified with 0.5 mL of HNO₃ Suprapur[®], brought up to 25 mL with ultrapure H₂O, stored and refrigerated.

2.4. Statistical Analysis

IBM SPSS Statistic software version 26.0 was used for statistical analysis. Soil homogeneity and its influence on the percentage of organic matter, cation exchange capacity, electrical conductivity and mercury concentration was assessed by applying a General Linear Model (GLM) followed by a Duncan test, three blocks and five replicates per block. The plant analysis has been made considering the interaction between mercury levels and plant species. Additionally, Person's correlation coefficient was obtained to measure the

strength of association and the direction of the relationship between organic matter content and cation exchange capacity, and electrical conductivity.

Data obtained from plant analysis (mercury concentration in aerial parts and bioaccumulation factors for the different species) were subjected to GLM and followed by a Duncan post hoc test to assess the significance of the differences among species and soil characteristics. Means were separated by using Duncan's test.

Principal component analysis (PCA) was applied for investigating potential relationships among the different soil parameters measured, including total mercury concentration and its value in the different fractions, and *Cistus* species.

3. Results

3.1. Assessment of Plot Homogeneity Assumptions

Soils were chemically characterised to assess the homogeneity of the experimental subplots. Table 2 shows the results of pH, organic matter content (OM), cation exchange capacity (CEC) and electrical conductivity (EC) for each soil sampled under each plant species measured in the experimental plots.

Table 2. Physicochemical characterisation of the soil in each for each subplot of the field experiment. Different lowercase means significant differences among subplots (Duncan's test, $p < 0.05$, mean \pm SE, $n = 5$). * Not applicable.

Soil Subplot	pH	OM (%)	CEC (cmol+/kg)	EC (μ S/cm)
SC_1	6.34 \pm 0.98 ^a	2.12 \pm 0.62 ^a	9.73 \pm 1.17 ^a	158 \pm 18 ^a
SC_2	6.26 \pm 0.08 ^a	2.66 \pm 0.65 ^a	10.08 \pm 2.01 ^a	158 \pm 2 ^a
SC_3	6.60 \pm 0.23 ^a	1.10 \pm 0.78 ^a	5.94 \pm 1.12 ^a	102 \pm 8 ^b
ANOVA				
F	1.39	2.036	2.352	3.754
Sig.	0.286	0.173	0.137	0.054
	n.s.	n.s.	n.s.	*

In view of the results, it can be stated that the pH is within the neutral range [36] and homogeneous throughout the plot. Therefore, this parameter is not expected to influence the plant species growing in the study area, as the differences between subplots are not statistically significant. Considering the extent of the field experiment, as can be seen in Figure 2, the pH data are very homogeneous, as can be seen in Table 2, which shows a significance of 0.286 for the ANOVA test. In general, soil pH can significantly affect vegetation growth and modify the availability of some essential elements for the plant. This detrimental effect is evident in soils with extreme acidity or alkalinity, where available nutrients can be drastically reduced. The soil used in this experiment ensures its suitability to support plant life by being close to neutral. As the soil is closed to neutrality and is also in oxidising conditions (well-oxygenated soils), mercury is mainly found in its mineral form of cinnabar (HgS), which is not bioavailable to plant species [45].

Organic matter is a parameter of great importance because mercury is retained mainly by organic matter due to its high affinity for sulfhydryl (-SH), carboxyl (-COOH) and hydroxyl (-OH) groups in their deprotonated form [14]. The results are also detailed in Table 2.

To corroborate the homogeneity of the "Fuente del Jardinillo" to the soil organic matter content in the field experiment, the data per plot were subjected to a statistical analysis of variance followed by Duncan's test. There are no significant differences between the three subplots as shown by the F-value, thus confirming the homogeneity of the area used in the field experiment. Consequently, it can be indicated that the field experiment has similar percentages of organic matter.

Cation exchange capacity (CEC) is a soil's ability to retain and release positive ions in accordance with its clay and organic matter content. The results of the cation exchange capacity measurement in this experiment are also shown in Table 2. The soil of this experiment is known to have a loamy texture (clay content 18.13%; silt 29.04%; sand 52.83%) so the CEC is mainly determined by the organic matter content. Therefore, these two variables (% OM-CEC) were subjected to a bivariate correlational analysis to find out whether the two variables were related to each other and, if so, to determine the direction of the relationship. The result of the analysis shows that OM (%)-CEC are 92% correlated (Pearson correlation, 0.920 bilateral significance 0.000). This implies that as the organic matter content of the soil increases, so does its cation exchange capacity. In any case, the statistical study confirms that the CEC remained homogeneous in the entire plot used in the field trial (Duncan's test, Table 2) and that the values are within the range for loam soils at pH > 6.0. (5–10 cmol +/kg). This parameter is homogeneous throughout the plot used in the field trial, so no differences in Hg sorption capacity are expected. Consequently, the absorption of Hg by the plant will not be affected, nor will the distribution of Hg in the soil-plant system be altered.

Another parameter evaluated to assess soil homogeneity is electrical conductivity (EC), which is an indicator of the total concentration of salts in the soil solution and provides information on the soil salinity. The main cations present in the soil are Ca^{2+} , Mg^{2+} , Na^{+} and K^{+} , and the main anions present in the soil are HCO_3^{2-} , Cl^{-} , SO_4^{2-} , CO_3^{2-} and NO_3^{-} . The interest of the parameter lies in the fact that the salinity of soil is decisive for the development of plant life. In view of the results (Table 2), it can be stated that these soils have a salinity within the normal range (0–200 $\mu\text{S}/\text{cm}$), which does not affect the vegetative development of the species in the study area [46]. A high percentage of organic matter in the soil increases its electrical conductivity, as shown by the high correlation between the parameters OM and EC, which correlate by 78% (Pearson correlation, 0.779 bilateral significance 0.001). The application of ANOVA to the data showed that there are significant differences in the EC values for the three subplots (Duncan's test, Table 2). However, the values are below 200 $\mu\text{S}/\text{cm}$ and are not expected to have any influence on the cultivated plant or to drive one species to another [47].

3.2. Assessment of Mercury Concentration in Soil

The total concentration of mercury in the soil samples is given in Table 3. Despite being one of the largest mining operations in the world, the total mercury concentration in the plot used in this work is low compared to the values observed elsewhere in Almadén [11,25]. It should be noted that the mean mercury concentration for the “Fuente del Jardinillo” ($7.5 \pm 1.8 \text{ mg}/\text{kg}$) is similar to the values found in previous work [11]. Again, the plot used as a field experiment is homogeneous in terms of total mercury concentration in the soil, as shown by the ANOVA tests (Table 3).

Table 3. Mercury concentration (total and fractionated) in soil samples in the selected sub-zones of the field experiment. Different lowercase means significant differences among subplots (Duncan's test, $p < 0.05$, mean \pm SE, $n = 5$).

Soil Subplot	Hg Total Soil (mg/kg)	Hg Soluble ($\mu\text{g}/\text{kg}$)	Hg Exchangeable ($\mu\text{g}/\text{kg}$)	Hg Available ($\mu\text{g}/\text{kg}$)
SC_1	7.07 ± 3.91^a	5.68 ± 1.71^a	14.4 ± 6.5^a	22.0 ± 7.2^a
SC_2	4.56 ± 1.13^a	5.67 ± 2.43^a	13.8 ± 5.7^a	19.6 ± 5.5^a
SC_3	10.8 ± 6.2^a	14.9 ± 7.8^a	16.8 ± 8.4^a	31.0 ± 11.5^a
ANOVA				
F	0.532	1.196	0.053	0.497
Sig.	0.600	0.336	0.949	0.620
	n.s.	n.s.	n.s.	n.s.

The concentration of mercury in the soluble, exchangeable and potentially available fractions are also given in Table 3. The mercury associated with the soluble fraction and the interchangeable fractions is very low compared to the values obtained for the total concentration of mercury in the soil. The data agree with the pH values measured on the plots, which are in a range where mercury is poorly soluble and mostly stabilised in the soil. Consequently, the soluble Hg/total Hg ratio in the plot will be very small, as will the exchangeable Hg/total Hg ratio.

Furthermore, it was found that the mercury concentration in soil is, to some extent, correlated with the mercury potentially available to plants. The fact that much of the mercury is in the form of cinnabar, an insoluble form of mercury, makes the correlation less than perfect.

3.3. Assessment of Mercury Concentration in *Cistus* Plants

In this work, an intragenus study was conducted to assess the mercury behaviour of *Cistus albidus*, *C. crispus*, *C. ladanifer*, *C. monspeliensis* and *C. salviifolius* and to determine if there were any differences between the species. The statistical study indicated that there was no plot effect on total Hg concentration and the samples, so the soil was found to be homogeneous. However, data were subjected to a second statistical treatment to check whether there was an influence of the plant on the level of mercury in the soil where plants are grown. Figures 3 and 4 show the average concentration of total and available mercury measured for each rockrose species evaluated.

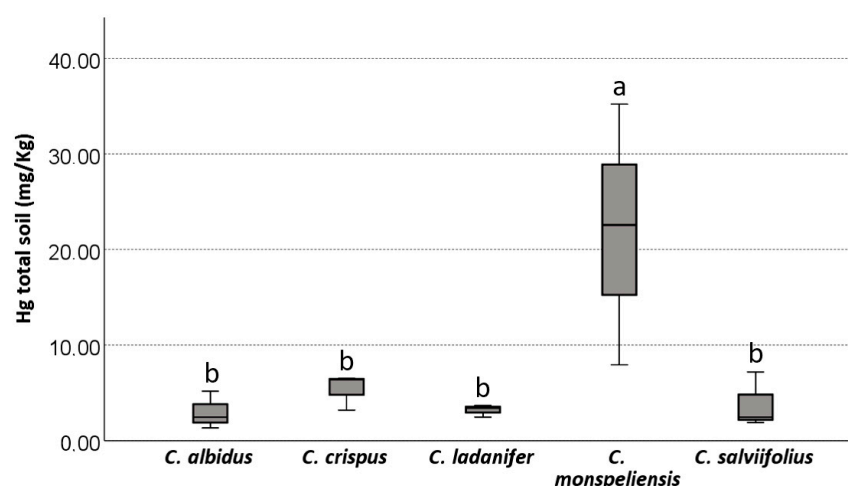


Figure 3. Total mercury concentration (mg/Kg) in soil samples from different *Cistus* sampled in “Fuente del Jardinillo” (Almadén, Spain). Distinct lowercase characters indicate statistically significant differences among species (Duncan’s test, $p < 0.05$, mean \pm SE, $n = 9$).

In the soil samples obtained under *Cistus monspeliensis*, these values increased significantly to total Hg concentration in the soils sampled for the other species studied. When compared to the fraction of available mercury, the differences are reduced for *Cistus monspeliensis* and *C. ladanifer*.

Figure 5 shows the total mercury concentration extracted by the plants. *Cistus albidus* showed the lowest values, and *C. monspeliensis* reached the highest, being significantly different from each other. The remaining species, *Cistus crispus*, *C. ladanifer* and *C. salviifolius* presented similar values without significant differences with *C. albidus* or *C. monspeliensis*.

To assess the mercury removal capacity of soils, the mercury concentration of each of the five species was measured on the shoots. In addition, the bioaccumulation factor was determined as the ratio of the total mercury concentration in the aerial part to the total concentration in the soil, which has been denoted as “BACtotal”. (Figure 6). Also, this factor was calculated as the coefficient of the total mercury in the aerial part and the concentration

in the available fraction (which is shown as the sum of Hg soluble and exchangeable in Table 2), which will be referred to as BAC available (Figure 7).

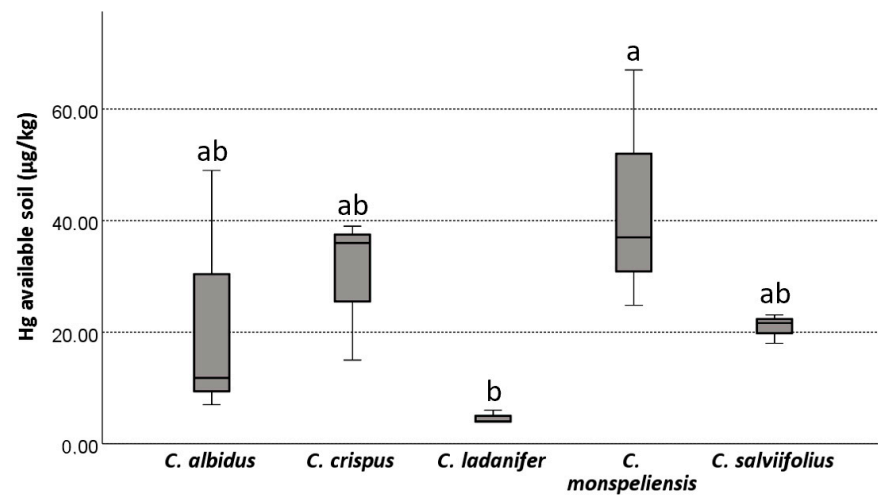


Figure 4. Available mercury concentration (mg/Kg) in soil samples from different *Cistus* sampled in “Fuente del Jardinillo” (Almadén, Spain). Distinct lowercase characters indicate statistically significant differences among species (Duncan’s test, $p < 0.05$, mean \pm SE, $n = 9$).

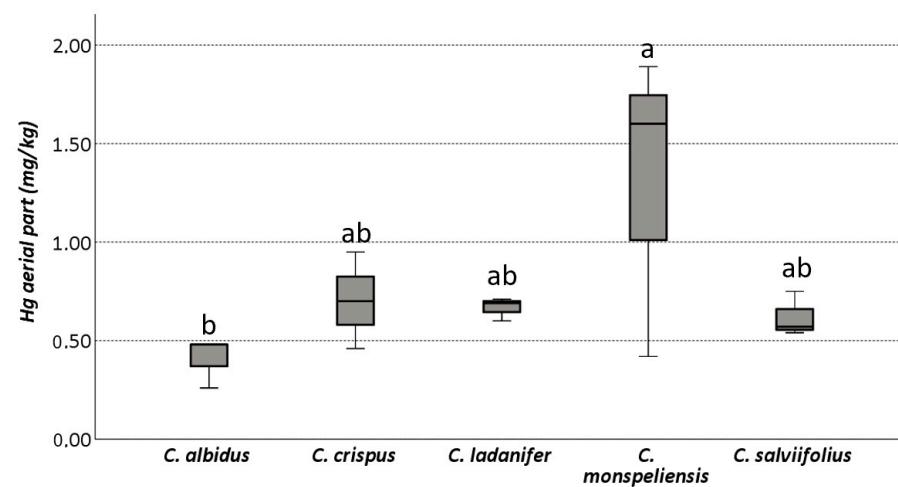


Figure 5. Mercury concentration (mg/Kg) in the aerial part of different *Cistus* sampled in “Fuente del Jardinillo” (Almadén, Spain). Distinct lowercase characters indicate statistically significant differences among species (Duncan’s test, $p < 0.05$, mean \pm SE, $n = 9$).

The effectiveness of phytoremediation can be evaluated using BAC total and BAC available as indicators of mercury mobilisation by the plants because they relate the mercury concentration (total or available) from the soil to the plant [39]. Both are represented in Figures 6 and 7. *Cistus ladanifer* reached the highest values in both parameters. The value is extremely high for the bioaccumulation factor related to available mercury (BAC_{avai}), and the differences were even statistically significant according to Duncan’s tests. However, the trend is different depending on whether BAC_{total} or BAC_{avai} is compared with the species. In the case of BAC available values, *Cistus ladanifer* shows significant differences with all the subgenera with the lowest value for *C. crispus*, but no significant differences among *C. albidus* > *C. monspeliensis* > *C. salviifolius*. In case of T-BAC, *C. ladanifer* > *C. salviifolius* > *C. albidus* > *C. crispus*, and the differences are only significant with *C. monspeliensis*, which showed the lowest values.

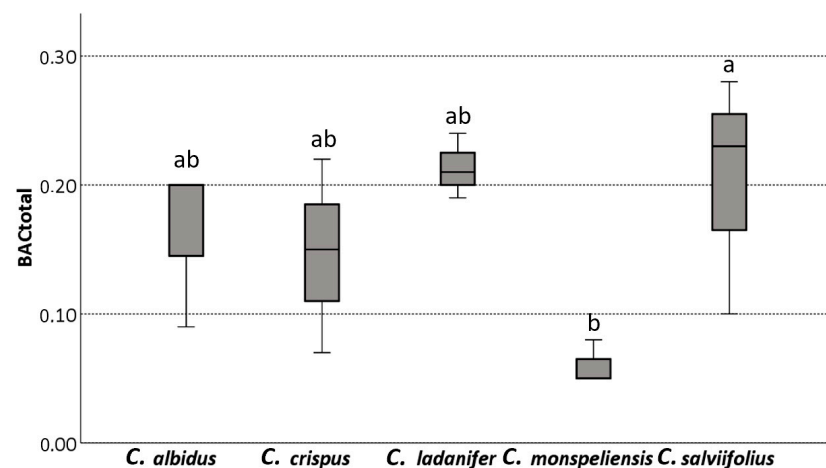


Figure 6. BAC related to total mercury concentration in soil for the different *Cistus* sampled in “Fuente del Jardinillo” (Almadén, Spain). Distinct lowercase characters indicate statistically significant differences among species (Duncan’s test, $p < 0.05$, mean \pm SE, $n = 9$).

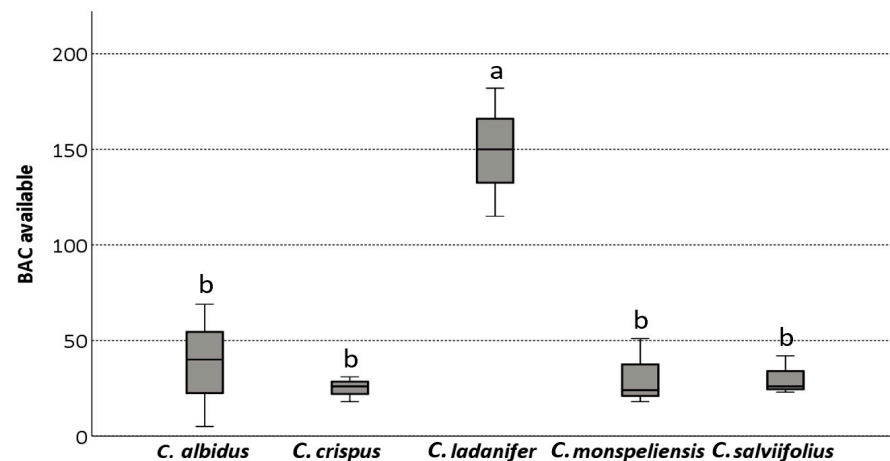


Figure 7. BAC related to available mercury concentration for the different *Cistus* sampled in “Fuente del Jardinillo” (Almadén, Spain). Distinct lowercase characters indicate statistically significant differences among species (Duncan’s test, $p < 0.05$, mean \pm SE, $n = 9$).

3.4. Principal Component Analysis

The analysis of the PCA (Figure 8) led to a reduction in the initial dimension of the dataset of two components, which explains 68.54% of the data variation (PC1 41.90%; and PC2 26.64% of the variance). The first component includes pH (92.7%), Hg soil total (93.2%), Hg soluble (85.5%), Hg available (74.6%), Hg aerial part (74.1%) and BAC related to total Hg (65.5%). The second component is associated with OM (75.8%), CEC (CIC) (75.5%), EC (CE) (86.2%), Hg interchangeable (51.3%) and BAC related to available Hg (65.5%). The PC1 value indicates that pH negatively affects the concentration of Hg in both the soil and aerial part, while the organic matter, CEC and EC of soil positively affect the concentration of Hg.

Through PCA analysis, it was also possible to obtain a clear separation of the studied species, as shown in the PCA ordination diagram (Figure 9).

The PC1 and PCA2 (Figure 9) values have been very effective in separating *Cistus monspeliensis* from the other three species. It is positively correlated to mercury concentration and negatively to soil characteristics. Similarly, *Cistus ladanifer* with high values of BCA_{tot} and BCA_{avai} are clearly grouped on the same side as BCA but negatively correlated to mercury concentration and soil characteristics. Finally, *Cistus albidus*, *C. salviifolius* and *C. crispus* are scattered along PC1 and PC2.

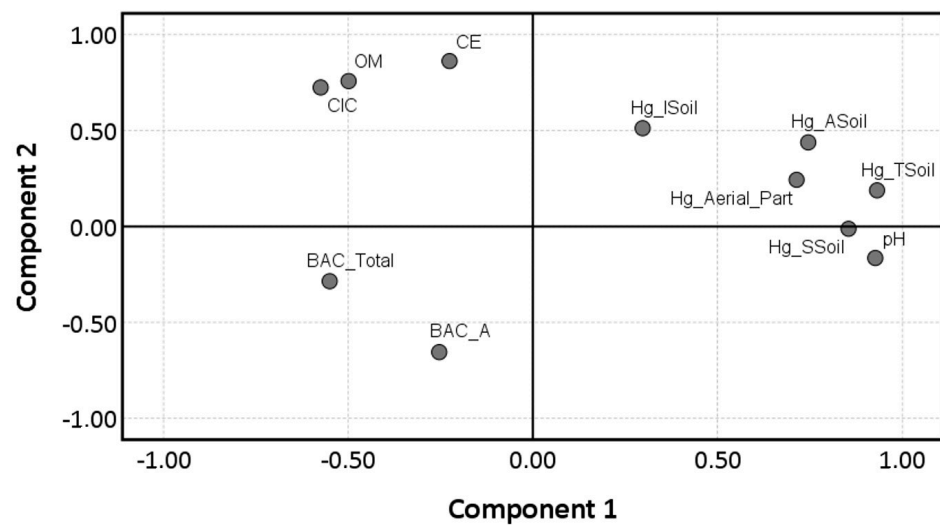


Figure 8. Principal component analysis (PCA) plot displaying the soil samples (points) spatially ordered in terms of their physicochemical characteristics.

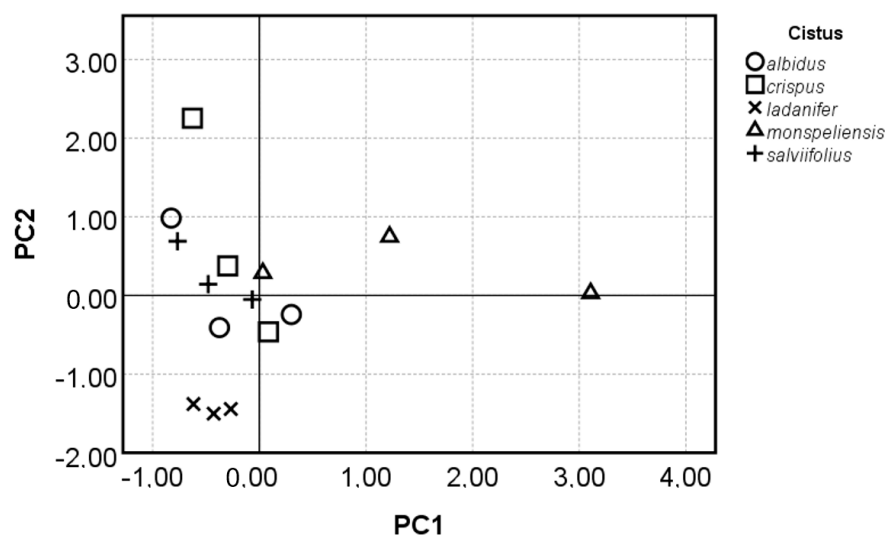


Figure 9. PCA ordination diagram showing the spatial arrangement of samples regarding the mercury concentrations in the aerial part, BAC, BAC available, genus, and soil characteristics.

4. Discussion

The aim of this work was to assess the potential of the five plant species, *Cistus albidus*, *C. crispus*, *C. ladanifer*, *C. monspeliensis* and *C. salviifolius*, that grow wild in “Fuente de Jardinillo” (Almaden) to be used in phytotechnologies. Previous studies assumed that the fraction of Hg potentially available to plants in the sampling area corresponded to soluble and easily exchangeable mercury [35]. The soluble mercury concentration obtained in the soil was 0.04 mg/kg, and the exchangeable mercury concentration in the soil was 0.29 mg/kg. Likewise, the analysis of mercury was carried out in the aerial part of the five plant species of the genus *Cistus* native to the area: *C. albidus* (1.29 ± 0.10 mg/kg); *C. crispus* (1.18 ± 0.12 mg/kg); *C. ladanifer* (0.79 ± 0.02 mg/kg); *C. monspeliensis* (0.87 ± 0.07 mg/kg) and *C. salviifolius* (0.52 ± 0.03 mg/kg) [11]. The differences found in the concentration of mercury in the aerial part of the different *Cistus* species suggest comparing the relationships between Hg uptake and gender, especially when these plants grow in the same area and under similar abiotic conditions.

Prior to plant sampling, the experimental field was characterised to select areas where the soil properties were the same or at least statistically minimally different. The plot was

divided into three zones where it was also possible to find at least three plants of each species to have replicates for robust analysis. After analysing the soil physicochemical parameters (pH, MO, CEC and EC), as well as the content of the different mercury fractions (total and potentially available) in the soil, it was concluded that the “Fuente del Jardinillo” plot can be considered homogeneous to achieve the objectives of this work. This implies that the five species of *Cistus* sampled in this study have grown under the same abiotic conditions. Consequently, they can be analysed and compared following the same criteria, as they are considered as having grown in a single plot.

The genus *Cistus* is well-adapted to extreme environments and can grow naturally in degraded environments without symptoms of toxicity [48]. The plants sampled in this work did not show toxicity symptoms in the leaves and the mercury concentration was like that found in a previous work [11], about 0.6–1.5 mg/kg DW, which points to a high concentration of this element. These data also agree with previous results, in which the concentration of total mercury (7.47 ± 2.47 mg/kg) is high compared to the values obtained for the different mercury fractions in the soil as available (24.2 ± 4.89 µg/kg), exchangeable (15.0 ± 3.84 µg/kg) or soluble (8.73 ± 2.93 µg/kg). Most of the total mercury is found as a part of cinnabar (HgS), a form that is neither very soluble nor bioavailable to plants, so the percentage of bioassimilable mercury, the key fraction for the present study, is very small. Consequently, the fraction of Hg potentially available for the plant species studied is also very small in relation to the values obtained for total mercury concentration in the soil.

It is noteworthy that although the plot was homogeneous in terms of mercury concentration for both the soil and its fractions, the soil samples where *Cistus monspeliensis* had grown reached the highest values. In this case, the concentration of mercury is above the soil mean, although only the value of total mercury is significant with respect to the other species. This may indicate that either *C. monspeliensis* can grow in areas where the metal concentration is higher, or that the plant is able to concentrate the metal in its root zone without transferring it to the aerial part. Almadén vegetation has been described as following four distinct patterns of Hg uptake [49]. Accordingly, *C. monspeliensis* has a complex behaviour, in which there is no relationship between Hg plant and Hg soil [50]. Uptake occurs but in unexpected ways. This tendency has been corroborated by the PCA included in this work.

The mercury concentration found by [49] in the aerial part of *Cistus monspeliensis* is similar to that presented in this experiment. In any case, *C. monspeliensis* is strongly recommended as a potential species for the reclamation of multi-element-polluted soils under Mediterranean conditions due to its high tolerance and adaptability [51]. The concentration of metals in shoots was below toxicity limits for domestic animals without any sign of phytotoxicity symptoms. Furthermore, the biological accumulation coefficient is not expected to reach hyperaccumulator values [52]. However, it is possible to consider this species as a phytoextractive plant if the coefficient of biological accumulation is related to the concentration of mercury in the available fraction since, in this case, the values reached for this parameter are higher than 1.

Cistus ladanifer is commonly found in metalliferous soils in the Iberian Peninsula and North Africa and exhibits a high potential to be used as a valuable tool in Mediterranean areas of low agronomic interest [53]. *C. ladanifer* bioextracts are known to have a potential role in the phytoremediation of trace-element-contaminated soils by phytoextraction or by phytostabilisation, which explains the fact that this plant is widely found in soils rich in metals [54]. Most studies concentrate on six significant trace elements, namely Cu, As, Mn, Pb, Zn and Ni. *Cistus ladanifer* (bioaccumulation factors < 1) has been reported to serve as an excluder for Cu, As, Pb and Ni. Consequently, these plants meet the requirements for phytostabilisation; however, it is not recommended to use these areas for grazing because the concentrations of these elements in the soil are all above the reference threshold, which is relevant because animals also consume the soil together with the grass [55]. Previously, the use of *C. ladanifer* has been also proposed in phytoremediation technologies such as the phytoextraction of Cr, Mn and Zn [56]. The addition of amendments, such as biochar, leads

to enhanced metal uptake in plant tissues without the reduction in biomass [57]. Resistance may be related to the balance between the generation of reactive oxygen species and their detoxification by CAT, POD and SOD, promoting the soluble and cell-wall-bound forms of these enzymes. This process may represent in *C. ladanifer* plants a form of toxic elements tolerance [58].

Hg uptake has been also evaluated in the Almadén area [49,50]. Following the criteria, the authors included *Cistus ladanifer* in Type 2 plants, which means that after an initial linear uptake, there is no increase in Hg in the plant. The concentration of mercury was similar that found in this experiment. Hence, *C. ladanifer* is the species that showed the highest phytoremediation potential by reaching the highest mean values of bioaccumulation factor in relation to available mercury (150 ± 24). This species also had the highest biomass compared to the other subgenera studied. In addition, the PCA showed a clear and independent clustering from the other species.

Cistus salviifolius is a shrub that can adapt to a wide range of environmental stresses [59]. According to [60], *C. salviifolius* is considered suitable for the phytostabilisation of mining waste in areas with semiarid characteristics. *C. salviifolius* translocated nutrients (Cu, Mn and Zn) to the aerial parts and stored mainly the phytotoxic elements (As, Pb and Sb) in roots. This species has also been identified in the Almadén district by [49] and [11] as siliceous scrub, as have *C. monspeliensis* and *C. crispus*. According to [49], *C. salviifolius* does not show a clear pattern for mercury unlike *C. crispus*, which follows a clear pattern. Initially, *C. crispus* does not show an increase in Hg in the plant until a threshold is exceeded; above this threshold, there is a linear relationship between Hg in the plant and Hg in the soil.

Cistus albidus has been suggested to be used in phytoremediation for phytostabilisation in pyritic mine soils [61] and can tolerate high concentrations of metals but without a bioaccumulation capacity [62]. In the case of mercury, *C. albidus* grows well in Almadén, especially in Fuente del Jaramillo and was able to accumulate $1.29 \pm 0.10 \text{ mg kg}^{-1}$ in its aerial part [11]. This pattern is not confirmed in this experiment.

For the use of plants in phytotechnologies, it would be interesting to determine how much mercury each plant could accumulate in its aerial part per Ha, in order to evaluate its phytoextractive capacity and the possibility of recovering mercury-contaminated soils. If 1 kg of adult shrubby plants, such as *Cistus albidus*, *C. ladanifer* and *C. monspeliensis*, are considered, by knowing the concentration of Hg they accumulate in the aerial part, the amount of mercury retained by each species per kilo of a plant used can be determined. *C. ladanifer* (0.40 mg/kg) and *C. monspeliensis* (0.60 mg/kg) have a similar phytoextractive capacity and are different to *Cistus albidus* (0.21 mg/kg). These values are hypothetical and calculated for very specific conditions. It is also important to note that the amount of phytoextracted mercury depends mainly on the concentration of this pollutant available in the soil, so action should be taken, as much as possible, to control and adapt the soil environment to allow for suitable conditions and ensure that mercury is readily available in the soil for the plant.

5. Conclusions

The district of Almadén (Ciudad Real) is an exceptional case of mercury concentration because it is an area affected by the mining of this heavy metal for thousands of years. This work has determined that the mean value of the total mercury concentration for the “Fuente del Jardinillo” plot is $7.5 \pm 1.8 \text{ mg/kg}$, a value in line with those reported by other authors. [11] ($5.0 \pm 0.4 \text{ mg/kg}$). Most of the total mercury is found as a part of cinnabar (HgS), a form that is neither soluble nor bioavailable to plants, and the percentage of bioassimilable mercury is very small. Consequently, the fraction of Hg potentially available to the plant species studied was found to be very small ($3.9\text{--}66 \text{ }\mu\text{g/kg}$) in relation to the values obtained for the total mercury content in the soil ($1.3\text{--}35 \text{ mg/kg}$). The plot under study can be considered unique and homogeneous, and therefore the five species of the genus *Cistus* under study have developed under the same abiotic conditions.

The species *Cistus ladanifer* and *C. monspeliensis* show a tendency to accumulate mercury in the aerial part, so they could be considered phytoextractive species. On the other hand, the species *Cistus crispus*, *C. albidus* and *C. salviifolius* have good perspectives to be used as phytostabilisers.

From the results obtained, the species with the greatest phytoremediation potential is *Cistus ladanifer*, with the highest mean values of bioaccumulation factor (85.5). Moreover, as the species with the greatest potential size, it has a greater biomass than the other two species, which means that its use can be optimised by minimising the number of specimens to be planted.

This study has been carried out with a view of the future application of phytoremediation techniques in areas affected by mercury contamination other than Almadén. Therefore, it is necessary to stress that due to the high natural mercury background, as well as anthropogenic mining activities, Almadén is only being used as a test scenario, as it does not make sense to apply phytotechnologies there. A future line of research would be to study the behaviour of the same species when subjected to different soil conditions to estimate whether the plant always behaves in the same way as it did in the plot at “Fuente del Jardinillo”.

Author Contributions: Conceptualisation, R.M. and A.P.-S.; methodology, R.M., M.J.S. and T.S.; software, A.P.-S.; validation A.P.-S., R.M. and G.G.; formal analysis, M.J.S., R.M. and T.S.; investigation, R.M., A.P.-S., M.J.S. and T.S.; resources, A.P.-S., R.M., M.J.S., T.S. and G.G.; data curation, A.P.-S. and R.M.; writing—original draft preparation, A.P.-S. and R.M.; writing—review and editing, A.P.-S., R.M. and G.G.; visualisation, A.P.-S., R.M. and G.G.; supervision, A.P.-S., R.M., M.J.S., T.S. and G.G.; project administration, R.M., A.P.-S. and G.G.; funding acquisition, R.M. and G.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Not applicable.

Acknowledgments: The authors are grateful for the support in infrastructure and technical assistance from CIEMAT (Spain). The authors also thank Sandra Carrasco-Gil for her assistance during the mercury analysis. An intensive revision of the manuscript has been carried out by a native English speaker.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Hernández, A.; Jébrak, M.; Higuera, P.; Oyarzun, R.; Morata, D.; Munhá, J. The Almadén mercury mining district, Spain. *Miner. Depos.* **1999**, *34*, 539–548. [\[CrossRef\]](#)
2. Barquero, J.I.; Lorenzo, S.; Esbrí, J.M.; Rivera, S.; González-Valoys, A.C.; García-Ordiales, E.; Higuera, P. Geochemical Assessment of Mineral Resource Potential in a Hg-Sb-Pb-Zn Mining Area: The Almadén and Guadalmez Synclines (South-Central Spain). *Appl. Sci.* **2022**, *12*, 11351. [\[CrossRef\]](#)
3. Elmayel, I.; Esbrí, J.M.; Efrén, G.-O.; García-Noguero, E.-M.; Elouear, Z.; Jalel, B.; Farieri, A.; Roqueñí, N.; Cienfuegos, P.; Higuera, P. Evolution of the Speciation and Mobility of Pb, Zn and Cd in Relation to Transport Processes in a Mining Environment. *Int. J. Environ. Res. Public Health* **2020**, *17*, 4912. [\[CrossRef\]](#)
4. Gray, J.E.; Pribil, M.J.; Higuera, P.L. Mercury isotope fractionation during ore retorting in the Almadén mining district, Spain. *Chem. Geol.* **2013**, *357*, 150–157. [\[CrossRef\]](#)
5. Bisquert, D.S.; Castejón, J.M.P.; Fernández, G.G. The impact of atmospheric dust deposition and trace elements levels on the villages surrounding the former mining areas in a semi-arid environment (SE Spain). *Atmos. Environ.* **2017**, *152*, 256–269. [\[CrossRef\]](#)
6. Higuera, P.; Esbrí, J.M.; Oyarzun, R.; Llanos, W.; Martínez-Coronado, A.; Lillo, J.; López-Berdones, M.A.; García-Noguero, E.M. Industrial and natural sources of gaseous elemental mercury in the Almadén district (Spain): An updated report on this issue after the ceasing of mining and metallurgical activities in 2003 and major land reclamation works. *Environ. Res.* **2013**, *125*, 197–208. [\[CrossRef\]](#)
7. Ferrara, R.; Maserti, B.E.; Anderson, M.; Edner, H.; Ragnarson, P.; Svanberg, S.; Hernández, A. Atmospheric mercury concentrations and fluxes in the Almadén district (Spain). *Atmos. Environ.* **1998**, *32*, 3897–3904. [\[CrossRef\]](#)
8. Berzas Nevado, J.J.; García Bermejo, L.F.; Rodríguez Martín-Doimeadios, R.C. Distribution of mercury in the aquatic environment at Almadén, Spain. *Environ. Pollut.* **2003**, *122*, 261–271. [\[CrossRef\]](#)

9. Higuera, P.; Oryazum, R.; Biester, H.; Lillo, J.; Lorenzo, S. A first insight into mercury distribution and speciation in soils from the Almadén mining district, Spain. *J. Geochem. Explor.* **2003**, *80*, 95–104. [\[CrossRef\]](#)
10. Gray, J.E.; Hines, M.E.; Higuera, P.L.; Adatto, I.; Lasorsa, B.K. Mercury speciation and microbial transformations in mine wastes, stream sediments and surface waters at the Almadén mining district, Spain. *Environ. Sci. Technol.* **2004**, *38*, 4285–4292. [\[CrossRef\]](#)
11. Millán, R.; Gamarra, R.; Schmid, T.; Sierra, M.; Quejido, A.; Sánchez, D.; Cardona, A.; Fernández, M.; Vera, R. Mercury content in vegetation and soils of the Almadén mining area (Spain). *Sci. Total. Environ.* **2006**, *368*, 79–87. [\[CrossRef\]](#)
12. Garcia-Ordiales, E.; Higuera, P.; Esbrí, J.M.; Roqueñí, N.; Loredó, J. Seasonal and spatial distribution of mercury in stream sediments from Almadén mining district. *Geochem. Explor. Environ. Anal.* **2018**, *19*, 121–128. [\[CrossRef\]](#)
13. Lominchar, M.A.; Sierra, M.J.; Jiménez-Moreno, M.; Guirado, M.; Rodríguez Martín-Doimeadios, R.M.; Millán, R. Mercury species accumulation and distribution in *Typha domingensis* under real field conditions (Almadén, Spain). *Environ. Sci. Pollut. Res.* **2019**, *26*, 3138–3144. [\[CrossRef\]](#)
14. Desauziers, V.; Castre, N.; Le Cloirec, P. Sorption of methylmercury by clays and mineral oxides. *Environ. Technol.* **1997**, *18*, 1009–1018. [\[CrossRef\]](#)
15. Reis, A.T.; Lopes, C.B.; Davidson, C.M.; Duarte, A.C.; Pereira, E. Extraction of available and labile fractions of mercury from contaminated soils: The role of operational parameters. *Geoderma* **2015**, *259–260*, 213–223. [\[CrossRef\]](#)
16. Rodríguez, L.; Alonso-Azcárate, J.; Gómez, R.; Rodríguez-Castellanos, L. Comparison of extractants used for the assessment of mercury availability in a soil from the Almadén mining district (Spain). *Environ. Sci. Pollut. Res.* **2017**, *24*, 12963–12970. [\[CrossRef\]](#)
17. Skyllberg, U.; Bloom, P.R.; Qian, J.; Lin, C.-M.; Blean, W.F. Complexation of Mercury(II) in Soil Organic Matter: EXAFS Evidence for Linear Two-Coordination with Reduced Sulfur Groups. *Environ. Sci. Technol.* **2006**, *40*, 4174–4180. [\[CrossRef\]](#)
18. Schuster, E. The behavior of mercury in the soil with special emphasis on complexation and adsorption processes—A review of the literature. *Water Air Soil Pollut.* **1991**, *56*, 667–680. [\[CrossRef\]](#)
19. Behra, P. Migration or retention of mercury II salts when percolating through a porous medium constituted of a natural quartz sand? *Environ. Contam.* **1986**, *2*, 318–320.
20. Huang, J.-H.; Shetaya, W.H.; Osterwalder, S. Determination of (Bio)-available mercury in soils: A review. *Environ. Pollut.* **2020**, *263*, 114323. [\[CrossRef\]](#)
21. Moreno-Jiménez, E.; Gamarra, R.G.; Carpena-Ruiz, R.O.; Millán, R.; Peñalosa, J.; Esteban, E. Mercury bioaccumulation and phytotoxicity in two wild plant species of Almadén area. *Chemosphere* **2006**, *63*, 1969–1973. [\[CrossRef\]](#)
22. Lindberg, S.E.; Jackson, D.R.; Huckabee, J.W.; Jansen, S.A.; Levin, M.J.; Lund, J.R. Atmospheric Emission and Plant Uptake of Mercury from Agricultural Soils near the Almadén Mercury Mine. *J. Environ. Qual.* **1979**, *8*, 572–578. [\[CrossRef\]](#)
23. Millán, R.; Gamarra, R.; Vera, R.; Schmid, T. Mercury uptake for plant species from an Almadén test plot. In *Proceedings of the 7th International Conference on the Biogeochemistry of Trace Elements*; Gobran, G.R., Lepp, L., Eds.; Swedish University of Agricultural Sciences: Uppsala, Sweden, 2003; Volume 4, pp. 15–19.
24. Millán, R.; Gamarra, R.; Schmid, T.; Vera, R.; Sierra, M.J.; Quejido, A.J. Mercury content in natural vegetation of three plots in the mining area of Almadén (Spain). *Mater. Geoenviron.* **2004**, *51*, 155–158.
25. Millán, R.; Schmid, T.; Sierra, M.J.; Carrasco-Gil, S.; Villadóniga, M.; Rico, C.; Ledesma, D.M.S.; Puente, F.J.D. Spatial variation of biological and pedological properties in an area affected by a metallurgical mercury plant: Almadenejos (Spain). *Appl. Geochem.* **2011**, *26*, 174–181. [\[CrossRef\]](#)
26. Hildebrand, S.G.; Huckabee, J.W.; Sanz Díaz, F.; Jansen, S.A.; Solomon, J.A.; Kumar, K.D. *Distribution of Mercury in the Environment of Almadén, Spain (ORNL/TM-7446)*; Oak Ridge National Laboratory: Oak Ridge, TN, USA, 1980; 87p.
27. Higuera, P.; Molina, J.A.; Oyarzún, R.; Lillo, J.; Esbrí, J.M. Identification of the plant-communities and hyperaccumulators in mercury contaminated sectors of the Almadén district Spain. *Mater. Geoenviron.* **2004**, *51*, 103–107.
28. Sierra, M.J.; Rodríguez-Alonso, J.; Millán, R. Impact of the lavender rhizosphere on the mercury uptake in field conditions. *Chemosphere* **2012**, *89*, 1457–1466. [\[CrossRef\]](#)
29. Millán, R.; Lominchar, M.A.; López-Tejedor, I.; Rodríguez-Alonso, J.; Schmid, T.; Sierra, M.J. Behaviour of mercury in soils from the Valdeazogues riverbank and transfer to *Nerium oleander* L. *J. Geochem. Explor.* **2012**, *123*, 136–142. [\[CrossRef\]](#)
30. Millán, R.; Lominchar, M.A.; Rodríguez-Alonso, J.; Schmid, T.; Sierra, M.J. Riparian vegetation role in mercury uptake (Valdeazogues River, Almadén, Spain). *J. Geochem. Expl.* **2014**, *140*, 104–110. [\[CrossRef\]](#)
31. Lominchar, M.; Sierra, M.; Millán, R. Accumulation of mercury in *Typha domingensis* under field conditions. *Chemosphere* **2015**, *119*, 994–999. [\[CrossRef\]](#)
32. Golia, E.; Angelaki, A.; Giannoulis, K.; Skoufogianni, E.; Bartzialis, D.; Cavalaris, C.; Vleioras, S. Evaluation of soil properties, irrigation and solid waste application levels on Cu and Zn uptake by industrial hemp. *Agron. Res.* **2021**, *19*, 92–99. [\[CrossRef\]](#)
33. Yan, A.; Wang, Y.; Tan, S.N.; Yusof, M.L.M.; Ghosh, S.; Chen, Z. Phytoremediation: A Promising Approach for Revegetation of Heavy Metal-Polluted Land. *Front. Plant Sci.* **2020**, *11*, 359. [\[CrossRef\]](#)
34. Gerhardt, K.E.; Gerwing, P.D.; Greenberg, B.M. Opinion: Taking phytoremediation from proven technology to accepted practice. *Plant Sci.* **2017**, *256*, 170–185. [\[CrossRef\]](#)
35. Millán, R.; Carpena, R.O.; Schmid, T.; Sierra, M.J.; Moreno, E.; Peñalosa, J.; Gamarra, R.; Esteban, E. Rehabilitación de suelos contaminados con mercurio: Estrategias aplicables en el área de Almadén. *Ecosistemas* **2007**, *16*, 56–66.
36. Rivas Martínez, S.; Loidi Arregui, J. Biogeography of the Iberian Peninsula. *Itinera Geobot.* **1999**, *13*, 49–67.

37. Rodríguez-Alonso, J.; Cabrales-García, C.; Millán, R. Factors influencing the cleaning of plant samples with ultrasonic technology. *Int. J. Phytoremediation* **2013**, *25*, 359–367. [\[CrossRef\]](#)
38. ISO 10390:2021; Soil, Treated Biowaste and Sludge—Determination of pH. Available online: <https://tienda.aenor.com/norma-iso-10390-2021-075243> (accessed on 30 July 2023).
39. EPA 9081 SW-846; Test Method 9081: Cation-Exchange Capacity of Soils (Sodium Acetate). Available online: <https://www.epa.gov/hw-sw846/sw-846-test-method-9081-cation-exchange-capacity-soils-sodium-acetate> (accessed on 30 July 2023).
40. ISO 11265:1994; Soil Quality—Determination of the Specific Electrical Conductivity. Available online: <https://www.iso.org/standard/19243.html> (accessed on 30 July 2023).
41. Sierra, M.; Millán, R.; Esteban, E. Mercury uptake and distribution in *Lavandula stoechas* plants grown in soil from Almadén mining district (Spain). *Food Chem. Toxicol.* **2009**, *47*, 2761–2767. [\[CrossRef\]](#)
42. Quejido, A.J.; Sánchez, D.M.; Fernández, M.; Millán, R.; Vera, R.; Schmid, T. Determination Of solid-phase associations of mercury in contaminated soils from Almadén area. In Proceedings of the CSI XXXIII Colloquium Spectroscopicum International, Granada, Spain, 7–12 September 2003; p. 362.
43. Sánchez, D.M.; Quejido, A.J.; Fernández, M.; Hernández, C.; Schmid, T.; Millán, R.; González, M.; Aldea, M.; Martín, R.; Morante, R. Mercury and trace element fractionation in Almadén soils by application of different sequential extraction procedures. *Anal. Bioanal. Chem.* **2005**, *381*, 1507–1513. [\[CrossRef\]](#)
44. Jones, B. *Laboratory Guide for Consulting Soil Test and Plant Analysis*; CRC Press: Boca Raton, FL, USA, 2001; ISBN 0-8493-0206-4.
45. Adriano, D.C. Trace elements in terrestrial environments. Chapter 11. In *Biogeochemistry, Bioavailability and Risks of Metals Mercury*; Springer: Berlin/Heidelberg, Germany, 2001; pp. 411–458.
46. González-Alcaraz, M.; Jiménez-Cárceles, F.; Álvarez, Y.; Álvarez-Rogel, J. Gradients of soil salinity and moisture, and plant distribution, in a Mediterranean semiarid saline watershed: A model of soil–plant relationships for contributing to the management. *Catena* **2014**, *115*, 150–158. [\[CrossRef\]](#)
47. Porta, J.; López-Acebedo, M.; Roquero, C. *Edafología Para la Agricultura y el Medio Ambiente. Capítulo 20: Salinización y Sodificación: Suelos de Regadío*; Mundi-Prensa: Madrid, Spain, 1999; p. 657.
48. Carvalho, L.C.; Santos, E.S.; Saraiva, J.A.; Magalhães, M.C.F.; Macías, F.; Abreu, M.M. The Potential of *Cistus salvifolius* L. to Phytostabilize Gossan Mine Wastes Amended with Ash and Organic Residues. *Plants* **2022**, *11*, 588. [\[CrossRef\]](#)
49. Molina, J.A.; Oyarzun, R.; Esbrí, J.M.; Higuera, P. Mercury accumulation in soils and plants in the Almadén mining district, Spain: One of the most contaminated sites on Earth. *Environ. Geochem. Health* **2006**, *28*, 487–498. [\[CrossRef\]](#)
50. Kovalevsky, A.L. *Biogeochemical Exploration for Mineral Deposits*, 2nd ed.; Brooks, R.R., Ed.; Trans. Russia; VNU Science Press: Utrecht, The Netherlands, 1987; Volume 224.
51. Arenas-Lago, D.; Santos, E.S.; Carvalho, L.S.; Abreu, M.M.; Andrade, M.L. *Cistus monspeliensis* L. as a potential species for rehabilitation of soils with multielemental contamination under Mediterranean conditions. *Environ. Sci. Pollut. Res. Int.* **2018**, *25*, 6443–6455. [\[CrossRef\]](#)
52. McGrath, S.P.; Zhao, F.-J. Phytoextraction of metals and metalloids from contaminated soils. *Curr. Opin. Biotechnol.* **2003**, *14*, 277–282. [\[CrossRef\]](#)
53. Raimundo, J.R.; Frazão, D.F.; Domingues, J.L.; Quintela-Sabarís, C.; Dentinho, T.P.; Anjos, O.; Alves, M.; Delgado, F. Neglected Mediterranean plant species are valuable resources: The example of *Cistus ladanifer*. *Planta* **2018**, *248*, 1351–1364. [\[CrossRef\]](#)
54. Frazão, D.F.; Raimundo, J.R.; Domingues, J.L.; Quintela-Sabarís, C.; Gonçalves, J.C.; Delgado, F. *Cistus ladanifer* (Cistaceae): A natural resource in Mediterranean-type ecosystems. *Planta* **2018**, *247*, 289–300. [\[CrossRef\]](#)
55. Batista, M.J.; Gonzalez-Fernandez, O.; Abreu, M.M.; Queralt, I.; Carvalho, M.L. Pioneer Mediterranean Shrub Species Revegetating Soils Developed on Mining Soils/Spoils. *Land Degrad. Dev.* **2016**, *28*, 718–730. [\[CrossRef\]](#)
56. Lázaro, J.D.; Kidd, P.; Martínez, C.M. A phytogeochemical study of the Trás-os-Montes region (NE Portugal): Possible species for plant-based soil remediation technologies. *Sci. Total Environ.* **2006**, *354*, 265–277. [\[CrossRef\]](#)
57. Duarte, B.; Pires, V.; Carreiras, J.; de Carvalho, R.C.; Ferreira, R.; Pereira, M.F.; Maurício, A.M.; Martins-Dias, S.; Caçador, I. *Cistus ladanifer* metal uptake and physiological performance in biochar amended mine soils. *S. Afr. J. Bot.* **2023**, *153*, 246–257. [\[CrossRef\]](#)
58. Santos, E.S.; Abreu, M.M.; Nabais, C.; Saraiva, J.A. Trace elements and activity of antioxidative enzymes in *Cistus ladanifer* L. growing on an abandoned mine area. *Ecotoxicology* **2009**, *18*, 860–868. [\[CrossRef\]](#)
59. Jiménez, M.N.; Bacchetta, G.; Navarro, F.B.; Casti, M.; Fernández-Ondoño, E. Native Plant Capacity for Gentle Remediation in Heavily Polluted Mines. *Appl. Sci.* **2021**, *11*, 1769. [\[CrossRef\]](#)
60. Abreu, M.; Santos, E.; Ferreira, M.; Magalhães, M. *Cistus salvifolius* a promising species for mine wastes remediation. *J. Geochem. Explor.* **2011**, *113*, 86–93. [\[CrossRef\]](#)
61. Parra, A.; Zornoza, R.; Conesa, E.; López, G.; Faz, A. Evaluation of the suitability of three Mediterranean shrub species for phytostabilization of pyritic mine soils. *Catena* **2016**, *136*, 59–65. [\[CrossRef\]](#)
62. Roca-Perez, L.; Boluda, R.; Rodríguez-Martín, J.A.; Ramos-Miras, J.; Tume, P.; Roca, N.; Bech, J. Potentially harmful elements pollute soil and vegetation around the Atrevida mine (Tarragona, NE Spain). *Environ. Geochem. Health* **2023**. [\[CrossRef\]](#)

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.