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Efficiency of white lupin in the removal of mercury from contaminated soils: Soil and hydroponic experiments

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Abstract

This study examined the ability of the white lupin to remove mercury (Hg) from a hydroponic system (Hg concentrations 0, 1.25, 2.5, 5 and 10 μmol/L) and from soil in pots and lysimeters (total Hg concentration (19.2 ± 1.9) mg/kg availability 0.07%, and (28.9 ± 0.4) mg/kg availability 0.09%, respectively), and investigated the accumulation and distribution of Hg in different parts of the plant. White lupin roots efficiently took up Hg, but its translocation to the harvestable parts of the plant was low. The Hg concentration in the seeds posed no risk to human health according to the recommendations of the World Health Organization, but the shoots should not be used as fodder for livestock, at least when unmixed with other fodder crops. The accumulation of Hg in the hydroponically-grown plants was linear over the concentration range tested. The amount of Hg retained in the roots, relative to the shoots, was almost constant irrespective of Hg dose (90%). In the soil experiments, Hg accumulation increased with exposure time and was the greater in the lysimeter than in the pot experiments. Although Hg removal was the greater in the hydroponic system, revealing the potential of the white lupin to extract Hg, bioaccumulation was the greatest in the lysimeter-grown plants; the latter system more likely reflects the true behaviour of white lupin in the field when Hg availability is a factor that limits Hg removal. The present results suggest that the white lupin could be used in long-term soil reclamation strategies that include the goal of profitable land use in Hg-polluted areas.

Key words: edible plant parts; hydroponic-soil-lysimeter experiments; *Lupinus albus* L.; mercury
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Introduction

Mercury (Hg) is a rare element on earth, ranking sixteenth from the bottom of the abundance list. It is, however, ubiquitous, being present in trace amounts throughout the lithosphere, the hydrosphere, the atmosphere, and the biosphere, as well as in igneous rocks of all classes (Goldwater, 1971). Anthropogenic activities, including Hg mining, have led to Hg contamination of the soil-plant system.

Almaden (Ciudad Real, Spain) was the largest Hg mining district in the world, producing more than 30% of all commercial Hg (Hernández et al., 1999). Mining activity ceased in May 2002, but significant quantities of ore were stockpiled and retorting of the ore did not end until February 2004 (Newman, 2002; Gray et al., 2004). Appropriate alternative uses of the soil now have to be found, and agricultural activities stand out as an environmentally-friendly and potentially profitable alternative to Hg mining in this area. Unfortunately, soils affected by mining activity are generally low in nutrients and organic matter, and although the inclusion of nitrogen-fixing plants in the stabilizing vegetation should help in ecosystem development by increasing the nitrogen content of the soil while promoting plant cover (Frérot et al., 2006), the problem of how these plants might deal with the Hg present also needs to be addressed.

Mercury accumulation in several leguminous plants has been studied, such as pea (Beauford et al., 1977), white clover (Greger et al., 2005), alfalfa (Ortega-Villasante et al., 2005), lentil, chickpea (Rodríguez et al., 2007) and common vetch (Sierra et al., 2008). Some grain legumes, such as lentil and chickpea, are currently cultivated in the Almaden area, but the literature contains little information on Hg accumulation in the edible parts of food crops. The use of such plants for fodder could also pose a health risk by allowing toxic elements such as Hg to enter the food chain. The distribution of Hg in the edible parts of food crops therefore needs to be carefully examined.

If lupin shoots are to be used as forage, sampling for analytical purposes should be performed at the beginning of the flowering stage (i.e., before seeds form). European legislation regarding forage use establishes an Hg limit of 0.1 mg/kg in raw material for animal nutrition (European Commission Directive, 2002). However, white lupin seeds

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are also used as human food, and the maximum amount a person can consume per day without incurring a risk of Hg intoxication is determined according to the recommendations given by the World Heath Organization (WHO, 1972; WHO-IPCS, 2004). In previous studies, the kinetic parameters of Hg uptake in short duration experiments have shown white lupin to have a high capacity to absorb Hg (Esteban et al., 2008), but no studies have investigated whether this behaviour is the same in long-term soil experiments. The aims of this study were therefore to determine (1) whether Hg can be efficiently removed and accumulated by white lupin plants growing on Hg-enriched media, and (2) whether Hg transferring to the edible parts of white lupin plants allows their use as human and animal foods according to current legislation. Such knowledge would help clarify the potential of white lupin as a crop for growth on Hg-rich soils.

1 Materials and methods

1.1 Plant growth

White lupin seeds (*Lupinus albus* L.) cv. Marta were surface-sterilised in 10% (*V/V*) sodium hypochlorite for 15 min, rinsed thoroughly with deionised water and germinated in darkness at 28°C for 3 days on water-moistened filter paper. The seedlings obtained were placed in plastic containers (8 L) with half strength, continuously aerated nutrient solution for 12 days. The composition of the nutrient solution was as follows (mmol/L): $Ca(NO_3)_2$ 1.5, KNO_3 4.0, KH_2PO_4 1.5 and $MgSO_4$ 1.0. Micronutrients were supplied as (μmol/L): Fe-EDDHA 36, $MnSO_4 \cdot H_2O$ 33, $ZnSO_4 \cdot 7H_2O$ 1.6, $CuSO_4 \cdot 5H_2O$ 1.6, H_3BO_3 46 and $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ 0.1. The pH of the nutrient solution ranged from 5.5 to 6.0.

1.2 Hydroponic experiments

After 12 days the plants (N = 30) were transferred to plastic Riviera pots (each representing an experimental unit with six plants) containing 10 L perlite in the upper compartment and 2 L solution in the lower compartment. Hg was added as $HgCl_2$ to the nutrient solutions to give concentrations of 0, 1.25, 2.50, 5 and 10 μmol/L Hg. Deionised water was used for preparing all solutions and was added to replace transpiration losses every two days. The entire nutrient solutions were changed weekly. All plants were grown in a glasshouse under the following environmental conditions: night/day temperature 12–28°C, relative humidity 50%–80% and a photosynthetic photon flux density of 500 μmol/(m^2·sec). All experiments were performed in triplicate following a randomised block design. Plants were harvested 5 and 10 weeks after beginning the Hg treatments. At harvest, the roots and shoots of each plant were separated and their fresh weight were recorded. They were then thoroughly washed, first with tap water, followed by rinsing three times with deionised water. To determine dry weights while avoiding Hg losses, all plant matter was dried at room temperature for at least two weeks until a constant weight was reached. The samples were then ground and sieved to a size of less than 50 μm for Hg determination.

1.3 Pot experiments

Twelve days after sowing, plants were transferred to 15 L plastic pots (density: 25 plants/pot; number of pots: 12) containing a mixture of soil, perlite and sand in equal proportions, to improve soil aeration. The soil was collected northeast of Almadenejos, in the Almaden District. Almadenejos lies in a river valley of mainly slate and sandstone lithology; the main land use is grassland pasture (Millán et al., 2006). Soil pH was slightly acid 6.33 ± 0.05, oxidable organic matter content was (1.66 ± 0.05)% and with a loam soil texture. This soil was classified as a Mollic Haploxeralf according to USDA Soil Taxonomy criteria. Only the horizons Ah and Bt1, i.e., to a depth of 40 cm (that of shallow ploughing), were used in these experiments. The aggregates in the substrate were broken down and the soil sieved to 2 mm. Plants were harvested 11 and 25 weeks after sowing, coinciding with the beginning of flowering (harvesting time for forage use) and full maturity (harvesting of seeds for human consumption). Only shoots were collected at the first sampling stage (11 weeks), while whole plants were collected at the second (25 weeks). These samples, where appropriate, were separated into roots, stems plus leaves, husks and grains, and the fresh weights were recorded. The aerial parts of the plants were washed as described for the hydroponic experiment, while roots were placed in individual beakers, rinsed several times with deionised water and cleaned using an ultrasonic bath to remove external contamination (5 min × 5 min). Dry weights were determined by drying the samples at room temperature for at least two weeks, until a constant weight was reached. The samples were then ground and sieved to a size of less than 50 μm for Hg determination. All plants were grown under the above-mentioned glasshouse conditions. This experiment was performed in quadruplicate.

1.4 Lysimeter experiments

Lysimeter experiments represent an intermediate stage between greenhouse and full-scale field experiments. The lysimeters used consisted of a cubic meter of an unaltered soil monolith placed within a metallic structure. Lysimeters were extracted northeast of Almadenejos; all lysimeter experiments were performed under cover at the CIEMAT research facilities in Madrid. The climatological conditions of the Almadén area and Madrid are similar. Physicochemical properties of the soil are those described above for pot experiments.

Seeds were directly sown in the lysimeter soil (30 seeds/m^2 in three rows) and the plants raised following the typical agricultural practices of the Almaden area. Samples were taken at the beginning of flowering (harvesting time for forage use; 19 weeks after sowing) and at full maturity (the time when seeds are harvested for human consumption; 31 weeks after sowing). The plants taken at the first sampling stage were separated into shoots and roots, while those taken at the second stage were separated into

roots, leaves plus stems, husks and grains. Fresh weights were recorded for all subsamples. Sample washing and processing was similar to that described for the pot assay. This experiment was performed in triplicate.

1.5 Hg determination

The available Hg in the soil (pot and lysimeter experiments) was extracted using the AB-DTPA method (Soltanpour and Schwab, 1977). Total and available Hg in soils and plant samples was measured using an Advanced Mercury Analyser (AMA-254, LECO Company, Czeck Republic). Five replicates of each sample were measured; the results are expressed as the mean and standard deviation. To test the accuracy of the results, certified reference material BCR-CRM 62 (olive leaves; (0.28 ± 0.02) mg Hg/kg) was also measured and a value of (0.29 ± 0.01) mg Hg/kg obtained.

1.6 Calculations and statistical analyses

The following variables were calculated to estimate Hg removal (μg Hg/plant) in shoots and accumulation by the plants growing under different experimental conditions:

$$F_b = \frac{C_s}{C_{as}} \quad (1)$$

where, F_b is the bioaccumulation factor, C_s (μg/g dw) is the Hg concentration in shoots, C_{as} (μg/g dw) is the available Hg concentration in soil.

$$T = \frac{A_{ts}}{A_{tp}} \quad (2)$$

where, T (%) is the translocation of Hg; A_{ts} (μg) is the total Hg accumulated in shoots; A_{tp} (μg) is the total Hg accumulated in whole plant.

$$R_{s/r} = \frac{C_s}{C_r} \quad (3)$$

where, $R_{s/r}$ is the ratio of Hg concentration of shoot to root; C_r (μg/g dw) is the Hg concentration in root.

Background concentrations of Hg in plants hydroponically grown without Hg were subtracted from the concentrations recorded for the treated plants prior to the calculations. The background concentration of Hg was due to the materials used in cultivation and Hg deposition. One-way ANOVA was used to compare the results obtained in the hydroponic and soil experiments, followed by a post-hoc multiple comparison of means using the Duncan test ($P < 0.05$). All calculations were made using SPSS v. 14.0 software.

2 Results and discussion

2.1 Effect of Hg on plant growth

In the hydroponic experiment, the most obvious symptom of Hg toxicity was the inhibition of plant growth. Compared to the control plants (0 μmol/L Hg), significant reductions of 13% and 21% in shoot length was seen in the plants treated with 5 and 10 μmol/L Hg (Fig. 1A). In addition, significant reductions in root length were seen as the Hg supply increased. The fresh shoot weight (Fig. 1b) was also significantly reduced by Hg exposure after 10 weeks of growth; reductions of 20% were seen in the 1.25 and 2.5 μmol/L Hg treatment plants compared to the controls, and of approximately 50% were seen in 5 and 10 μmol/L Hg treatments. The fresh root weight of the Hg-treated plants was reduced within a range of 37%–63% (Fig. 1B). Yet despite spit these results, the Hg-treated plants showed no visible toxicity symptoms, e.g., leaf yellowing or root browning.

Plants that accumulate Hg in the roots convert the pollutant into less available forms. As the roots are probably the first to suffer Hg injury, the inhibition of root growth might be the first symptom of Hg stress. Thus, the roots of plants treated with the highest Hg doses under hydroponic conditions might be expected to be at particular at risk – as was the case.

No Hg toxicity symptoms were observed in the plants of the soil experiments. All completed their growth cycles adequately with biomass productions were similar to those reported in the Almaden area (data not shown).

2.2 Hg accumulation in plants in the hydroponic and soil experiments

Plants grown without Hg in the nutrient solutions showed low Hg concentrations in their shoots and roots (< 0.4 and < 1.2 mg/kg dw, respectively). However, the Hg

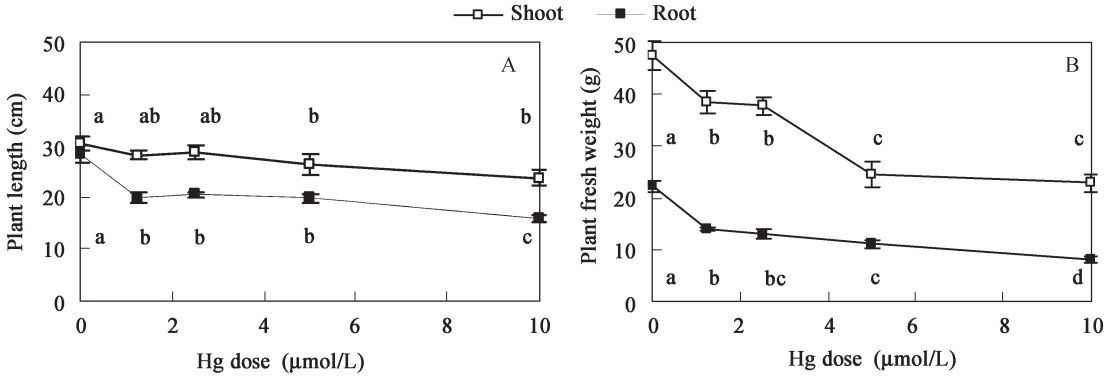

Fig. 1 Effect of Hg supply on plant growth at 10 weeks in hydroponic experiments. (A) shoot and root lengths; (B) shoot and root weights. Vertical bars indicate relative standard error of the mean ($n = 3$). Different letters indicate significant differences among Hg ($P < 0.05$).

concentrations of the shoots and roots of plants grown for 5 and 10 weeks in the presence of Hg increased significantly with Hg concentration increasing (Fig. 2). At 5 weeks, the Hg concentration in the roots of plants treated with the highest Hg dose (10 μmol/L) was 23 times that of the shoots; after 10 weeks accumulation it was 10 times that of the shoots. The results for Hg accumulation in the roots agreed with those reported in other plant species (Patra and Sharma, 2000). Irrespective of sampling time, a linear relationship was seen between Hg concentration in the shoots with the Hg supplied to the plants (R^2 = 0.98; $P < 0.01$), and between the Hg concentration of the roots and the Hg supplied (R^2 = 0.97 and R^2 = 0.99, $P < 0.01$). Therefore, in the shoots, Hg tended to accumulate with exposure. The slope at 10 weeks was twice that at 5 weeks in the Hg-treated plants (Fig. 2a). The trend in Hg accumulation in the roots was the opposite; longer exposures were associated with reductions in the Hg concentration (Fig. 2b). A higher root growth rate between 5 and 10 weeks of Hg exposure could cause a dilution effect in the roots, explaining this reduction in root Hg concentration.

Table 1 shows the Hg concentrations of the plants in the soil experiments. In the pot experiment, the Hg concentration almost doubled in the shoots from the beginning of flowering (plants for forage use) to seed maturation (plants for human consumption). The root Hg concentration at the second sampling point (human consumption) was significantly higher than the shoot Hg concentration, and both were significantly higher ($P < 0.05$) than the grain and husk Hg concentrations. No significant differences were seen between the grain and husk Hg concentrations. These results were obtained for a total Hg concentration in the soil of (19.2 ± 1.9) mg/kg, of which just 0.07% was available to the plant.

In the lysimeter experiment (Table 1), the shoot Hg concentration was 2.4 times at the second sampling point than at the first, while the roots showed the opposite behaviour (as in the hydroponic experiment). The husk Hg concentration was similar to the shoot Hg concentration. The lowest concentration was that of the seeds. These results were obtained for a total Hg concentration in the soil of (28.9 ± 0.4) mg/kg, of which just 0.09% was available to the plant. The total Hg concentrations for the soil in the pot and lysimeter experiments were within the range found by Lindberg et al. (1979) and Millán et al. (2004) for the Almaden area. In both soil experiments, the percentage of available Hg for the plants was very low (< 0.1%); in the study area, Hg is mainly found in its cinnabar form (HgS), which is poorly soluble. Accordingly, risk assessments regarding pollutants in contaminated soils should place emphasis on the availability of heavy metals rather than their total concentration, which does not always accurately inform about the real risk (Adriano, 2001).

The shoot Hg concentration was significantly higher in the pot-grown plants than the lysimeter-grown plants at both sampling times. No differences were seen for the roots and grains, but the Hg concentration of the husks was 1.5 times that seen in the lysimeter-grown plants (Table 1). Figure 3 shows the percentage contribution of Hg to each fraction of the shoots. The percentage of Hg in the shoots (leaves plus stems) in the lysimeter-grown plants was higher than in the pot-grown plants, while the husks showed similar percentages. Hg concentration in the grains was the highest in the pot-grown plants.

In the present soil experiments, the Hg concentration

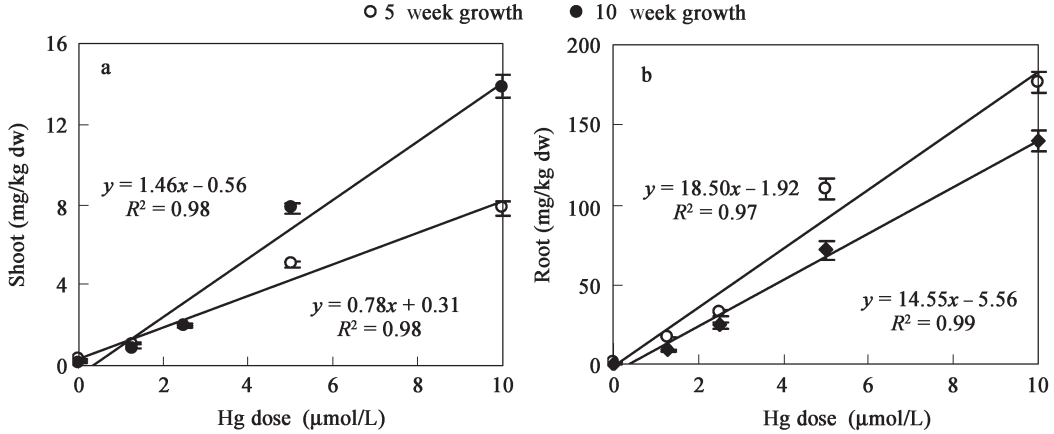

Fig. 2 Effect of Hg supply on Hg concentration in hydroponic experiments. (a) shoots; (b) roots. Vertical bars indicate (±) standard error of the mean (n = 3).

Table 1 Plant Hg concentration (μg/kg dw) in the soil experiments

	Forage use		Human consumption			
	Shoots	Roots	Shoots	Roots	Grains	Husks
Pot experiment	118.8 ± 8.9 a	n.a.	207.0 ± 25.8 a	293.3 ± 23.2 a	30.5 ± 6.5 a	95.4 ± 5.8 a
Lysimeter experiment	60.7 ± 3.7 b	455.1 ± 24.3	143.7 ± 7.6 b	284.7 ± 12.9 a	31.0 ± 2.0 a	142.1 ± 6.2 b

n.a.: not analysed.

Data represent the mean ± standard error of the mean (n = 4); means within a column (in each use compartment) followed by the same letters do not differ significantly according to the Duncan test ($P < 0.05$).

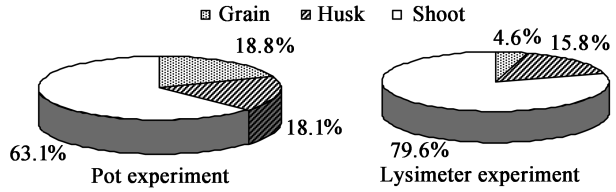

Fig. 3 Hg distribution (%) in the different aerial parts of the plant in the soil experiments.

of the roots was significantly higher than in the shoots, grain and husks (Table 1). Similar Hg values in shoots have previously been reported for white lupin grown on Almaden soils (Rodríguez et al., 2007), but no information was provided on the Hg concentration of the roots and edible plant parts of this crop. European legislation regarding forage use establishes an Hg limit of 0.1 mg/kg in the raw material for animal nutrition (European Commission Directive, 2002). The Hg concentration in the shoots of the pot-grown plants (Table 1) was just above this value, suggesting these plants should not be used as fodder for livestock, at least when unmixed with other fodder crops. However, in the lysimeter experiment, which much better reflects true field conditions, this limit was not surpassed. This suggests these plants could, therefore, be used for fodder.

White lupin grains are used in human nutrition. The maximum amount of the present white lupin grains a person could consume per day without risk of intoxication was calculated according to the recommendations of the World Heath Organization (WHO, 1972; WHO-IPCS, 2004). The tolerable daily intake of total Hg for adults is 0.71 μg/kg body weight per day; thus, considering a medium body weight of 60 kg, an adult would be able to take in 42.6 μg Hg per day. According to Table 1 and the results of the pot experiment, an adult could therefore consume a maximum of 0.60 kg of the present grain per day, or 1.4 kg of grain per day according to the results of the lysimeter experiment. These amounts greatly surpass recommendations regarding the amount of legumes that should be included in the human diet. Therefore, the present grains could be eaten without fear of causing Hg intoxication.

2.3 Hg accumulation in the hydroponic and soil experiments

Table 2 shows the values of a number of variables related to Hg accumulation in the plants grown hydroponically for 10 weeks. The bioaccumulation factor ranged from 0.08 to 0.23, the highest value was seen for plants grown in the presence of 5 μmol/L Hg. A significant increase in the efficiency of Hg removal was observed as the amount of Hg supplied increased. With the Hg dose increasing, the percentage of Hg translocated to the shoots decreased lightly, although no significant differences were seen for the Hg range between 2.50 and 10 μmol/L Hg. The shoot:root Hg concentration ratio did not change with different Hg doses.

Table 3 shows that the ability of the plants to remove Hg in both the pot and lysimeter experiments increased with time, although still in only very small amounts. The bioaccumulation factor was significantly higher for the lysimeter-grown plants, probably because the roots could explore a larger soil volume, leading to more Hg being taken up by the plant. The percentage of Hg translocated to the aerial part of the plant was also higher in the lysimeter-grown plants than in the pot- or hydroponically-grown plants. Translocation also increased with time in the lysimeter experiment; indeed, almost 80% of the total root Hg was passed to the shoot. However, the extraction of the whole root in soil experiments is not easy, and this has to be taken into account when comparing the results with experiments performed in other cultivation media. The shoot:root Hg concentration ratio was very similar in both soil experiments at the second sampling stage,

Table 2 Values of variables related to Hg accumulation and Hg removal in the hydroponic experiment

	Hg dose			
	1.25 μmol/L	2.50 μmol/L	5 μmol/L	10 μmol/L
Bioaccumulation factor	0.08 ± 0.01 a	0.13 ± 0.01 b	0.23 ± 0.02 c	0.16 ± 0.01 b
Hg removal	3.49 ± 0.53 a	10.83 ± 0.86 b	38.01 ± 1.66 c	54.53 ± 2.58 d
Translocation of Hg (%)	13.05 ± 0.51 a	10.17 ± 0.73 b	9.64 ± 0.55 b	8.87 ± 0.31 b
Shoot:root Hg concentration ratio	0.10 ± 0.01 a	0.08 ± 0.01 a	0.11 ± 0.01 a	0.10 ± 0.01 a

Data represent means ± standard error (n = 3). Means within a row followed by the same letter do not differ significantly according to the Duncan test ($P < 0.05$).

Table 3 Values of variables related to Hg accumulation and Hg removal in the soil experiments

	Pot experiment		Lysimeter experiment	
	Forage use	Seed consumption	Forage use	Seed consumption
Bioaccumulation factor	0.09 ± 0.01 a	0.09 ± 0.01 a	2.46 ± 0.15 b	5.82 ± 0.31 c
Hg removal	0.27 ± 0.04 a	0.65 ± 0.14 b	0.17 ± 0.01 a	0.93 ± 0.05 b
Hg translocation (%)	n.c.	53.31 ± 1.21 a	48.90 ± 0.05 a	79.60 ± 0.12 b
Shoot: root Hg concentration ratio	n.c.	0.45 ± 0.08 b	0.13 ± 0.01 a	0.50 ± 0.01 b
Phytoextraction (mg Hg/ha)	106.40 ± 14.50 a	137.21 ± 29.11 ab	68.00 ± 4.11 a	195.40 ± 10.30 b

n.c.: not calculated.

Data represent the mean ± standard error (n = 4 for the pot experiment and n = 3 for the lysimeter experiment); means within a row followed by the same letter do not differ significantly according to the Duncan test ($P < 0.05$).

increasing significantly from the first sampling stage. The values recorded were similar to those obtained by Moreno-Jiménez et al. (2006) for *Marrubium vulgare* grown on Almaden soils. In contrast, the bioaccumulation factor for the pot-grown plants was much lower than that of the wild native plants studied by Moreno-Jiménez et al. (2006). The bioaccumulation factor was lower in both the pot and lysimeter experiments than for common vetch grown in a similar soil by Sierra et al. (2008). The shoot:root ratio was similar for both species.

The phytoextraction capacity of the plants was calculated taking into account biomass production, the usual sowing density for white lupin under field conditions, and the plant Hg concentrations were obtained. Phytoextraction increased with time, the highest value at the second sampling stage in the lysimeter experiment–that with the experimental conditions, was the most similar to the field. Such phytoextraction would allow the progressive reduction of available Hg in the soil, and at the same time, the use of the seed for consumption.

The results of the hydroponic experiments show the plants retained Hg in their roots, but the shoot:root ratio was higher in the soil-grown plants (both pot- and lysimeter-grown plants). Since the roots of the hydroponically-grown plants accounted for 90% of the total plant Hg, it is possible that the retention process is enhanced when all the Hg is available to the plants. Progressive accumulation of Hg in the shoots with increasing Hg exposure time might help explain the higher Hg shoot:root ratios recorded in the soil experiment. A similar effect was observed when comparing the shoot:root ratios of wild plants grown in soil from Almadén area and in hydroponic conditions (Moreno-Jiménez et al., 2006, 2007). The Hg removal efficiency of the hydroponically-grown plants was greater than that of the soil-grown plants (both types), but the bioaccumulation factor of the lysimeter-grown plants was higher. These findings show that Hg removal efficiency is mainly limited by the amount of available Hg in the substrate.

3 Conclusions

The present study shows that, despite most Hg in soils being present in a non-available form, it is taken up by white lupin plants and translocated to their edible parts. Nonetheless, this crop could be cultivated in the soils of the Almaden area and used for human consumption, but it could not be used without mixing as a fodder crop. When Hg is completely available (as in the hydroponic experiment), it is mainly retained in the roots. As part of soil remediation strategies these plants could also provide food for human or animal consumption (with appropriate mixing), allowing profitable use be made of these contaminated areas. According to the present results, the white lupin might be a good choice for use in strategies that combine long-term soil reclamation and the goal of profitable land use. Further research is needed to see whether the present results are maintained under different field conditions.

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