Absorption and distribution of cadmium of three maize hybrids in three environments

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Abstract

Anthropogenic soil contamination with heavy metals has received much attention in recent years, especially cadmium (Cd), which is a very toxic element for human health and its exposure is mainly through contaminated food. Maize (*Zea mays* L.) is one of the most important cereals in the human diet that is characterized as species whose cultivars differ in Cd accumulation. Therefore, identifying and selecting low Cd-accumulating genetic material will contribute to reducing its ingestion. Among the agricultural crops that are important for Cd in the human diet is maize. Cadmium contents in three maize cultivars were grown under different environments conditions in Chile where soils were enriched with increasing Cd rates, were evaluated. Grain yield, Cd concentration in different plant tissues, and soil post-harvest, were evaluated. Results showed that grain yield was not affected by soil Cd; however, plant tissues generally exhibited differences in Cd concentration associated with the environment, La Serena showed the highest grain Cd accumulation ($30 \mu\text{g kg}^{-1}$; $P < 0.05$). In addition, among cultivars, Pioneer showed the highest grain Cd concentration ($19.5 \mu\text{g kg}^{-1}$; $P < 0.05$). Grain Cd concentration of the three maize cultivars were within the range cited in the bibliography as not toxic.

Keywords: Cadmium, maize, Chilean soils, *Zea mays*

1. Introduction

There is a growing concern regarding food security because of environmental pollution. Anthropogenic soil contamination by heavy metals has been produced mainly by industry or agriculture, e.g., foundries, metalliferous mine waste, pesticides, fertilizers, and municipal organic waste (Siebers *et al.*, 2014). Cadmium is one of the heavy metals that is usually found in low concentrations in the soil (Rothbaum *et al.* 1986; Quezada-Hinojosa *et al.*, 2015; Seshadri *et al.*, 2015). It is toxic for living organisms and carcinogenic for human beings (Liu *et al.*, 2015; Seshadri *et al.*, 2015). Although it is not an essential plant nutrient, Cd can be absorbed in larger quantities than other elements, such as cobalt and nickel, with no adverse effects on growth (Eshghi and Ranjbar, 2014; Seshadri *et al.*, 2015). Cadmium also interacts with the metabolism of three essential metals: Ca, Zn, and Fe, generating their low intake by means of substitution and increase of competitive mobility with other elements (Goyer, 1997; Mora *et al.*, 2016). The human body can absorb Cd through food, especially leaves and grains, water, or air: it accumulates and persists for a long time causing health problems (Liu *et al.*, 2015). The World Health Organization (WHO) has considered a daily Cd intake of $0.83 \mu\text{g kg}^{-1}$ body weight or $58.1 \mu\text{g Cd}$ per person as toxic for human beings (WHO, 2010). Cadmium accumulates in the liver and kidneys and has a long biological half-life of 17 to 30 y in humans. Its toxicity involves two organ systems, kidney and skeleton, and is largely the result of interactions between Cd and essential metals, particularly generating low Ca intake (Goyer, 1997). The European Union has identified maximum Cd concentrations of some agricultural products as 50, 100, 200, and $200 \mu\text{g kg}^{-1}$ for fruit, root vegetables, wheat, and lettuce, respectively (Berg and Litch, 2002).

Crop and cultivar species differ widely in their ability to absorb, accumulate, and tolerate Cd (Yang *et al.*, 2014). Among the agricultural crops that are important for Cd in the human diet are, durum wheat (*Triticum turgidum* L. var. durum), maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), oat (*Avena sativa* L.), barley (*Hordeum vulgare* L.), rice (*Oryza sativa* L.), and pea (*Pisum sativum* L.); these have exhibited Cd concentrations over the limits permitted for human health (Fahad *et al.*, 2015; Quezada-Hinojosa *et al.*, 2015).

The amount of soil Cd mainly depends on its origin, and physical and chemical properties, such as clay, acidity, salinity, Zn, and organic matter (OM) content. Phosphorus and N fertilization, organic amendment applications, exposure to contamination sources, crop rotation, and management practices are the main sources of increased soil Cd content (Siebers *et al.*, 2014). As for the soil Cd concentration considered as a risk, various authors point out that the value ranges from 0.8 to $1.0 \text{ mg Cd kg}^{-1}$ (Quezada-Hinojosa *et al.*, 2015). A similar value is indicated by Segura *et al.* (2006) for soils in Chile.

Another factor affecting both Cd availability and uptake by plants is root exudation of organic acids, such as citrate and malate (Adeniji *et al.*, 2010; Seshadri *et al.*, 2015). White lupin (*Lupinus albus* L.) is defined as a species that exudes elevated levels of organic acids that can increase soil Cd availability, but restricts Cd transport from the roots to the stems and grains (Tejo *et al.*, 2016).

Given that the world population and its demand for food is increasing over time (FAO, 2015), a larger area is needed for agricultural use. Some of the available soils could have total Cd concentrations close to or greater than the 1 mg kg^{-1} critical level; this will require species and cultivars exhibiting lower Cd

accumulation and translocation. To contribute in providing maize cultivars that exhibit low Cd accumulation in both grain and straw, the objective of the present study was to evaluate the response of three maize cultivars grown with increasing Cd rates in three different agroclimatic zones of Chile.

2. Materials and Methods

2.1. Climatic and soil characteristics of each environment

The environments in which the field experiments were conducted, located in three contrasting agro-

climatic zones: i) La Serena (30°3' S, 71°14' W), colluvial-alluvial soil (Typic Haplocambids; USDA, 2014), arid climate with maritime influence, and 40 mm precipitation concentrated in winter; ii) Los Tilos (33°34' S, 70°37' W), alluvial soil (Haploxeroll; USDA, 2014), semi-arid and temperate Mediterranean climate with hot and dry summer, cold winter, and 163 mm precipitation; and iii) Chillán (36°31' S, 71°54' W), volcanic soil (Melanoxerand; USDA, 2014), temperate Mediterranean climate with hot and dry summer, cold and humid winter, and 672 mm precipitation concentrated in winter and beginning of spring (Table 1) (Red Agrometeorológica de INIA, 2013).

Table 1. Climatic characteristics of each environment during the 2013 season.

	La Serena			Los Tilos			Chillán		
	Tm	pp	Ev	Tm	Pp	Ev	Tm	pp	Ev
January	19.5	0.0	60.0	20.7	0.0	138.1	19.9	1.2	134.4
February	19.8	0.0	47.9	18.8	0.0	105.8	18.5	19.3	101.9
March	16.6	0.0	103.6	17.0	0.0	88.4	15.1	4.1	78.5
April	14.1	0.0	62.5	13.4	0.0	36.6	12.4	6.1	40.6
May	12.3	61.1	37.1	11.4	0.0	20.9	9.4	183.0	21.5
June	10.4	8.2	22.9	7.3	39.1	16.5	7.3	123.7	12.4
July	9.9	5.7	28.7	8.2	4.5	20.3	7.1	110.1	16.1
August	11.3	0.5	47.2	9.3	35.8	34.6	8.2	128.0	20.8
September	12.9	0.3	71.1	11.3	5.7	45.6	9.7	49.9	52.6
October	13.5	0.2	102.0	14.3	0.1	74.1	12.7	35.7	86.4
November	15.6	7.8	78.7	16.4	0.0	125.9	15.2	11.0	123.1
December	17.8	0.0	142.6	19.2	0.0	146.9	19.1	0.0	156.7
Total accumulation	--	83.8	804.3	--	85.2	853.7	--	672.1	845.0

Tm, mean temperature (°C); pp, precipitation (mm); Ev, evaporation (mm)

The physico-chemical properties of each soil analyzed at the start of the experiment are shown in Table 2. The samples were collected at two depths (0 - 0.2 and 0.2 - 0.4 m) and the physico-chemical analyses were performed according to the methodology indicated by Sadzawka *et al.* (2006). Soil pH was measured in a 1:2.5 (soil/water) ratio. Organic matter was estimated by the Walkley-Black wet digestion method. Soil available N ($\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$) was previously extracted with 1 M KCl and determined by colorimetry in a Skalar Auto Analyzer (segmented flow spectrophotometer). Available P was extracted with 0.5 M NaHCO_3 (Olsen-P) and determined by the ascorbic acid-molybdate method. Exchangeable Ca, Mg, K, and Na were extracted with 1 M ammonium acetate and measured using flame emission spectrometry (K and Na) and atomic absorption (Ca and Mg) spectrometry (AAS). The soil exchangeable Al concentration was determined by extraction with 1 M KCl and AAS. Concentrations of DTPA extractable Fe, Mn, Zn, and Cu were determined (Lindsay and Norvell, 1978) and by AAS. Boron was determined by colorimetry after acid digestion. 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 maize 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 (Tejo *et al.*, 2016).

Table 2. Soil physical and chemical properties (0 - 0.2 and 0.2 - 0.4 m depths).

Parameters	Environments and depths (m)					
	La Serena		Los Tilos		Chillán	
	0-0.2	0.2-0.4	0-0.2	0.2-0.4	0-0.2	0.2-0.4
Clay (%)	20.2	20.3	21.5	27.3	20.7	15.9
Silt (%)	30.2	31.2	50.0	49.3	43.6	45.4
Sand (%)	49.6	48.5	28.5	23.4	35.7	38.7
Bulk density (g cm^{-3})	1.76	1.80	1.20	1.24	1.00	1.05
pH (soil:water 1:5)	6.94	6.87	8.25	8.19	5.74	5.76
Organic matter (g kg^{-1})	11.6	11.3	19.6	21.7	63.0	56.2
EC (dS m^{-1})	0.15	0.23	0.11	0.15	0.11	0.07
Available N (mg kg^{-1})	18.0	20.0	11.0	14.0	40.0	38.0
Olsen-P (mg kg^{-1})	51.3	44.9	3.9	5.1	35.2	25.3
Exchangeable K (cmolc kg^{-1})	0.85	0.67	0.35	0.41	0.65	0.39
Exchangeable Ca (cmolc kg^{-1})	8.12	8.22	20.70	19.66	6.74	5.89
Exchangeable Mg (cmolc kg^{-1})	2.41	2.61	0.92	0.86	0.95	0.72
Exchangeable Na (cmolc kg^{-1})	0.59	0.69	0.49	0.40	0.16	0.19
Exchangeable Al (cmolc kg^{-1})	0.05	0.05	0.04	0.04	0.21	0.10
Available Fe (mg kg^{-1})	21.5	20.8	16.7	17.0	59.8	46.5
Available Mn (mg kg^{-1})	36.3	34.3	11.8	11.5	9.8	5.4
Available Zn (mg kg^{-1})	4.5	4.4	1.1	1.2	0.7	0.6
Available Cu (mg kg^{-1})	9.2	9.3	8.9	8.8	1.4	1.2
Available B (mg kg^{-1})	2.3	2.4	0.8	0.8	0.5	0.4
Available S (mg kg^{-1})	40.8	64.9	11.9	13.5	14.2	15.4
Total Cd (mg kg^{-1})	1.33	1.49	0.52	0.51	0.21	0.18

EC, electrical conductivity

2.2. Cadmium rates and maize cultivars

Cadmium was applied as CdCl_2 (61.3% Cd) at rates of 0, 1, and 2 mg kg^{-1} adjusted for a 0 - 0.2 m soil depth and considered the bulk density of each soil (Table 2). The equivalent amount of Cd applied at the 1 and 2 mg kg^{-1} CdCl_2 rates in each environment was: i) La Serena 2157.76 and 4315.52 g ha^{-1} , respectively; ii) Los Tilos 1593.8 and 3187.6 g ha^{-1} , respectively, and iii) Chillán 1226.0 and 2452.0 g ha^{-1} , respectively.

The maize cultivars used in the study were Syngenta NK 703 in La Serena and Los Tilos and Syngenta NK Exp in Chillán; Pioneer P 32 D12 in La Serena and Los Tilos and Pioneer P 37 W 05 in Chillán; Dekalb DK 627 in La Serena and Los Tilos and Dekalb DK 469 in Chillán. These cultivars were

selected because the Syngenta, Pioneer, and Dekalb genotypes exhibit different genetic characteristics, and different responses would be expected.

2.3. Agronomic management of the experiment

Agronomic management practices were standardized for all the environments using Chilean norms for this crop. The N, P, and K fertilization rates were 360, 120, and 120 kg ha⁻¹, respectively, and fertilizer sources were urea, triple superphosphate, and potassium chloride. Nitrogen was applied 30% and 70% at sowing and the sixth leaf stage, respectively. Both P and K were applied 100% at sowing.

Each experimental unit consisted of five 3-m long rows with 0.6 m row spacing (9 m²). The cultivated area at each environment was 223 m² considering three Cd rates, three maize cultivars, and three replicates for each experimental unit. Experiments were sown on 8, 17, and 24 October in La Serena, Los Tilos, and Chillán, respectively. The sowing rate was 8 seeds m⁻¹ at the three environments. The seedbed was prepared by ploughing at a depth of 0.3 m followed by the surface cultivator and two crosswise harrowings at 45°.

Six or seven irrigation events were applied after sowing up to the milk stage of kernel development to complement precipitation accumulated between October and December 2013 (8.0, 0.1, 46.7 mm in La Serena, Los Tilos, and Chillán, respectively, Table 1). From 50 to 60 mm were applied during each irrigation event; this maintained adequate soil moisture to ensure good crop development. The pre-emergent herbicide was a mixture of atrazine and s-metolachlor (Primagran Gold 660 SC) that was applied at 6.0 L ha⁻¹ at the post-emergence stage to control dicotyledonous weeds. The chlorpyrifos insecticide (Pirinec 48 EC) was applied at 5.0 L ha⁻¹ before sowing to control larvae in the soil. The incidence of diseases and

insects was very low and leaf fungicides and insecticides were not used.

Plots were harvested on 22, 24, and 29 April 2014 in La Serena, Los Tilos, and Chillán, respectively.

2.4. Grain yield, and Cd analysis in soil and plant tissue

When grains reached 15% moisture content, the crop was harvested and GY determined. Samples of plants with roots were collected from 1.0 m² in each experimental plot; these were then separated into grain, straw, and root. The separated samples were washed with distilled water and oven-dried at 70 °C for 72 h. At the end of the experiment, 10 soil samples were collected from each plot at the 0 - 0.2 and 0.2 - 0.4 m depths; these were air-dried and passed through a 2 mm sieve to determine total Cd.

2.5. Statistical analysis

A split-split-plot experimental design was used where main plot was environment (3), split plots were Cd rates (3), and split-split-plots were maize cultivars (3); each treatment was triplicated.

The results were analyzed by ANOVA. A mean separation test was then performed (Tukey, $P = 0.05$) using the SAS general model procedure (SAS Institute, 1989).

3. Results

Grain yield fluctuated between 5.5 and 15.5 Mg ha⁻¹; the highest value was obtained in La Serena followed by Chillán and both had higher GY than Los Tilos. Grain yield was affected only by environment ($P < 0.05$, Figure 1) and by the interaction between the Cd rate and maize cultivars ($P < 0.05$, Table 3), while the Cd rate, cultivars, and resulting interactions with environments did not affect this parameter (Table 3).

Figure 1. Maize grain yield at three environments as a mean of different cultivars and Cd rates.

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

Table 3. Significance levels of evaluated parameters for experiments conducted with three maize hybrids fertilized with three CdCl₂ rates at three environments.

Parameter	Li	Rii	Ciii	L×R	L×C	R×C	L×R×C
Grain yield	**	NS	NS	NS	NS	*	NS
Straw DM production	**	NS	NS	NS	**	NS	NS
Root DM production	**	NS	**	NS	**	NS	NS
Grain Cd content	**	**	NS	*	**	NS	NS
Straw Cd content	**	**	NS	**	NS	**	NS
Root Cd content	**	**	NS	**	NS	NS	NS
Soil Cd from 0-0.2 m	**	**	NS	**	*	NS	NS
Soil Cd from 0.2-0.4 m	**	**	NS	*	NS	NS	NS

Li, environments (3) La Serena, Los Tilos, and Chillán; Rii, Cadmium rates (3) 0, 1, and 2 mg kg⁻¹; Ciii, maize hybrids (3) Syngenta, Pioneer, and Dekalb.

NS, nonsignificant, * $p < 0.05$, ** $p < 0.01$.

Grain Cd content was affected by the environment, Cd rate, and the Environment × Cd Rate and Environment × Cultivar interactions (Table 3). For environment or environment, the highest mean value for grain Cd content was obtained in La Serena (30.0 µg kg⁻¹) ($P < 0.05$), which was greater than in Los Tilos (12.7 µg kg⁻¹) and Chillán (12.0 µg kg⁻¹), which were not different ($P > 0.05$) (Figure 2a). When comparing Cd rates (mean values of environments and cultivars), the highest grain Cd content was obtained with 2 mg kg⁻¹ CdCl₂ (21.2 µg kg⁻¹) and 1 mg kg⁻¹ CdCl₂ (15.5 µg kg⁻¹) rates; there were nonsignificant differences one from the other ($P > 0.05$) and both were higher than the control where Cd was not applied (4.3 µg kg⁻¹) ($P < 0.05$) (Figure 2b). Regarding the cultivars (mean values of environments and Cd rates), it was observed that grain Cd contents in Pioneer (14.6 µg kg⁻¹), Dekalb (13.1 µg kg⁻¹), and Syngenta (13.0 µg kg⁻¹) were non-significantly different one from the other ($p > 0.05$) (Figure 2c). The interactions that affected grain Cd content variability had a greater effect in La Serena (Figure 2). Straw Cd content was affected by environment, Cd rate, and the Environment × Cd Rate and Cd Rate × Cultivar interactions (Table 3). For agroclimatic zones (mean values of cultivars and Cd rates), the highest straw Cd content was obtained in Los Tilos (3356.4 µg kg⁻¹) followed by Chillán (2037.5 µg kg⁻¹) and there were non-significant differences between the two ($p > 0.05$); however, both were significantly higher than La Serena (1283.7 µg kg⁻¹) ($p < 0.05$) (Figure 2a).

Figure 2. Cadmium content in the maize plant for a) different environments, b) cadmium rates, and c) hybrids.

For each figure, different uppercase letters over the bars indicate significant differences in the same plant structure (grain, straw, roots) compared for a) environments, b) cadmium rates, and c) maize hybrids according to Tukey's test ($p < 0.05$).

For each figure, different lower-case letters over the bars indicate significant differences in the same plant structure (grain, straw, roots) for each a) environment, b) cadmium rates, and c) maize hybrid according to Tukey's test ($p < 0.05$).

For the Cd rates (mean values of the environment and cultivars), the highest straw Cd content was obtained with 2 mg kg⁻¹ CdCl₂ rate that was significantly higher than 1 mg kg⁻¹ CdCl₂ rate ($p < 0.05$); both were significantly higher than the control with no Cd ($p < 0.05$). For the 2, 1, and 0 mg kg⁻¹ CdCl₂ rates, values were 3395.6, 1883.2, and 203.8 µg kg⁻¹, respectively (Figure 2b). For cultivars (mean values of environments and Cd rates), values of straw Cd content sorted in descending order were Dekalb (2017.4 µg kg⁻¹), Pioneer (1947.2 µg kg⁻¹), and Syngenta (1618.0 µg kg⁻¹) and these were non-significantly different one from the other ($p > 0.05$) (Figure 2c). The interactions in straw Cd content indicated that the highest variability associated with using different Cd rates was 5895.8 µg kg⁻¹ of Cd in stem residue in Los Tilos (data not shown). Root Cd content was affected by environment, Cd rate, and the Environment × Cd Rate interaction (Table 3). When considering the environment (mean values of cultivars and Cd rates), the highest root Cd content was found in La Serena (2252.8 µg kg⁻¹) and it was significantly different from the other two environments ($p < 0.05$); Chillán (439.8 µg kg⁻¹) and Los Tilos (283.8 µg kg⁻¹) were non-significantly different one from the other ($p > 0.05$) (Figure 2a). For Cd rates (mean values of environments and cultivars), the highest root Cd contents were obtained with the 2 and 1 mg kg⁻¹ CdCl₂ rates; there were non-significant differences between the two ($p > 0.05$) with values of 1215.6 and 945.3 µg kg⁻¹, respectively, which significantly surpassed ($p < 0.05$) the control (273.0 µg kg⁻¹, Figure 2b). Regarding the cultivars (mean values of environments and Cd rates), root Cd concentrations were 907.8 µg kg⁻¹ in Dekalb, 816.9 µg kg⁻¹ in Pioneer, and 709.3 µg kg⁻¹ in Syngenta

and there was non-significant difference among them ($p > 0.05$) (Figure 2c). The interactions in the root Cd concentration exhibited higher variability associated with the environment where the mean concentration found in La Serena was 5.1 and 7.9 times higher than in Chillán and Los Tilos, respectively (Figures 2a, 2b).

For differences in the tissues of a single plant, usually from the same agroclimatic zone, the highest Cd concentration was in the straw, with the exception of La Serena where the root had the highest Cd concentration; grain and root Cd concentrations were non significantly different ($P > 0.05$) among environments (Figure 2a). For the same Cd rate, the effect was similar to the abovementioned observations (Figure 2b). Finally, the highest concentration for any one cultivar was also in the straw, but the values were non-significantly different one from the other (Figure 2c).

Total soil Cd at harvest in both the first and second soil layers (0 - 0.2 and 0.2 - 0.4 m) was affected by the environment, Cd rate, and Environment $\times$ Cd Rate interaction; only the first layer was affected by the Environment $\times$ Cultivar interaction (Table 3). When comparing environments for the first soil layer (0 - 0.2 m), the highest total Cd concentrations were obtained in Los Tilos and La Serena and there were significant differences between the two ($P > 0.05$) with values of 1.97 and 1.95 mg kg⁻¹, respectively; these values were also significantly higher ($p < 0.05$) than in Chillán where total Cd concentration was 0.81 mg kg⁻¹ (Table 3 and Figure 3a). Different Cd rates (Table 3 and Figure 3b) showed that total soil Cd concentration was significantly equal for the two applied Cd rates (2 and 1 mg kg⁻¹ CdCl₂) ($P > 0.05$) whose values were 2.13 and 1.48 mg kg⁻¹, respectively, while the control value (0.5 mg kg⁻¹ Cd) was only significantly different at the highest Cd rate ($P < 0.05$) (Figure 3b).

Figure 3. Soil total cadmium content in two soil depths at maize crop harvest for different a) environments, b) cadmium rates, and c) hybrids.

For each figure, different uppercase letters over the bars indicate significant differences at the same soil depth compared for a) environments, b) cadmium rates, and c) maize hybrids according to Tukey's test ($p < 0.05$).

For each figure, different lower-case letters over the bars indicate significant differences between soil depths for each a) environment, b) cadmium rates, and c) maize hybrid according to Tukey's test ($p < 0.05$).

When comparing environments for the second soil layer (0.2 - 0.4 m), the highest total Cd concentrations were obtained in La Serena (1.82 mg kg⁻¹) and Los Tilos (0.81 mg kg⁻¹); there were non-significant differences between the two ($p > 0.05$). On the other hand, the value in Chillán (0.45 mg kg⁻¹) was quite lower than the other environments, but only showed significant differences with La Serena ($p < 0.05$) (Figure 3a). Mean values obtained by applying the different Cd rates, 0, 1, and 2 mg kg⁻¹ CdCl₂ were 0.47, 0.97, and 1.27 mg kg⁻¹, respectively. The highest total Cd concentration in the second soil layer occurred, as expected, when applying the 2 mg kg⁻¹ CdCl₂ rate, which only surpassed the control value ($p > 0.05$); the value obtained with the 1 mg kg⁻¹ rate showed nonsignificant differences when compared to either the control or the highest applied CdCl₂ rate ($p > 0.05$) (Figure 3b).

The concentration in the second soil depth showed non-significant differences ($P > 0.05$) among cultivars (Figure 3c); this is similar to the result obtained in the first soil layer (0 - 0.20 m).

4. Discussion

Maize grain yields (GY) obtained in La Serena and Chillán (Figure 1) were similar to GY found by other authors for this species under adequate agronomic management conditions. However, GY obtained in Los Tilos was lower than the value recorded by these authors and lower than expected in the study area (Liu *et al.*, 2013; Shah *et al.*, 2016). The limitations of GY for this environment are mainly due to the lack of heat accumulation during the development period (Table 1); this coincides with findings described by Liu *et al.* (2013) who observed a significant correlation between GY and mean temperature and accumulation of degree-days.

Maize grain Cd concentration (Figures 2a, 2b, 2c) showed values within the range cited by Yang *et al.*

(2014); this is the mean value of Cd found in the present study, 0.015 mg kg⁻¹ (mean of 27 values that consider three environments, three Cd rates, and three cultivars). Yang *et al.* (2014) obtained a mean grain Cd concentration of 0.03 mg kg⁻¹ (mean of nine Cd rates). Grain Cd concentration values for all the treatments in the present study did not surpass the allowable limits of 0.2 mg kg⁻¹ pointed out by several authors (Arduini *et al.*, 2014). Maize grain Cd concentration values, obtained by averaging cultivars and environments, were lower in all cases than those pointed out by Yang *et al.* (2014) for different maize cultivars (Figures 2a, 2b, 2c). Several authors have pointed out that maize grain Cd concentration is below the detection limit of 0.002 mg kg⁻¹ (Wahsha *et al.*, 2014). Therefore, the low grain Cd accumulation could be due to several factors: 1) uptake and translocation limitations generated in the root (Adeniji *et al.*, 2010; Yang *et al.*, 2014), which suggests that maize plants appear to have more efficient defence mechanisms than other crops to deal with Cd toxicity, including its accumulation in the root (Adeniji *et al.*, 2010); 2) soil available Zn concentration since Zn is an antagonist to plant Cd uptake (Tanwir *et al.*, 2015); and 3) low Cd concentration could be attributable to the high agronomic efficiency of nutrient use (kg DM produced per kg of applied nutrient) obtained in this experiment (data not shown) compared with other studies cited in the literature, which implied an overall nutrient dilution effect (Fahad *et al.*, 2015). However, applying increasing Cd rates produced an increase of more than 100% in maize grain Cd accumulation when compared with the control with no applied Cd (Arduini *et al.*, 2014; Yang *et al.*, 2014). Although differences among environments were found in the controls, this fact could be related to the initial total soil Cd concentration (Table 2); this has been reported by numerous authors (Degryse *et al.*, 2009; Yang *et al.*, 2014). On the other hand, differences in grain Cd concentration

detected among environments could be due to higher mineral use efficiency (Arduini *et al.*, 2014). When comparing cultivars, grain Cd concentration in the controls, in addition to being low, was similar in the different evaluated cultivars (Syngenta, Pioneer and Dekalb); this contributes in selecting genetic material with low Cd accumulation for the agroclimatic conditions in Chile.

Regarding the straw Cd concentration, Wahsha *et al.* (2014) cite values of 0.021 and 0.058 mg kg⁻¹ as the mean Cd concentration in stems and leaves, respectively. Mean straw Cd values obtained in the present study in La Serena, Los Tilos, and Chillán were higher than those reported by Wahsha *et al.* (2014), which was probably due to the initial soil Cd concentration as indicated by Putwatana *et al.* (2015). Maize straw Cd concentration for the mean of cultivars was also higher than those mentioned by Wahsha *et al.* (2014); however, Cd was applied in two of the three treatments in the present study, which could have affected the abovementioned mean concentration values (Putwatana *et al.*, 2015; Yang *et al.*, 2014).

The highest root Cd concentration (mean of environments) was obtained in La Serena (Figure 2a), which can be associated with the initial soil Cd concentration (Table 2) (Degryse *et al.*, 2009). Results at the other two environments (Los Tilos and Chillán) did not coincide with those found by other authors, who indicate that roots always exhibit higher Cd concentrations than other parts of the plant (Wahsha *et al.*, 2014; Putwatana *et al.*, 2015; Tanwir *et al.*, 2015); this suggests that Cd distribution in the plant could be related to the cultivar (Yang *et al.*, 2014). Likewise, root Cd concentration in Los Tilos would have been expected to be higher than in Chillán (Figure 2a) because it was associated with higher soil Cd concentration (Table 2). However, soluble Cd compounds could have been immobilized by increases in pH (Degryse *et al.*, 2009), especially in values higher than

8.2 such as those found in Los Tilos (Table 2). It has also been observed that roots can exude compounds that increase environment pH, decrease availability, and restrict Cd uptake (Tanwir *et al.*, 2015). On the contrary, another factor that would explain low Cd accumulation in Chillán for grain, stem, and root could be due to greater competition between the H⁺ and Cd⁺² cations in the uptake sites on the root surface (Larsson and Asp, 2013). A directly proportional response was found between the applied Cd rate and root Cd concentration (Figure 2b); results were similar to those reported by Liu *et al.* (2013), and values corresponded to an increase equivalent to 3.5 and 4.5 times compared with the control when applying 1 and 2 mg kg⁻¹ CdCl₂, respectively. In general, the residue had higher Cd concentration compared to grain and root (Figure 2a, 2b, and 2c). In this regard, similar results were observed in other crops as higher Cd concentrations in stems of lettuce (*Lactuca sativa* L.) and amaranth (*Amaranthus caudatus* L.) by Egwu and Agbenin (2013) and wheat (*Triticum aestivum* L.) by Siebers *et al.* (2014). Also, these results were similar to those of Liang *et al.* (2005), he found higher Cd concentration in residue than in the rest of maize plants. This higher Cd concentration in residue would indicate a higher Cd transference rate to the aerial part influenced by a decrease of sap flow, which would generate an increase of Cd concentration in xylem by exposure of this crop to high concentrations of this metal, as stated by Liang *et al.* (2005). These higher concentrations in aerial parts expose animals and human beings to this heavy metal. So, these results would help us to discriminate these cultivars for animal feeding decreasing the transference risk of this heavy metal to human being. For cultivars, the lowest root Cd concentration was obtained in Syngenta; Pioneer surpassed Syngenta and Dekalb by 15% and 11%, respectively (Figure 2c); however, there was nonsignificant difference among cultivars ($p < 0.05$).

Total Cd concentrations in the first soil layer (0 - 0.2 m) of the present study were similar to those cited by some authors for soils with different agricultural use (Rothbaum *et al.*, 1986; Quezada-Hinojosa *et al.*, 2015) (Figures 3a, 3b, 3c), and also similar to those cited by Segura *et al.* (2006) for different soils in Chile. Values were lower at 1 mg kg^{-1} , which is identified as the critical soil Cd level (Quezada-Hinojosa *et al.*, 2015). When comparing environments, higher total Cd concentration was found in La Serena (Figure 3a), which could be associated with its initial Cd content (Table 2) and available Zn concentration (Degryse *et al.*, 2009; Fahad *et al.*, 2015). Even though initial soil total Cd concentration in La Serena was higher than in Los Tilos and Chillán (Table 2), this order was not maintained when concentrations were analyzed at the end of crop growth (Figure 3a). This situation could be explained by the formation of complexes between the metal and OM (Degryse *et al.*, 2009); taking into account that Chillán had a higher OM concentration than the other environments, Cd availability is reduced (Table 2). When comparing Los Tilos and La Serena, the former would also be influenced by higher OM content than La Serena, which would reduce Cd availability (Degryse *et al.*, 2009). As expected, the Cd rate used was directly proportional to soil concentration (Figure 3b), which coincides with findings reported in the literature (Arduini *et al.*, 2014; Fahad *et al.*, 2015); both applied CdCl_2 rates were higher than the critical threshold of this element in soils (1 mg kg^{-1}). Regarding the maize cultivars under study, no effect was recorded on soil total Cd concentration (Figure 3c).

In general, the second soil layer (0.2 - 0.4 m) exhibited a similar effect as the one found in the first soil layer when comparing environments and Cd rates. However, concentrations and the magnitude of the differences were lower (Figures 3a, 3b). This effect could be attributable to the characteristics of

this element in the soil, which is mainly located at the surface (Rothbaum *et al.*, 1986). When comparing the environments, the Cd concentration was higher in Los Tilos than Chillán (Figure 3a), unlike the result in the first soil layer. This could be due to the higher salinity found in Los Tilos (Table 2).

5. Conclusions

Results show that the presence of Cd in the soil (baseline and applied) did not affect maize yield. In turn, the Cd concentration in both maize grain and straw was affected by crop environment and applied soil Cd. The Cd concentration in the roots was affected by the environment and also by applied soil Cd. In roots, Cd concentration was affected by environment and also by soil Cd application. The cultivars evaluated in the present study showed no differences in Cd concentration in none of the analyzed tissues, which does not allow the selection of a cultivar with a lower Cd accumulation for contaminated environments or at risk of being contaminated with this metal. The highest grain Cd concentration was found in La Serena environment ($30 \text{ } \mu\text{g kg}^{-1}$; $P < 0.05$) and in Pioneer cultivar ($19.5 \text{ } \mu\text{g kg}^{-1}$; $P < 0.05$), but in no case corresponded to the value considered as the limit (0.2 mg kg^{-1}). Finally, once the maize was harvested, soil total Cd concentration depended on the environment and the Cd rate used, and higher accumulation occurred in the first soil layer.

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