Cadmium phytoextraction capacity of white lupine (*Lupinus albus* L.) and narrow-leafed lupine (*Lupinus angustifolius* L.) in three contrasting agroclimatic conditions of Chile

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ABSTRACT

The phytoextraction process implies the use of plants to promote the elimination of metal contaminants in the soil. In fact, metal-accumulating plants are planted or transplanted in metal-contaminated soil and cultivated in accordance with established agricultural practices. The objective of the present study was to evaluate the productivity and Cd phytoextraction capacity of white lupine (*Lupinus albus* L.) and narrow-leafed lupine (*Lupinus angustifolius* L.), as well as the effect on residual Cd concentration in the soil. Both species of lupines were grown at three $CdCl_2$ rates (0, 1, and 2 mg kg^{-1}), under three agroclimatic conditions in Chile in 2013. In the arid zone (Pan de Azúcar, 73 mm precipitation), narrow-leafed lupine production was significantly ($P < 0.05$) higher than white lupine (4.55 *vs*. 3.26 Mg DM ha^{-1}, respectively). In locations with higher precipitation (Santa Rosa, 670 mm; Carillanca, 880 mm), narrow-leafed lupine DM production was slightly higher than in Pan de Azúcar, but white lupine was approximately three times higher. Total plant Cd concentrations in white and narrow-leafed lupine increased as Cd rates increased in the three environments, but they were much higher in narrow-leafed lupine than white lupine; 150%, 58%, and 344% higher in Pan de Azúcar, Santa Rosa, and Carillanca, respectively. Cadmium uptake (g Cd ha^{-1}) and apparent recovery were also higher ($P < 0.05$) in narrow-leafed lupine in two environments (Pan de Azúcar and Carillanca). These results suggest that narrow-leafed lupine present higher potential as phytoremediation species than white lupine.

Key words: Cd recovery, Cd retention, Cd uptake, legumes, phytostabilization, soil properties.

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INTRODUCTION

Cadmium is one of the most important soil contaminants because it is toxic for many living organisms, including cultivated plants. Human health is therefore threatened when Cd is bioaccumulated in the food chain (UNEP, 2008). The World Health Organization (WHO) considers toxic a daily intake of 1 μg kg^{-1} body weight or 70 μg Cd for an average person (WHO, 2010). Cadmium exposure and accumulation mostly affects kidneys and liver in the human body and it can also affect the skeletal system through a disease called Itai-Itai that was first detected in Japan in individuals living near Cd contaminated rice fields (Phillips and Tudoreanu, 2011).

Industrial and mining activities are the main sources of Cd liberated into the air, water, and soil (UNEP, 2008). Some agricultural practices, such as sustained application of P and N fertilizers with ammonium sources have also led to Cd accumulation and increase availability on the soil surface (Chen et al., 2008; Bhargava et al., 2012). Other factors affecting Cd availability and mobility in the soil are pH, moisture, organic matter, type and quantity of clay (Bhargava et al., 2012).

When soils and aquifers are contaminated with high concentrations of heavy metals, bioremediation is one of the natural alternatives for its restoration, as well as being the cleanest and most economical (Sarma, 2011; Bhargava et al., 2012). Phytoremediation uses the capacity of certain plant species to survive in contaminated environments with synthetic organic compounds, xenobiotics, pesticides, hydrocarbons, and heavy metals as they extract, accumulate, immobilize, or transform the contaminants (Sarma, 2011). The quantities of Cd that can be absorbed by plants depend on species, age, and root development (Lux et al., 2011). Similarly, Cd concentration is not the same in different plant parts; the sequence is generally roots > stems > leaves > fruits > seeds (Lux et al., 2011; Bhargava et al., 2012). The four phytoremediation technologies are phytostabilization, phytofiltration, phytovolatilization, and phytoextraction; they are based on plants and each one has a different action mechanism on soils, sediments, or water (Sarma, 2011).

Plants can absorb metals from the soil through the root system and translocate them toward other structures where they are accumulated; the most commonly used plants are called hyperaccumulators. More than 450 species of vascular plants have been reported with these characteristics in 45 angiosperm families, including members of *Asteraceae*, *Brassicaceae*, *Caryophyllaceae*, *Cyperaceae*, *Cunoniaceae*, *Fabaceae*, *Flacourtiaceae*, *Lamiaceae*, *Poaceae*, *Violaceae*, and *Euphorbiaceae* (Padmavathiamma and

Li, 2007). Many studies have indicated that certain varieties of the genus *Lupinus* (Fabaceae) have a tolerance non-specific to heavy metals similar to that of hyperaccumulator plants despite not being considered as such (Zornoza et al., 2002; Lambers et al., 2013; Fumagalli et al., 2014).

White lupine (*Lupinus albus* L.; Fabaceae) is an annual species that fixes N, is drought-tolerant, tolerant to excessive salinity, and able to extract moderate levels of heavy metals, such as Zn, Cd, Cu, Pb, Mn, Cr, and Hg (Ximénez-Embún et al., 2002). When it grows in soils that are deficient in available nutrients, white lupine plants exude chelating agents (organic anions and enzymes, such as phosphatase and probably phytase) and hydrogen ions through proteiform roots in order to improve acquisition of P, Fe, Mn, and Zn (Lambers et al., 2013). The white lupine root system has a high Cd retention capacity through cell walls (Zornoza et al., 2002), thus reducing its available concentration in the soil. White lupine effectively decreases the soluble fraction of As and Cd in acidic soils at the same time it accumulates high concentrations of As and Cd in its roots (Vázquez et al., 2006). Meanwhile, narrow-leafed lupine (*Lupinus angustifolius* L.; Fabaceae) does not produce specialized clusted or cluster-like roots, but do release large amounts of carboxylates from nonspecialized roots (Egle et al., 2003).

Several reports can be found about Cd tolerance in white lupine (Ximénez-Embún et al., 2002; Zornoza et al., 2002; Fumagalli et al., 2014). However, little information exists about the response of narrow-leafed lupine as a Cd phytoremediation species (Römer et al., 2000; Brennan and Bolland, 2003). The present work is part of a larger study in which the phytoextraction and phytoremediation capacity of white and narrow-leafed lupine is assessed as total plant (aerial part) and its effect on later Cd absorption by two species for human consumption characterized by its Cd accumulation capacity in grains, durum wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.) and maize (*Zea mays* L.); therefore, the Cd compartmentalization study in lupine plant structures was not considered. The objective of this study was therefore to determine the Cd uptake capacity of lupine on different soil environments in Chile when applying increasing Cd rates, as well as evaluate the effect of residual Cd concentration in the soil.

MATERIALS AND METHODS

Location description

Experiments were established in experimental stations of the Instituto de Investigaciones Agropecuarias (INIA), Chile, located in three contrasting agroclimatic zones: Pan de Azúcar Station (30°06' S, 71°23' W) in the Coquimbo Region with arid climate and alluvial-colluvial soils (Typic Haplocambids; USDA, 2014), Santa Rosa Station (36°31' S, 71°54' W) in the Biobío Region with humid Mediterranean climate and volcanic soil (Melanoxerand; USDA, 2014), and Carillanca Station (32°49' S, 70°35' W) in La Araucanía Region with rainy temperate climate and volcanic soil (Melanudands; USDA, 2014). The meteorological characteristics of each station in 2013 are shown in Table 1 (Red Agrometeorológica de Chile, http://agromet.inia.cl/estaciones.php).

Treatments and crop management

The two species of lupines were grown at three $CdCl_2$ rates: 0, 1, and 2 mg kg^{-1}. For each station, each experimental plot was 27 m^2 (3 × 9 m) and there were six replicates per treatment. Since the bulk density of the 0-20 cm soil depth were different at the three experimental sites (Table 2), the application $CdCl_2$ to the soil, for the treatments 1 and 2 mg kg^{-1}, were 3.17 and 6.34 g $plot^{-1}$ at Pan de Azúcar, 1.8 and 3.6 g $plot^{-1}$ at Santa Rosa, and 1.44 y 2.88 g $plot^{-1}$ at Carillanca, respectively. Application of $CdCl_2$ was performed in solution with distilled water at the rate of 20 mL m^{-2}. Sowing dates for both white and narrow-leafed lupine was 15 May 2013 at Pan de Azúcar and 17 July 2013 at Santa Rosa and Carillanca. Seed rates were 140 and 120 kg ha^{-1} for white and narrow-leafed lupine, respectively, in order to reach populations that maximize yield (Tay and Vidal, 2009). Agronomic practices were in accordance with commercial use; only elemental S was applied to reduce pH in soils at Pan de Azúcar at rate of

Table 1. Mean temperature (Mean T, °C), precipitation (PP), and evapotranspiration (EV) in three environments in 2013.

	Pan de Azúcar			Santa Rosa			Carillanca		
Month	Mean T	PP	EV	Mean T	PP	EV	Mean T	PP	EV
	°C	mm		°C	mm		°C	mm	
January	18.6	0.0	129.7	19.9	1.2	134.4	17.2	5.5	124.4
February	18.9	0.4	126.1	18.5	19.3	101.9	15.9	38.1	77.1
March	15.7	0.1	99.0	15.1	4.1	78.5	13.2	29.8	62.6
April	13.4	0.2	67.2	12.4	6.1	40.6	11.5	38.5	34.5
May	12.5	57.1	43.8	9.4	183.0	21.5	9.1	158.0	12.3
June	10.6	1.0	27.8	7.3	123.7	12.4	6.5	116.3	9.8
July	9.7	1.5	32.9	7.1	110.1	16.1	6.5	123.6	13.1
August	10.9	0.3	57.9	8.2	128.0	20.8	6.3	170.1	21.8
September	11.9	0.0	89.1	9.7	49.9	52.6	8.2	103.4	32.6
October	12.5	0.1	87.0	12.7	35.7	86.4	10.5	39.5	56.0
November	14.5	6.0	106.5	15.2	11.0	123.1	11.9	48.9	45.9
December	16.9	6.0	122.8	19.1	0.0	156.7	15.1	6.6	0.0
Total	-	72.7	989.8	-	672.1	845.0	-	878.3	490.1

-: Do not exist accumulate value.

Table 2. Soil physical and chemical properties (0-20 and 20-40 cm depths) in three environments in 2013.

	Pan de Azúcar		Santa Rosa		Carillanca	
Parameters	0-20	20-40	0-20	20-40	0-20	20-40
Clay, %	20.20	20.30	20.70	15.90	25.30	23.10
Silt, %	30.20	31.20	43.60	45.40	38.50	41.50
Sand, %	49.60	48.50	35.70	38.70	36.20	35.40
Bulk density, g cm^{-3}	1.76	1.80	1.00	1.05	0.80	0.82
$pH_{(soil:water\ 1:5)}$	6.94	6.87	5.74	5.76	5.96	5.92
Organic matter, g kg^{-1}	11.60	11.30	63.00	56.20	155.60	143.70
EC, dS m^{-1}	0.15	0.23	0.11	0.07	0.09	0.09
Available N, mg kg^{-1}	18.00	20.00	40.00	38.00	30.00	28.00
P Olsen, mg kg^{-1}	51.30	44.90	35.20	25.30	24.50	20.90
Exchangeable K, cmol+ kg^{-1}	0.85	0.67	0.65	0.39	0.67	0.41
Exchangeable Ca, cmol+ kg^{-1}	8.12	8.22	6.74	5.89	7.40	7.77
Exchangeable Mg, cmol+ kg^{-1}	2.41	2.61	0.95	0.72	0.71	0.76
Exchangeable Na, cmol+ kg^{-1}	0.59	0.69	0.16	0.19	0.03	0.05
Exchangeable Al, cmol+ kg^{-1}	0.05	0.05	0.21	0.10	0.06	0.05
Available Fe, mg kg^{-1}	21.50	20.80	59.80	46.50	55.90	59.90
Available Mn, mg kg^{-1}	36.30	34.30	9.80	5.40	3.50	2.80
Available Zn, mg kg^{-1}	4.50	4.40	0.70	0.60	0.80	0.60
Available Cu, mg kg^{-1}	9.20	9.30	1.40	1.20	1.00	0.90
Available B, mg kg^{-1}	2.30	2.40	0.50	0.40	0.30	0.30
Available S, mg kg^{-1}	40.80	64.90	14.20	15.40	14.60	9.60
Total Cd, mg kg^{-1}	1.33	1.49	0.21	0.18	0.14	0.14

EC: Electrical conductivity.

200 kg ha^{-1}, respectively. Fertilization with N and P was not applied because the two lupine species do not respond to these nutrients (Tay and Vidal, 2009); the level of K was adequate in the soils, so there was no need to apply it (Table 2).

Measurements and determination of Cd

In May 2013, prior to sowing, initial soil samples were collected from the 0-20 and 20-40 cm depths for chemical characterization by methods indicated by the National Accreditation Commission for laboratory analyses (Sadzawka et al., 2006). Each soil sample was characterized for pH, organic matter (OM), available N and P, Ca, Mg, Na, and K, exchange bases, micronutrients as Cu, Zn, Fe, and Mn. Soil total Cd was determined in an HNO_3-HCl (*aqua regia*) extract and analyzed by inductively coupled plasma-optical emission spectrometry (ICP-OES). Soil texture was analyzed by the hydrometer of Bouyoucos method. Bulk density was determined by the cylinder method (Table 2).

At maturity four frames of 0.5 × 1 m (2 m^2) per plot of plant material (aerial part) were collected for DM determination. The material was homogenized, oven-dried at 70 ºC for 72 h, and a subsample ground for Cd determination. In addition, soil samples were collected in each experimental unit, at two depths: 0-20 and 20-40 cm.

Soil and plant Cd was quantified by electrothermal atomic absorption spectrophotometry (graphite furnace technique) with Thermo Elemental Solaar M5 equipment coupled to a Model GF95 graphite furnace. Samples were digested in a microwave oven (MARS-Xpress, CEM Corporation, Matthews, North Carolina, USA) before the spectrophotometry reading. For each soil sample, 0.5 g was weighed and 10 mL HNO_3 (nitric acid 65%, Suprapur Nitric Acid, Merck Millipore, Darmstadt, Germany) were added, whereas 1 g lupine DM in digestion tubes was weighed and 10 mL Suprapur HNO_3 + 1 mL 30% H_2O_2 were added. Quality control for the analysis was based on certified reference material (ISE 979 for soil and IPE 981 for plant tissue), comparing samples between laboratories, internal control samples, and duplicates of the analyses.

The amount of Cd extracted (g Cd ha^{-1}) by white and narrow-leafed lupine at the three applied Cd rates (g ha^{-1}) were calculated as: DM (Mg ha^{-1}) × Cd concentration (g kg^{-1}) in the plant tissue. The percentage of Cd recovery was determined by dividing Cd uptake (g ha^{-1}) by the applied Cd rate (g ha^{-1}) and multiplying by 100.

The percentage of Cd fixation was calculated by considering the increase of Cd at the 0-40 cm depth compared to the control and applied Cd (g ha^{-1}), which were associated with the bulk density of each depth and each soil as expressed in the following equation:

$$\text{Cd fixation (\%)} = \left[1 - \frac{\textit{Soil Cd concentration treatment}_{0-40\ cm} - \textit{control (g ha}^{-1}\textit{)}}{\textit{Cd rate applied in the treatment (g ha}^{-1}\textit{)}}\right] * 100$$

Experimental design and treatments

The experimental design was a randomized complete block in a split-split plot arrangement with six replicates; the three environments (Pan de Azúcar, Santa Rosa, and Carillanca) were the main plots, the two lupine species the split-plots (*L. albus* and *L. angustifolius*), and the three $CdCl_2$ (0, 1, and 2 mg kg^{-1}) rates the split-split plots. Results were analyzed by an ANOVA and Tukey's test ($P \leq 0.05$) using the general linear model from the SAS System version 6.0 (1989) statistical program (SAS Institute, Cary, North Carolina, USA).

RESULTS

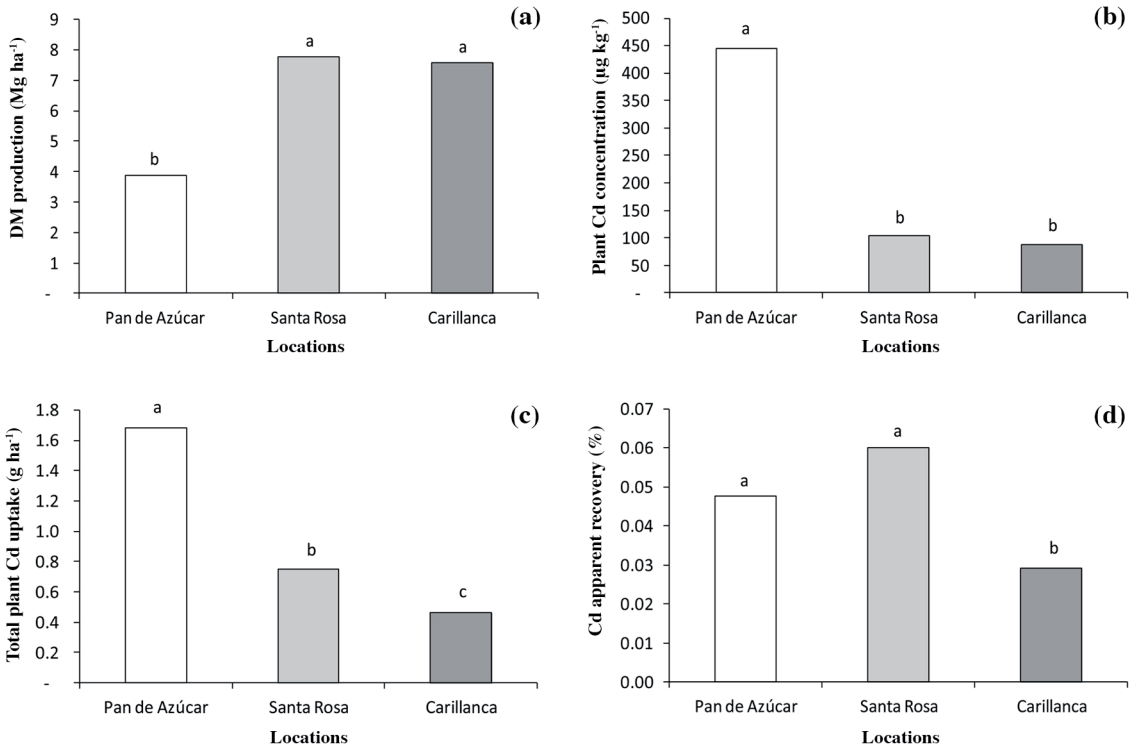
Dry matter production

Plant DM is an important factor in Cd phytoextraction of contaminated soils. Dry matter fluctuated between 3.88 and 7.78 Mg ha^{-1}; Santa Rosa and Carillanca produced the highest DM with nonsignificant difference between the two ($P > 0.05$), whereas Pan de Azúcar had a lower DM production (Figure 1a). White lupine produced higher DM (57%) than narrow-leafed lupine in Santa Rosa and Carillanca; however, narrow-leafed lupine attained 39% more DM than white lupine in Pan de Azúcar (Table 4). The influence of the Cd rates was nonsignificant ($P > 0.05$) on DM production in none of the three environments (Tables 3 and 4). The Species × Rate and Location × Rate interactions were nonsignificant (Table 3).

Total plant Cd concentration, uptake, and recovery

The Cd concentration was influenced by location, species, rate, and their interactions were significant (Table 3). On the average, plant Cd concentrations in the arid zone (Pan de Azúcar) were much higher than those detected in Santa Rosa and Carillanca (Figure 1b). Total plant Cd concentrations in white and narrow-leafed lupine increased as Cd rates increased in the three environments, but it was much higher in narrow-leafed lupine than in white lupine, especially in Pan de Azúcar and Carillanca (Table 4).

Figure 1. (a) Dry matter production, (b) total plant Cd concentration, (c) total plant Cd uptake, and (d) apparent Cd recovery of lupine (white and narrow-leafed) in three environments.



Different letters over the bars indicate significant differences among treatments according to Tukey's test ($P < 0.05$).

Table 3. Significance levels for dry matter (DM), Cd concentration, Cd uptake, and apparent Cd recovery of two lupine species under three $CdCl_2$ rates in Pan de Azúcar, Santa Rosa, and Carillanca.

	Source of variation						
Variable	Location (L)	Species (S)	Rate (R)	L x S	L x R	S x R	L x S x R
DM, Mg ha^{-1}	*	*	ns	*	ns	ns	ns
Cd concentration, µg kg^{-1}	*	*	*	*	*	*	*
Cd uptake, g ha^{-1}	*	*	*	*	*	*	*
Apparent Cd recovery, %	*	*	ns	*	ns	ns	ns

*$P < 0.05$; ns: nonsignificant ($P > 0.05$).
[a]Locations: (1) Pan de Azúcar, (2) Santa Rosa, and (3) Carillanca.
[b]Species: (1) *Lupinus albus* and (2) *Lupinus angustifolius*.
[c]Rates: (1) 0 mg kg^{-1}, (2) 1 mg kg^{-1}, and (3) 2 mg kg^{-1}.

Table 4. Dry matter, Cd concentration, Cd uptake, and Cd apparent recovery in Pan de Azúcar, Santa Rosa, and Carillanca for two lupine species, and three $CdCl_2$ rates.

Treatment	DM production	Cd concentration	Cd uptake	Apparent Cd recovery
	Mg ha^{-1}	µg kg^{-1}	g ha^{-1}	%
Pan de Azúcar				
Species				
White lupine	3.26 ± 0.267b	255.99 ± 41.81b	0.78 ± 0.14b	0.013 ± 0.005b
Narrow-leafed lupine	4.55 ± 0.267a	641.36 ± 41.81a	2.64 ± 0.14a	0.049 ± 0.005a
Rates of $CdCl_2$				
0 mg kg^{-1}	3.83 ± 0.327a	170.44 ± 51.21c	0.64 ± 0.17c	-
1 mg kg^{-1}	3.83 ± 0.327a	421.82 ± 51.21b	1.52 ± 0.17b	0.041 ± 0.006a
2 mg kg^{-1}	4.06 ± 0.327a	753.76 ± 51.21a	2.97 ± 0.17a	0.054 ± 0.006a
Santa Rosa				
Species				
White lupine	9.79 ± 0.219a	81.14 ± 10.27b	0.73 ± 0.06a	0.031 ± 0.003a
Narrow-leafed lupine	5.78 ± 0.219b	127.35 ± 10.27a	0.77 ± 0.06a	0.032 ± 0.003a
Rates of $CdCl_2$				
0 mg kg^{-1}	7.61 ± 0.268a	23.1 ± 12.58c	0.18 ± 0.08c	-
1 mg kg^{-1}	7.82 ± 0.268a	116.82 ± 12.58b	0.83 ± 0.08b	0.042 ± 0.004a
2 mg kg^{-1}	7.92 ± 0.268a	172.82 ± 12.58a	1.22 ± 0.08a	0.052 ± 0.004a
Carillanca				
Species				
White lupine	10.50 ± 0.332a	31.55 ± 8.93b	0.31 ± 0.04b	0.008 ± 0.002b
Narrow-leafed lupine	4.65 ± 0.332b	141.7 ± 8.93a	0.61 ± 0.04a	0.030 ± 0.002a
Rates of $CdCl_2$				
0 mg kg^{-1}	7.99 ± 0.406a	32.42 ± 10.94b	0.19 ± 0.05c	-
1 mg kg^{-1}	7.61 ± 0.406a	95.45 ± 10.94a	0.51 ± 0.05b	0.032 ± 0.003a
2 mg kg^{-1}	7.13 ± 0.406a	132.01 ± 10.94a	0.70 ± 0.05a	0.026 ± 0.003a

[a]Different letters in the columns for a given location, species, or rates indicate significant differences according to Tukey's test ($P < 0.05$).

Cadmium uptake was significantly ($P < 0.05$) different among environments (Table 3). Although the lowest DM production was found in Pan de Azúcar, Cd uptake was 224% and 365% higher than in Santa Rosa and Carillanca, respectively (Figure 1c). Just as for total plant Cd concentration, the rate increased Cd uptake, as compared to the control, by 182% and 371% when applying 1 and 2 mg kg^{-1} $CdCl_2$, respectively (Table 4). Narrow-leafed lupine averaged 214% more Cd uptake than white lupine in the three environments, however, significant differences ($P < 0.05$) were observed between species in Pan de Azúcar and Carillanca (Table 4).

Apparent Cd recovery was influenced by location, species, and their interaction (Table 3). With a difference of 0.012%, Pan de Azúcar and Santa Rosa were not significantly different ($P > 0.05$) for Cd recovery (Figure 1d), but these two locations differed from Carillanca (0.029%) by 65% and 106% more, respectively. Narrow-leafed lupine averaged 107% more Cd recovery than white lupine in the three environments. Unlike Santa Rosa, Pan de Azúcar and Carillanca exhibited a statistical difference between lupines (Table 4).

Residual Cd concentration in the soil

In each of the soil conditions, a higher residual Cd concentration was recorded in the 0-20 than the 20-40 cm horizon. Pan de Azúcar was the environment with the highest residual Cd concentration in the soil at both depths (Table 5). For the 0-20 cm depth at the 1 mg kg^{-1} rate, Pan de Azúcar exhibited 3.0 times more residual Cd concentration in the soil than Santa Rosa and 1.8 times more than Carillanca (Table 5), whereas concentrations at 2 mg kg^{-1} were 2.1 and 2.3 times more, respectively (Table 5). These differences are associated with the initial concentrations of the metal in the soil (Table 2), which mainly depend on the origin of each soil. For the 20-40 cm depth at the 1 mg kg^{-1} rate, Pan de Azúcar exhibited 3.0 and 2.5 times higher concentration than Santa Rosa and Carillanca, while concentrations at 2 mg kg^{-1} were 3.6 and 3.0 times higher, respectively (Table 5). For species, its behavior was different among locations, at Pan de Azúcar and Carillanca narrow-leafed lupine was 1.15 and 1.33 times higher than white lupine, while in Santa Rosa white lupine was 1.06 times higher than narrow-leafed lupine (Table 5).

Table 5. Residual Cd concentration in Pan de Azúcar, Santa Rosa, and Carillanca for two lupine species, three $CdCl_2$ rates, and two depths.

Treatment	Pan de Azúcar	Santa Rosa	Carillanca
	mg kg^{-1}		
Species			
White lupine	1.24 ± 0.126a	0.51 ± 0.066a	0.51 ± 0.057b
Narrow-leafed lupine	1.43 ± 0.126a	0.48 ± 0.066a	0.68 ± 0.057a
$CdCl_2$ rates			
0 mg kg^{-1}	1.25 ± 0.154a	0.50 ± 0.081a	0.62 ± 0.069a
1 mg kg^{-1}	1.33 ± 0.154a	0.44 ± 0.081a	0.62 ± 0.069a
2 mg kg^{-1}	1.42 ± 0.154a	0.54 ± 0.081a	0.54 ± 0.069a
Depth			
0-20 cm	1.43 ± 0.126a	0.58 ± 0.066a	0.71 ± 0.056a
20-40 cm	1.24 ± 0.126a	0.41 ± 0.066a	0.48 ± 0.056b

Different letters in the columns for a given location, species, rates or depths indicate significant differences according to Tukey's test ($P < 0.05$).

Cadmium fixation in the soil

The highest percentage of Cd fixation in the soil was found in Santa Rosa at both applied rates (Table 6). Compared with 1 mg kg^{-1} $CdCl_2$, the percentage of fixation in the soil at 2 mg kg^{-1} rate was 1.7, 1.2, and 1.9 times higher for Pan de Azúcar, Santa Rosa, and Carillanca, respectively (Table 6) although at Santa Rosa there was nonsignificant difference. When comparing among species, at Pan de Azúcar and Carillanca, Cd fixation in white lupine was 1.1 and 1.2 times higher respectively than narrow-leafed lupine, slightly only at Santa Rosa, narrow-leafed lupine showed 1.04 times greater Cd fixation than white lupine when applying 1 and 2 mg kg^{-1} $CdCl_2$ (Table 6). Nevertheless at any location there were significant differences among species.

DISCUSSION

Dry matter production

Narrow-leafed lupine is an early- maturating crop compared to white lupine and thus is better adapted to low rainfall areas. In fact, DM production of narrow-leafed lupine was significantly ($P < 0.05$) higher than white lupine (4.55 *vs*. 3.26 Mg ha^{-1}) in Pan de Azúcar with 73 mm of precipitation. At the locations with higher precipitation (Santa Rosa, 670 mm; Carillanca, 880 mm), narrow-leafed lupine production was slightly higher than in Pan de Azúcar, but did not surpass white lupine, which was approximately three times higher.

Soil properties are also important factors to understand the growth of lupine species. According to (Funayama-Noguchi et al., 2014) both narrow-leafed and white lupines grow poorly on alkaline soils, probably as a consequence of their sensitivity to Fe deficiency, high bicarbonate concentrations in the soil solution, excess Ca, and high pH. Other study indicate that white lupine grows better in soils with neutral to alkaline pH (Martínez-Alcalá et al., 2010). Narrow-leafed lupine preferably thrives in sandy soils because its root system is characterized by few secondary roots compared with other *Lupinus* species (Funayama-Noguchi et al., 2014). Furthermore, it does not tolerate oxygen deficiency and high clay content (Lambers et al., 2013).

The applied Cd rates in the present study did not affect DM production (Table 3). A similar finding was reported by Zornoza et al. (2002) where changes in the white lupine growth parameters occurred only in the highest Cd concentration under study (45 µM), which reduced DM production in the shoots more than in the roots. This same study reported that the Cd supply altered nodules formation and decreased plant N content, which suggests that white lupine has developed several defense strategies against Cd, such as high Cd retention by cell walls and the formation of complex thiol groups, which both contribute to decrease the level of free Cd.

Table 6. Soil Cd fixation (%) in Pan de Azúcar, Santa Rosa, and Carillanca for two lupine species and three $CdCl_2$ rates.

Treatment	Pan de Azúcar	Santa Rosa	Carillanca
Species			
White lupine	56.01 ± 7.15a	70.25 ± 5.10a	61.38 ± 7.11a
Narrow-leafed lupine	50.40 ± 7.15a	73.19 ± 5.10a	48.37 ± 7.11a
$CdCl_2$ rates			
1 mg kg^{-1}	39.05 ± 7.15a	65.13 ± 5.10a	37.02 ± 7.11a
2 mg kg^{-1}	67.36 ± 7.15b	78.31 ± 5.10a	72.73 ± 7.11b

Different letters in the columns for a given location, species or rates indicate significant differences according to Tukey's test ($P < 0.05$).

Total plant Cd concentration and uptake

White and narrow-leafed lupine showed Cd concentrations of 123 and 303.5 µg kg^{-1}, respectively, which are considered as low compared to hyperaccumulator species; Cd hyperaccumulator plants are defined as species that accumulate at least 100 mg Cd kg^{-1} (0.01% dry weight) (Sarma, 2011). Total plant Cd concentration in white lupine (Table 3) is lower than those found by Ximénez-Embún et al. (2002), who reported concentrations of up to 4.9 g kg^{-1} DM when the crop was cultivated in sand contaminated with a 50 mg L^{-1} $Cd(NO_3)_2$ solution. However, narrow-leafed lupine concentration is higher than in a study reported by Brennan and Bolland (2003), who evaluated yellow lupine (*Lupinus luteus* L. 'Wodjil') and narrow-leafed lupine 'Kalya' with different sources of phosphate fertilizers and different Cd concentrations as an impurity and found up to 24 µg kg^{-1} DM in narrow-leafed lupine.

The Cd uptake in white and narrow-leafed lupine was 0.62 and 1.33 g ha^{-1}, respectively, in the above-ground biomass. It is known that white lupine retain up to 88% of total Cd extracted in the roots (Zornoza et al., 2002). A study conducted with radionuclides (^{109}Cd) in white lupine shows that Cd distribution persisted 75% in the root 28 d after treating the plants, while Cd that was translocated to the shoots was uniformly distributed among the leaves and was mainly concentrated in the central vein of the leaflets (Page et al., 2006). The above indicates that probably both lupine species have higher phytostabilization than phytoextraction potential, as was previously suggested by Vázquez et al. (2006) for white lupine.

White lupine, narrow-leafed lupine, and ryegrass Cd mobilization and uptake in the shoots were also evaluated by Römer et al. (2000), who found that in all three plant species, the Cd concentrations in root were much higher than in the shoot. In white lupine, only 6% Cd was apparently translocated to the shoot, whereas in the case of narrow-leafed lupine it was 29%-36%, and perhaps due to this, in our study, the Cd uptake in narrow-leafed lupine was higher compared with white lupine. Roots of both lupines secrete citrates and these compounds possibly form a complex with soil Cd that reduces Cd uptake in both lupine species. Actually Egle et al. (2003) when evaluated the influence of P fertilizer application on the quality and quantity of organic acid exuded, reported that narrow-leafed lupine excreted higher amounts of organic acids per root length and per hour than white lupine which could explain why Cd uptake was higher in narrow-leafed lupine than in white lupine.

White lupine is also highly tolerant to heavy metals and has low absorption and translocation of metals toward the shoots (Fumagalli et al., 2014); these results partly coincide with Cd absorption found in the present study. Ximénez-Embún et al. (2002) reported that the Cd distribution relationship between root/shoot in a study with white lupine indicated poor Cd translocation when compared to other evaluated metals, such as Pb(II), Cr(III), Cr(VI), CH_3Hg, and Hg(II).

Apparent Cd recovery and residual concentration in the soil

Cadmium recovery in both white and narrow-leafed lupine is low when compared with other species such as *Thlaspi caerulescens* J. Presl. & C. Presl. (McGrath et al., 2006) or *Brassica juncea* (L.) Czern. (Belimov et al., 2005), which are examples of hyperaccumulator plants with a high phytoextraction potential for Cd. For example, *T. caerulescens* can uptake up to 21% Cd found in the soil (McGrath et al., 2006). Results obtained in this study therefore indicate that narrow-leafed lupine exhibits interesting Cd recovery capacity that should be investigated for phytoremediation purposes in a larger number of soil and environmental conditions.

Residual soil Cd levels were higher in the present study in Pan de Azúcar, including control plots without Cd application (Table 6); this was associated with its higher initial soil Cd content, higher sand content, and lower OM content (Table 2). Loganathan et al. (2012) studied soil Cd distribution and uptake influenced by OM and soil type in a greenhouse experiment with ryegrass (*Lolium multiflorum* Lam.); the increase in exchangeable Cd fraction was directly proportional to adding OM at the expense of the oxide fraction bound to Fe-Mn. Adding OM to the soils reduced Cd concentration in ryegrass. At any level of added OM, the decrease in Cd concentration in ryegrass was sand > sandy loam > clay loam. Pan de Azúcar had a lower OM content than Santa Rosa and Carillanca, which could explain why Pan de Azúcar had the highest plant Cd concentration (Figure 1b), Cd uptake (Table 4), and the lowest percentage of soil Cd fixation (Table 6). However, it must also be considered that this location exhibited the highest initial soil Cd concentration (Table 2), which is directly associated with plant Cd absorption (Lux et al., 2011).

Cadmium fixation in the soil

Given that pH is the main characteristic influencing solubility and availability of elements for plant absorption, it could influence soil Cd fixation because lower soil pH increases heavy metal concentration in the solution by decreasing it adsorption. Many metal cations, such as Cd, Cu, Hg, Pb, and Zn, are reported to be more soluble and available in the low pH soil solution (< 5.5) (Bhargava et al., 2012).

Buekers et al. (2007) studied the effect of time and soil properties on Cd, Cu, Zn, and Ni fixation in soils (increased fraction of added metals that were not isotopically interchangeable) after different incubation times; they found that Cd fixation was higher at pH > 7 (20% to 60%), and fixation was explained because pH diffusion depended on oxides and/or carbonate co-precipitation. However, this is contradictory because Santa Rosa, with pH 5.7 (Table 2), was the environment that exhibited the highest Cd fixation for the evaluated rates in spite of had uptake a larger quantity of Cd, and it even showed a higher Cd apparent recovery than Carillanca and Pan de Azúcar (Figure 1). The explanation of why Santa Rosa fixed more Cd could be explain due to in acid soils, carboxylates may bound to Fe(Al)- surfaces and may increase the negative surface charge as well as Cd sorption whilst in calcareous soils, where the Cd sorption capacity is high, Cd complexation by citrate may increase the Cd solubility (Jones, 1998; Römer et al., 2000). Due to the above Cd fixation in Pan de Azúcar was less and its fixation could be partly explained by soil OM content, and by the lower soil Ca concentration for Carillanca (Table 2) (Lux et al., 2011). Other explanation for the lower Cd fixation in the first soil layer obtained in Carillanca is the lower bulk density (Table 2) associate to its volcanic origin and to higher presence of amorphous clays and oxides-hydroxides of Fe and Al, which could increase the positive surface charge and decrease the Cd sorption (Neall, 2006).

Temminghoff et al. (1995) studied the effects of ionic strength and Ca concentration in Cd adsorption in sandy soil; they found that Cd adsorption was 60% to 80% lower in a 0.01 M $Ca(NO_3)_2$ solution than in a $NaNO_3$ solution with the same ionic strength, and this was attributed to the competition existing between Cd and Ca. This competition between Cd and Ca could explain the lower Cd fixation obtained in Pan de Azúcar when compared with the other environments (Table 2). Degryse et al. (2009) evaluated the effect of changing the Ca concentration (at constant ionic strength) on metal concentrations in a soil extract (L/S = 10 L kg^{-1}) with pH 4.3 and 7.1; they also pointed out that Zn and Cd concentrations increased as Ca concentrations increased, which suggests that there was competition of Ca absorption on the surface of the exchange sites.

CONCLUSIONS

Applying Cd to the soil in three contrasting environments did not affect aerial DM production in both studied lupine species. Narrow-leafed lupine exhibited higher Cd uptake than white lupine. Cadmium uptake capacity in both lupines and in the evaluated environments was very low and corresponded to 0.027% of applied Cd in white lupine and 0.056% in narrow-leafed lupine, however this quantity allows to use this species in Cd phytoremediation because of its low risk to the food chain. The effect of residual applied Cd in the three environments, lupine species, and Cd rates indicated that high soil Cd fixation exists, which fluctuated between 37% and 78.3% for the three soils under study.

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