Universidad de Concepción
Dirección de Postgrado
Facultad de Agronomía - Programa de Doctorado en Ciencias de la Agronomía

Absorción y bioacumulación de Cadmio en tres cultivares de maíz (*Zea mays* L.) para diferentes medioambientes de Chile

Cadmium absorption and bioaccumulation in three cultivars of maize (*Zea mays* L.) for different environments of Chile

Tesis para optar al grado de Doctor en Ciencias de la Agronomía

JORGE ANDRÉS RETAMAL SALGADO
CHILLÁN-CHILE
2017

Profesor Guía: Dr. Juan Hirzel Campos
Departamento de Recursos Naturales
Instituto de Investigaciones Agropecuarias

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Aprobada por:

Juan Hirzel Campos
Ing. Agrónomo, Mg. Sc., Dr. Cs.
Profesor Guía

Erick Zagal Venegas
Ing. Agrónomo, Mg. Sc., Ph. D.
Profesor Co-Guía

Neal Stolpe Lau
Ing. Agrónomo, Mg. Sc., Ph. D.
Evaluador interno

Gonzalo Silva Aguayo
Ing. Agrónomo, Mg. Cs., Dr. Cs.
Director de Programa

DEDICATORIA

Al amor de mi vida, Cecilia, quien me apoyo durante toda mi carrera, entregándome preocupación, aliento, entusiasmo, energía y la confianza necesaria para poder concretar con éxito esta meta. A mi hijo Camilo, mi nueva energía para seguir en este camino.

A mi mamá Dori y segunda madre Eliana que jamas olvidare, por sus apoyos incondicionales, sin ellas jamás hubiese logrado concretar con éxito esta meta. A mi hermano Sebastian y mi sobrina Paula, por su cariño y apoyo.

A todos ellos, gracias por su amor, tolerancia y respeto.

AGRADECIMIENTOS

A mi profesor guía el Dr. Juan Hirzel Campos, por la entrega de conocimiento y exigencia rigurosa en el proceso de formación académica y científica, además por su valiosos consejos y sincera amistad. Por creer en mí y apoyar durante todo el proceso estudiantil.

A Fondecyt Chile por el apoyo financiero de la investigación, atraves del proyecto Nº 1120992.

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RESUMEN

La contaminación del suelo con metales pesados antropogénicos liberados desde la industria o la agricultura, ha recibido mucha atención en los últimos años. El Cadmio (Cd) es uno de los metales pesados que suele estar presente en los suelos, siendo tóxico para los organismos vivos, y es cancerígeno para los seres humanos. La Organización Mundial de la Salud (OMS) ha considerado tóxico para los seres humanos una concentración de Cd correspondiente a una ingesta diaria de 0.83 µg kg^{-1} de peso corporal o 70 µg Cd por persona. La severidad y el daño de estos metales dependen del tiempo, nivel de exposición, susceptibilidad de la persona. A pesar que el Cd no es un nutriente esencial para las plantas, este metal puede ser absorbido en mayor cantidad que otros elementos, sin efectos adversos en el crecimiento. Las especies de cultivos y cultivares difieren ampliamente en su capacidad de absorber, acumular y tolerar Cd. Dentro de los alimentos mas utilizados en la dieta humana y que han presentado una concentración de Cd en el grano por sobre los límites permitidos para la salud humana, destacan el trigo duro (*Triticum turgidum* L. var. Durum), maíz (*Zea mays* L.), trigo (*Triticum aestivum* L.), avena (*Avena sativa* L.), entre otros. En cuanto a la concentración de Cd en el suelo considerados de riesgo, diversos autores señalan que este valor corresponde a 0,8 – 1,0 mg kg^{-1} de C. La acidez o basicidad del suelo (pH) también afecta a la disponibilidad de Cd en el suelo para el cultivo. El enriquecimiento de los suelos pueden dar lugar a la transferencia involuntaria y acelerada de elementos traza a través de la cadena alimentaria. En granos de cultivos para el consumo humano se han mostrado diferentes concentraciones de Cd, con algunos de ellos sobre el límites para la salud humana. Dentro de la disponibilidad de suelos se podría encontrar algunos con concentraciones cercanas o mayores al nivel crítico de 1 mg kg^{-1} de Cd total, donde será necesario disponer de especies y cultivares que presenten una menor acumulación y translocación de este metal. En este trabajo se plantean las siguientes hipótesis: a) La absorción Cd por parte de la planta de maíz es afectada por la concentración de este metal presente en el suelo. b) La absorción y acumulación de Cd es diferencial según la estructura de la planta de maíz. c) La tasa acumulación de Cd en la planta de maíz dependerá del cultivar y la interacción con el medioambiente. Para dar respuesta a las hipótesis antes planteadas, se establecieron los siguientes objetivos específicos: a) Identificar los suelos utilizados para la agricultura que presentan riesgo de contaminación de Cd. b) Seleccionar híbridos de maíz que presentan menor riesgo tanto de

absorción y acumulación de Cd. c) Identificar cultivares de maíz de baja acumulación de Cd para las distintas zonas agroclimáticas de Chile.

Durante la temporada 2013-15 se realizaron tres experiencias de campo en diferentes medioambientes agrícolas de Chile. Los medioambientes fueron los siguientes; i) La Serena (30º3' S ; 71º14' W), suelo de origen aluvial-coluvial (Typic Haplocambids); ii) Los Tilos (33º34' S; 70º37' W), suelo de origen aluvial (Haploxeroll), de clima mediterráneo semiárido y templado; iii) Chillán (36º31' S; 71º54' W), suelo de origen volcánico (Melanoxerand), de clima mediterráneo templado. Para cada medioambiente se analizaron las propiedades físico-quimicas a dos profundidades antes de implementar los experiemntos (0 – 0.2 y 0.2 – 0.4 m), estos análisis se realizaron según la metodología indicada por Sadzawka et al. (2006). El Cd total del suelo y la planta se determinó por espectrofotometría de absorción atómica electrotermica (Técnica de horno de grafito) con el equipo Thermo elemental solar M5 acoplado a un horno de grafito modelo GF95. Las muestras fueron digeridas en un horno de microondas (MARS-Xpress, CEM Corporation, Matthews, Carolina del Norte, EE.UU.) antes de las lecturas por espectrofotometría. Para cada muestra de suelo, se pesó 0.5 g y se añadió 10 mL (Acido nítrico 65%, Acido Nítrico Suprapur, Merck Millipore, Darmstadt, Germany), en tanto, para las muestras de tejido vegetal, que se colocó en un tubo de digestión 1 g DM de maíz, y se le añadió 10 mL Suprapur HNO_3, + 1 mL al 30% de H_2O_2. El control de calidad para los análisis se basó en el material de referencia certificado (ISE 979 para suelo y IPE 981 para tejido vegetal), comparación de las muestras entre laboratorios, las muestras de control interno, y los duplicados (Trejo et al., 2016). El Cd fue aplicado a la forma de $CdCl_2$ (61.3% of Cd), cuya dosis de esta sal correspondió a 0, 1 y 2 mg kg^{-1}, ajustado para una profundidad de suelo de 0 a 0.2 m considerando la densidad aparente de cada suelo. Se utilizaron tres cultivares para cada mediambiente (Singenta, Pionner, Delkab), que poseen características genéticas diferentes, por lo cual se esperaría diferentes respuestas. Las practicas de manejo agronómico se estandarizaron para todos los lugares. Cuando los granos alcanzaron 15% de contenido de humedad, se realizó la cosecha del cultivo y se determinó la materia seca (MS) del grano, en raíz y residuo (tallo + hoja), y siguiendo la misma segmentación se determino el Cadmio total. Al final del experimento se colectaron 10 muestras de suelo de cada parcela a dos profundidades (0 – 0.2 y 0.2 – 0.4 m), estas se secaron al aire, posteriormente se molieron y se pasó por un tamiz de 2 mm, para determinar el Cd total.

Posteriormente con los resultados obtenidos, se realizaron correlaciones lineales simple y se

calculó el coeficiente de correlación para evaluar la relación entre el contenido de cadmio en el suelo y el desplazamiento relativo del cadmio desde las raíces a la parte aérea de la planta, para cada una de las medioambientes. Además se calculó los siguientes factores: a) Factor Bioconcentración y acumulación biológica. B) Factor translocación, c) Índice de tolerancia. El diseño experimental fue una parcela sub sub dividida, donde la parcela principal era el medioambiente (3), el de parcelas divididas fueron las dosis de Cd (3), y la parcela sub sub dividida fueron los cultivares de maíz (3), con 3 repeticiones. Los resultados fuerón analizados por ANOVA y la prueba de Tukey ($P = 0.05$) usando procedimiento de modelo general SAS (SAS Institute, Cary, North Carolina, USA).

Dentro de los principales resultados destacan que la producción de Materia seca (MS) en el residuo fue afectada significativamente por el medioambiente y la interacción de ésta con el cultivar, fluctuando entre 7.0 y 14.7 Mg ha^{-1}. El consumo de Cd en planta entera sólo fue afectado por la dosis de Cd. El mayor consumo de Cd en planta entera fue obtenido con el uso de 2 mg kg^{-1} de $CdCl_2$ (34.4 g ha^{-1}) ($P<0.05$). La distribución de Cd entre las tres estructuras de la planta de maíz, fue mayor en el residuo ($P<0.05$), independiente del medioambiente, dosis de Cd y cultivar evaluado. Según los resultados obtenidos en este estudio, se puede concluir que la producción de materia seca no varió según los distintos grados de contaminación de cadmio en el suelo en los distintos medioambientes evaluados. En todos los tratamientos, los mayores niveles de concentración y extracción de cadmio obtubierón en la parte aérea, principalmente en el residuo (tallo y hojas). Entre los ambientes evaluados, la mayor concentración de Cd en el grano se observó en La Serena, pero en ningún caso fue superior a los limites establecidos. Según los valores obtenidos de TF ($TF > 2$) y BAF ($BAF > 1$) en el medioambiente de Los Tilos y Chillán, estos medioambientes se clasificarían con alta capacidad de contaminación de la cadena alimentaria para los cultivares evaluados y los distintos grados de contaminación de cadmio en el suelo, por lo cual, en dichos medioambientes se debería realizar mayores estudios sobre la absorción y la translocación de metales pesados a la parte aérea del cultivo, principalmente de cultivares utilizados como fuente de alimentos para los animales destinados a consumo humano.

ABSTRACT

Soil contamination with anthropogenic heavy metals released from industry or agriculture has received much attention in recent years. Cadmium (Cd) is one of the heavy metals that is usually present in soils, being toxic to living organisms, and is carcinogenic to humans. The World Health Organization (WHO) has considered a concentration of Cd corresponding to a daily intake of 0.83 μg kg^{-1} body weight or 70 μg Cd per person to be toxic to humans. The severity and damage of these metals depends on the time, level of exposure, susceptibility of the person. Although Cd is not an essential nutrient for plants, this metal can be absorbed in more amounts than other elements, without adverse effects on growth. Crop species and cultivars differ widely in their ability to absorb, accumulate and tolerate Cd. Among the foods most used in the human diet and which have presented a concentration of Cd in the grain above the limits allowed for human health, stand out durum wheat (*Triticum turgidum* L. var. Durum), maize (*Zea mays* L .), Wheat (*Triticum aestivum* L.), oats (*Avena sativa* L.), among others. Concerning the concentration of Cd in the soil considered of risk, several authors indicate that this value corresponds to 0,8 - 1,0 mg kg-1 of Cd. The acidity or basicity of the soil (pH) also affects the availability of Cd in the soil for the crop. The enrichment of soils can lead to the involuntary and accelerated transfer of trace elements through the food chain. In grain crops for human consumption different concentrations of Cd have been shown, with some of them on the limits of human health. Within the availability of soils one could find some with concentrations close to or greater than the critical level of 1 mg kg^{-1} of total Cd, where it will be necessary to have species and cultivars that have a lower accumulation and translocation of this metal. Therefore, the following hypotheses are considered: a) Cd absorption by the maize plant is affected by the concentration of this metal presented by the soil. B) The absorption and accumulation of Cd is differential according to the organ of the corn plant. C) The accumulation rate of Cd in the maize plant will depend on the cultivar and the interaction with the environment. In order to respond to the above hypotheses, the following specific objectives were established: a) Identify the soils used for agriculture that present a risk of contamination of Cd. B) Select maize cultivars that present a lower risk of both absorption and Accumulation of Cd. C) To identify cultivars of low accumulation Cd maize for the different agroclimatic zones of Chile. During the 2013-15 season, three field experiences were carried out in different agricultural environments in Chile. The

environment was as follows; I) La Serena (30º3 'S; 71º14' W), soil of alluvial-coluvial origin (Typic Haplocambids); Ii) Tilos (33º34 'S; 70º37' W), soil of alluvial origin (Haploxeroll), with semi-arid and temperate Mediterranean climate; Iii) Chillán (36º31 'S; 71º54' W), soil of volcanic origin (Melanoxerand), temperate Mediterranean climate. For each environment the physico-chemical properties were analyzed at two depths prior to the implementation of the experiments (0 - 0.2 and 0.2 - 0.4 m), these analyzes were performed according to the methodology indicated by Sadzawka et al. (2006). The total soil and plant Cd was determined by electrothermal atomic absorption spectrophotometry (graphite furnace technique) with the thermo elemental solar M5 equipment coupled to a graphite furnace model GF95. The samples were digested in a microwave oven (MARS-Xpress, CEM Corporation, Matthews, North Carolina, USA) prior to spectrophotometric readings. For each soil sample, 0.5 g was weighed and 10 mL (65% nitric acid, Suprapur Nitric Acid, Merck Millipore, Darmstadt, Germany) was added, whereas, for plant tissue samples, it was placed in a digestion tube 1 g DM of corn, and 10 mL Suprapur HNO_3, + 1 mL at 30% H_2O2 was added. Quality control for the analyzes was based on the certified reference material (ISE 979 for soil and IPE 981 for plant tissue), comparison of samples between laboratories, internal control samples, and duplicates. The Cd was applied to the form of $CdCl_2$ (61.3% of Cd), whose dose of this salt corresponded to 0, 1 and 2 mg kg^{-1}, adjusted for a soil depth of 0 to 0.2 m considering the bulk density of each soil. Three cultivars were used for each environment (Syngenta, Pioneer, Dekalb), which have different genetic characteristics, which would allow different responses. Agronomic management practices were standardized for all locations. When the grains reached 15% of moisture content, the crop was harvested and the dry matter (DM) of the grain was determined in root and residue (stem + leaf), and following the same segmentation the total cadmium was determined. At the end of the experiment 10 soil samples from each plot were collected at two depths (0 - 0.2 and 0.2 - 0.4 m), dried in the air, then ground and passed through a 2 mm sieve to determine the Cd total. Subsequently with the results obtained, simple linear correlations were made and the correlation coefficient was calculated to evaluate the relationship between the cadmium content in the soil and the relative displacement of cadmium from the roots to the aerial part of the plant, for each one of the environment. In addition, the following factors were calculated: a) Factor Bioconcentration and biological accumulation. B) Translocation factor, c) Tolerance index. The experimental design was a sub sub divided plot, where the main plot was the environment (3), the split plots were the

Cd (3) doses, and the sub sub split plot were maize cultivars (3), with 3 repetitions.

Results were analyzed by ANOVA and Tukey's test ($P = 0.05$) using SAS general model procedure (SAS Institute, Cary, North Carolina, USA).

Among the main ones, the production of dry matter (DM) in the residue was significantly affected by the environment and its interaction with the hybrid, fluctuating between 7.0 and 14.7 Mg ha^{-1}. Cd consumption in whole plant was only affected by the Cd dose. The highest Cd consumption in whole plant was obtained with the use of 2 mg kg^{-1} of $CdCl_2$ (34.4 g ha^{-1}) ($P <0.05$). The distribution of Cd among the three structures of the maize plant, it was observed that, in general, the residue concentrates the greatest accumulation of this metal ($P <0.05$), independent of the environment, dose of Cd and hybrid evaluated. According to the results obtained in this study, it can be concluded that dry matter production does not vary according to the different levels of cadmium contamination in the soil in the different evaluated environments. In all treatments, the highest levels of concentration and cadmium extraction were observed in the aerial part, mainly in the residue (stem and leaves). Among the evaluated environments, the highest concentration of Cd in the grain was observed in La Serena, but in no case was higher than the established limits.

According to the values obtained from TF (TF> 2) and BAF (BAF> 1) in the environment of Los Tilos and Chillán, these environments would be classified with high contamination capacity of the food chain for the evaluated cultivars and the different degrees of contamination Of cadmium in the soil. Therefore, in these environments, further studies should be carried out on the absorption and translocation of heavy metals to the aerial part of the crop, mainly cultivars used as a food source for animals for destination human consumption

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I. INTRODUCCIÓN GENERAL

Existe una creciente preocupación respecto a la seguridad alimentaria debido a la contaminación del medio ambiente, donde la contaminación del suelo con metales pesados antropogénicos liberados desde la industria o la agricultura, tales como las industrias de fundición, residuos de minas metalíferas, pesticidas, fertilizantes, abonos municipales, ha recibido mucha atención en los últimos años (Wuana and Okieimen, 2011; McDowell et al., 2013; Siebers et al., 2014; Wang et al., 2014). El Cadmio (Cd) es uno de los metales pesados que suele estar presente en los suelos (McDowell et al., 2013). Es tóxico para los organismos vivos, y es cancerígeno para los seres humanos (Fowler, 2009; Chang et al., 2012; McDowell et al., 2013; Liu et al., 2015a) e incluso letal (Chang et al., 2012).

El cuerpo humano puede absorber el Cd en porciones menores a través de la ingesta de alimentos, en especial las hojas y granos, el agua o el aire (Chang et al., 2012), donde se acumulan y permanecen durante mucho tiempo, causando algunos problemas a la salud (Goyer, 1997; Grant et al., 1998; Wångstrand et al., 2007; Chang et al., 2012; Liu et al., 2015a). La Organización Mundial de la Salud (OMS) ha considerado tóxico para los seres humanos una concentración de Cd correspondiente a una ingesta diaria de 0.83 µg kg^{-1} de peso corporal o 70 µg Cd por persona (OMS, 2010), y la Agencia para Sustancias Tóxicas y de Registro de Enfermedades de los Estados Unidos (ATSDR), señala una dosis de Cd de referencia de 5x10^{-4} mg kg^{-1}día^{-1} en el agua y 1x10^{-3} mg kg^{-1}día^{-1} en alimentos (ATSDR, 2012). El Cd se acumula en el hígado y los riñones, y tiene una vida media biológica larga, 17-30 años en el hombre. La toxicidad implica dos sistemas de órganos, renales y esqueléticas, y es en gran parte la consecuencia de las interacciones entre el Cd y metales esenciales, en particular calcio (Goyer, 1997; Wångstrand et al., 2007; Chang et al., 2012). La severidad y el daño de estos metales dependen del tiempo, nivel de exposición, susceptibilidad de la persona y además de la ruta por la cual el metal sea absorbido (Chang et al., 2012).

A pesar que el Cd no es un nutriente esencial para las plantas, este metal puede ser absorbido en mayor cantidad que otros elementos, sin efectos adversos en el crecimiento (Grant et al. 1998; Wang et al., 2014), y puede interactuar con el metabolismo de tres metales esenciales como el calcio, zinc y hierro (Goyer, 1997). Las concentraciones máximas Cd identificados por la Unión Europea de algunos productos agrícolas fueron de 50, 100, 200 y 200 mg kg^{-1} para las frutas, hortalizas de raíz, el trigo y la lechuga, respectivamente (Berg y Litcht, 2002). Las especies de cultivos y cultivares difieren ampliamente en su capacidad de absorber, acumular y tolerar Cd (Grant et al., 1998; Grant et al., 2008; Zhao et al., 2013; Yang et al., 2014). Entre los cultivos agrícolas importantes en la dieta humana, las principales especies que absorben y translocan Cd al grano son trigo duro (*Triticum turgidum* L. var. Durum), maíz (*Zea mays* L.), trigo (*Triticum aestivum* L.), avena (*Avena sativa* L.), cebada (*Hordeum vulgare* L.), arroz (*Oryza sativa* L.) y arveja (*Pisum sativum* L.), estos han presentado una concentración de Cd sobre los límites permitidos para la salud humana (Greger y Löfstedt, 2004; Cajuste et al., 2006;. Tanaka et al., 2007;. Tsyganov et al., 2007; Wångstrand et al., 2007).

En el suelo la cantidad de Cd depende principalmente de las propiedades físicas del suelo y las propiedades químicas, como el contenido de arcilla, acidez, salinidad, zinc (Zn) y materia orgánica (MO); la fertilización con fósforo (P) y nitrógeno (N); aplicaciones de enmiendas orgánicas; la exposición a fuentes de contaminación como la actividad industrial; la rotación de cultivos y prácticas de manejo (Chaudri et al., 1995; McLaughlin et al., 1997; Grant et al., 1998; Vásquez et al., 2006; Wångstrand et al., 2007; Chen et al., 2008; Grant et al., 2008; Bao et al., 2011; McDowell et al., 2013; Zhao et al., 2013; Siebers et al., 2014). En cuanto a la concentración de Cd en el suelo considerados de riesgo, diversos autores señalan que este valor corresponde a 0.8 - 1.0 mg kg^{-1} de Cd (Lehoczky et al., 2006; Quezada-Hinojosa et al., 2015) y no debe exceder 1.5 mg kg^{-1} de Cd total en los suelos (Liu et al., 2015b). Valor similar está indicado para suelos chilenos por Villanueva (2003) y Segura et al. (2006).

Otro factor que afecta tanto a la disponibilidad y absorción de Cd por las plantas es la exudación de la raíz de ácidos orgánicos tales como citrato y malato (Han et al., 2006; Grant et al., 2008; Adeniji et al., 2010; Bao et al., 2011; Xu et al., 2015). Existen especies que se caracterizan por su alta exudación de ácidos orgánicos, lo que puede aumentar la disponibilidad de Cd en el suelo, pero este restringe el transporte de Cd de las raíces hacia los tallos y granos (Page et al., 2006; Xu et al., 2015). Al respecto, Oliver et al. (1993), indican que la concentración de Cd en el grano de trigo fue mayor cuando se cultivan en rotación después de Lupino blanco (especie que se caracteriza por exudación de ácidos orgánicos) que después de cereales.

Dentro de los fertilizantes y enmiendas empleadas como fuentes de nutrientes a los cultivos, y que a su vez contribuyen al ingreso de Cd al sistema suelo-planta, una de los principales son los fertilizantes fosforados (Chaudri et al., 1995; Chen et al., 2008; Lehoczky et al, 2006; Pál et al., 2005), como también lodos y biosólidos urbanos (Chaudri et al., 1995; Pál et al., 2005; Walter et al., 2006).

El uso de fertilizantes nitrogenados con fuentes de amonio (reacción ácido), aumenta la disponibilidad de Cd en el suelo para los cultivos (Wångstrand et al., 2007). Por el contrario, el uso de fertilizantes con reacción básica como nitratos y sales de calcio contribuyen a reducir la disponibilidad de este metal para los cultivos.

El Cd en el suelo se fija por óxidos e hidróxidos a arcilla y materia orgánica, y su disponibilidad se puede cuantificar con extracciones utilizando HNO_3-HCl (agua regia). La fracción extraíble (Cd intercambiables, complejo de Cd con compuestos orgánicos y sales de carbonato) se determina con el agente quelante ácido dietilentriaminapentacético (DTPA), que representan la mayor fracción de la Cd disponible para la planta (Walter y Cuevas, 1999; Walter et al, 2002; Walter et al., 2006). Otro complejo importante para estimar la biodisponibilidad de Cd corresponde a la complejación-Cloro en la solución del suelo que podría conducir a aumentar la captación de Cd por los cultivos ya sea a través de una mayor difusión de Cd a las raíces a través del suelo (McLaughlin et al., 1997).

La acidez o basicidad del suelo (pH) también afecta a la disponibilidad de Cd en el suelo para el cultivo (Larson Jönson y Asp, 2013). En suelos con pH básico, y con aplicaciones de lodos compostados con alta concentración de Cd, como se ha señalado por Cuevas et al. (2003), producen una reducción de la disponibilidad Cd.

El enriquecimiento de los suelos pueden dar lugar a la transferencia involuntaria y acelerada de elementos traza a través de la cadena alimentaria (Chen et al., 2008). En granos de cultivos para el consumo humano se han mostrado diferentes concentraciones de Cd, con algunos de ellos sobre el límites para la salud humana (Greger y Löfstedt, 2004; Tanaka et al., 2007; Tsyganov et al., 2007). Chaudri et al. (1995) indican que la concentración de Cd en varias muestras de granos de trigo fluctuó entre 0,004 y 0,31 mg kg^{-1} con un valor medio de 0,03 mg kg^{-1}, lo que resulta en una ingesta diaria de aproximadamente 8 g Cd por persona o sobre 11% del límite indicado por la OMS.

Para los granos de trigo de invierno Wångstrand et al. (2007) han reportado concentraciones de Cd entre 0,01 y 0,09 mg kg^{-1}, muy por debajo del límite de 0,2 mg kg^{-1} indicado por la Comunidad Europea. A la vez, este autor señala concentraciones de Cd de 0,005 a 0,1 mg kg^{-1} en grano de avena, y de 0,05 a 0,06 mg kg^{-1} en la cebada. Además, el aumento en la aplicación de nitrógeno (N) al cultivo, generó un incremento en la concentración de Cd en los granos de trigo. Al respecto, Wångstrand et al. (2007) informó de concentraciones de Cd en granos de cereales que fluctuaron entre 0,01 y 0,09 mg kg^{-1}. Por otra parte, un experimento llevado a cabo en Suecia demostró que existen diferencias entre cultivares en la capacidad de acumular Cd en los granos (Greger y Löfstedt, 2004).

El grano de arroz tiene bajas concentraciones de zinc (Zn), hierro (Fe) y calcio (Ca) para la dieta humana, pero promueve la acumulación de Cd en el grano (Liu et al., 2015b).

En las plantas cultivadas, la absorción de Cd en altas concentraciones puede causar problemas en el crecimiento y el desarrollo. En un experimento realizado en macetas con tres tipos de suelo y plantas de maíz, la mayor concentración de Cd en el suelo, tanto fracciones solubles en agua e intercambiables, generó una menor producción de materia seca total (Cajuste et al., 2006). En cultivos de maravilla (*Helianthus annus* L), Cd en baja concentración inhibe el crecimiento de raíces y tallos, afectando la absorción de agua y nutrientes, el proceso de fotosíntesis y la actividad de varias enzimas, y también induce estrés oxidativo (Tsyganov et al., 2007). Al mismo tiempo, reduce la concentración de calcio (Ca), fósforo (P), sodio (Na) y manganeso (Mn) en las raíces. Por otro lado, se obtuvo aumento de la concentración de Cd en las raíces de las plantas no tratadas con este elemento desde 1,2 - 1,7 mg g^{-1} (Cd), hasta 427 a 520 mg g^{-1} en las plantas tratadas. En los tallos, la concentración de Cd aumentó desde 0,07-0,1 mg g^{-1} en planta sin tratar, hasta 21,4 a 51,9 mg g^{-1} en las plantas tratadas. Esto evidencia que en algunas especies existe menos translocación de Cd hacia las partes aéreas de la planta, ayudando a reducir el riesgo de contaminación del producto consumido por los organismos superiores (animales y humanos). Un efecto similar se observó en el maíz, que al aumentar la concentración de Cd de la solución nutritiva, genera una mayor concentración de este metal en todos los tejidos evaluados (Han et al., 2006).

En cultivos agrícolas la especie, variedad o cultivar también afecta a la absorción de Cd y la tolerancia a este metal, como se ha demostrado para arveja (*Pisum sativum* L.) (Tsyganov et al., 2007), maní (*Arachis hypogea* L.) (McLaughlin et al., 1997), tabaco (*Nicotiana tabacum* y *Nicotiana rustica*), el maíz (*Zea mays* L.) (Hinesly et al., 1978), el arroz (*Oryza sativa* L.) y soja (*Glisine max* L.) (Arao y Ishikawa, 2006), y el trigo duro (Grant et al., 2008; Stolt et al., 2006). Además, en los estudios de cultivares de trigo duro (*Triticum turgidum* L. var durum) difieren en la concentración de Cd absorbido, atribuible a diferencias en la translocación de la raíz a la parte area de la planta, en lugar de las diferencias en la absorción de la raíz (Greger y Löfstedt, 2004; Hirzel et al., 2016).

Una vez que el Cd es absorbido por la planta, la translocación puede ocurrir a través del xilema y el floema. Distribución de Cd dentro de la planta se ve influenciada por el transporte de las raíces a los brotes a través del xilema (Gallego et al., 2012). En este sentido, Tanaka et al. (2007) indicaron que la translocación de Cd (Cd^{109}) en plantas de arroz se produce principalmente a través del floema (91-100%), y que está asociado con aumentos en la concentración de sacarosa (azúcar transportado en el floema). A su vez, la capacidad de absorción de Cd desde el suelo y la tasa de translocación desde el suelo a la raíz, y luego a las partes aéreas de las plantas es diferente entre las especies (Lehoczky et al., 2006). Por ejemplo, la lechuga de cultivo (*Lactuca sativa* L.) presenta una mayor proporción de transferencia (velocidad de absorción) de Cd y una mayor concentración en los tejidos en comparación con las ballicas (*Lollium perenne* L.). Lupino blanco cultivado presenta una mayor concentración de Cd en las raíces en comparación con los tallos, aunque mostraron cuantitativamente una distribución similar, dado el aumento de la producción de materia seca en los tallos (Vásquez et al., 2006). Además, lupino presenta restricciones de transporte de Cd desde las raíces a los brotes, reduciendo la concentración de Cd en los granos, como se indicó para lupino blanco por Page et al. (2006).

Se ha observado en plantas de maíz (Han et al., 2006), que la adición de Zn a la solución disminuye la absorción de Cd. A su vez estos autores sugieren que hay sistemas similares de transporte entre el Zn y Cd en las raíces del maíz cultivado. En este sentido, Pál et al. (2005) señaló que el aumento las tasas de absorción de Cd en plantas de maíz, genera un estrés oxidativo, con aumentos en el ácido salicílico (molécula de síntesis endógena debido al estrés abiótico) y reducciones tanto en la eficiencia cuántica a nivel del fotosistema II, como en la síntesis de clorofila.

Considerando que la población mundial y su demanda de alimentos son crecientes en el tiempo (FAO, 2015), será necesario contar con mayor superficie de uso agrícola. Dentro de la disponibilidad de suelos se podría encontrar algunos con concentraciones cercanas o mayores al nivel crítico de 1 mg kg^{-1} de Cd total, donde será necesario disponer de especies y cultivares que presenten una menor acumulación y translocación de este metal. El objetivo del presente estudio será evaluar la respuesta de tres cultivares de maíz sembrados bajo diferentes dosis de Cd, en tres medioambientes de Chile.

HIPOTESIS

1. La absorción Cd por parte de la planta de maíz es afectada por la concentración de este metal presente en el suelo.
2. La absorción y acumulación de Cd es diferencial según la estructura de la planta de maíz.
3. La tasa acumulación de Cd en la planta de maíz dependerá del cultivar y la interacción con el medioambiente.

OBJETIVOS ESPECIFICOS

1\. Identificar el riesgo de contaminación de Cd en tres suelos de Chile usados para el cultivo de maíz..

2\. Seleccionar cultivares de maíz que presentan menor riesgo tanto de absorción y acumulación de Cd.

3\. Identificar cultivares de maíz de baja acumulación de Cd para las distintas zonas agroclimáticas de Chile.

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III. CAPITULO I

ABSORPTION AND DISTRIBUTION OF CADMIUM OF THREE MAIZE HYBRIDS IN THREE ENVIRONMENTS

Jorge Retamal-Salgado[a], Ivan Matus[b], Ingrid Walter[c] and Juan Hirzel[b*]

[a]Universidad de Concepción, Facultad de Agronomía, Avenida Vicente Méndez 595, Chillán, Chile.

[b]Instituto de Investigaciones Agropecuarias, INIA Quilamapu, Avenida Vicente Méndez 515, Chillán, Chile. [*]Corresponding author: jhirzel@inia.cl

[c]Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Apdo. Correos 8111, 28080 Madrid, España.

Publicado en Journal of Soils Science and Plant Nutrition, 2017, Volumen 17, número 2, pagínas 266-278.

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 is 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 µg kg^{-1}; $P < 0.05$). In addition, among cultivars, Pioneer showed the highest grain Cd concentration (19.5 µg kg^{-1}; $P < 0.05$).. Grain Cd concentration of the three maize cultivars were within the range cited in the bibliography as not toxic.

Key words: 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; Wang *et al*., 2014). Cadmium is one of the heavy metals that is usually found in low concentrations in the soil (McDowell *et al*., 2013). It is toxic for living organisms and carcinogenic for human beings (Chang *et al*., 2012; McDowell *et al*., 2013; Liu *et al*., 2015a). 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 (Jayakumar and Vijayarengan, 2014; Eshghi and Ranjbar, 2014). 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). 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 (Chang *et al*., 2012; Liu *et al*., 2015a). The World Health Organization (WHO) has considered a daily Cd intake of 0.83 µg kg^{-1} body weight or 58.1 µg Cd per person as toxic for human beings (WHO, 2010). The Agency for Toxic Substances and Disease Registry (ATSDR) of the United States establishes a Cd reference rate of $5\text{x}10^{-4}$ mg kg^{-1} d^{-1} in water and 1×10^{-3} mg kg^{-1} d^{-1} in food (ATSDR, 2012). 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; Chang *et al*., 2012). The European Union has identified maximum Cd concentrations of some agricultural products as 50, 100, 200, and 200 µg kg^{-1} for fruit, root vegetables, wheat, and lettuce, respectively (Berg and Litcht, 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 (Tanaka *et al*., 2007).

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 (McDowell *et al*., 2013; 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 mg Cd kg^{-1} (Clemens *et al*., 2013; Quezada-Hinojosa *et al*., 2015) and should not exceed 1.5 mg kg^{-1} total soil Cd (Liu *et al*., 2015b). 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). 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). Oliver *et al*. (1993) indicate that Cd concentration in the wheat grain was higher (until 0.02 mg kg^{-1}) when this crop followed white lupin in the rotation instead of cereals.

Given that the world population and its demand for food is increasing over time (Oxfam, 2013; 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 agroclimatic zones: i) La Serena (30º3’ S, 71º14’ W), colluvial-alluvial soil (Typic Haplocambids; USDA, 2014), arid climate with maritime influence, and 40 mm precipitation concentrated in winter; ii) Los Tilos (33º34’ S, 70º37’ 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 (36º31’ S, 71º54’ 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). 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 (NO_3-N and NH_4-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).

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 32D12 in La Serena and Los Tilos and Pioneer P 37W05 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 m^2). The cultivated area at each environment was 223 m^2 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 45º.

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 (Pirinex 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 m^2 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 ºC 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).

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} $CdCl_2$ (21.2 µg kg^{-1}) and 1 mg kg^{-1} $CdCl_2$ (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). For the Cd rates (mean values of the environment and cultivars), the highest straw Cd content was obtained with 2 mg kg^{-1} $CdCl_2$ rate that was significantly higher than 1 mg kg^{-1} $CdCl_2$ 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} $CdCl_2$ 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} $CdCl_2$ 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 × Cd Rate interaction; only the first layer was affected by the Environment × 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} $CdCl_2$) ($P > 0.05$) 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).

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} $CdCl_2$ 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} $CdCl_2$ 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 $CdCl_2$ 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). 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) and Xu *et al*. (2015); 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; Putwattana *et al*., 2015) or the limits allowed by the different health organizations, such as the Ministry of Health of the People's Republic of China (MSRPCH, 2012). 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) and Xu *et al*. (2015) 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; Xu *et al*., 2015). 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; McDowell *et al*., 2013; 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; Xu *et al*., 2015).

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; McDowell *et al*., 2013). 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 (Lysenko *et al*., 2014; 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 Du *et al*. (2014), 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} $CdCl_2$, 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) and Stritsis *et al*. (2012), who 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;

McDowell *et al.*, 2013) (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; Liu *et al.*, 2015b). 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 (Du *et al.*, 2014); 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 µg kg^{-1}; $P < 0.05$) and in Pioneer cultivar (19.5 µg 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.

ACKNOWLEDGEMENTS

This study was funded by Chile FONDECYT Project 1120992.

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Tables and figures

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 (ºC); pp, precipitation (mm); Ev, evaporation (mm).

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 ($cmol_c$ kg^{-1})	0.85	0.67	0.35	0.41	0.65	0.39
Exchangeable Ca ($cmol_c$ kg^{-1})	8.12	8.22	20.70	19.66	6.74	5.89
Exchangeable Mg ($cmol_c$ kg^{-1})	2.41	2.61	0.92	0.86	0.95	0.72
Exchangeable Na ($cmol_c$ kg^{-1})	0.59	0.69	0.49	0.40	0.16	0.19
Exchangeable Al ($cmol_c$ 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.

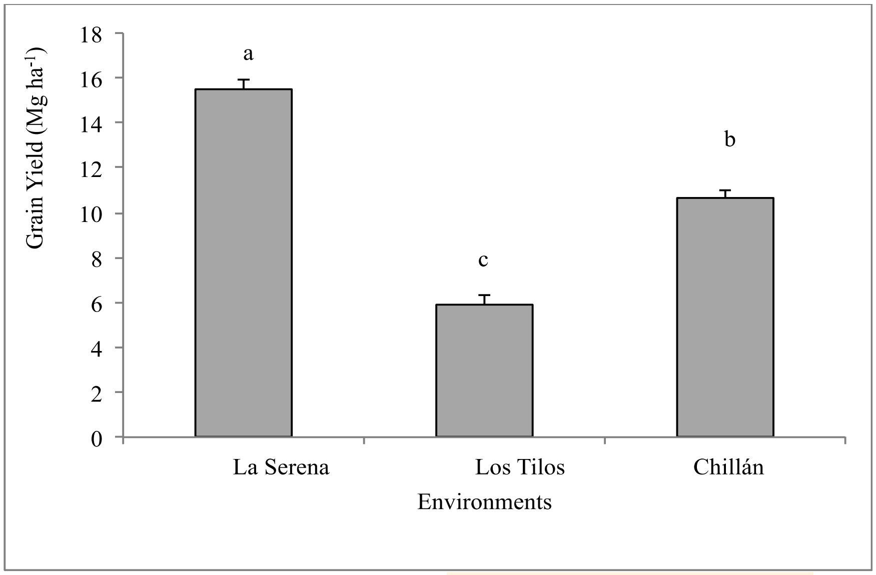
Table 3 Significance levels of evaluated parameters for experiments conducted with three maize hybrids fertilized with three $CdCl_2$ rates at three environments.

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.

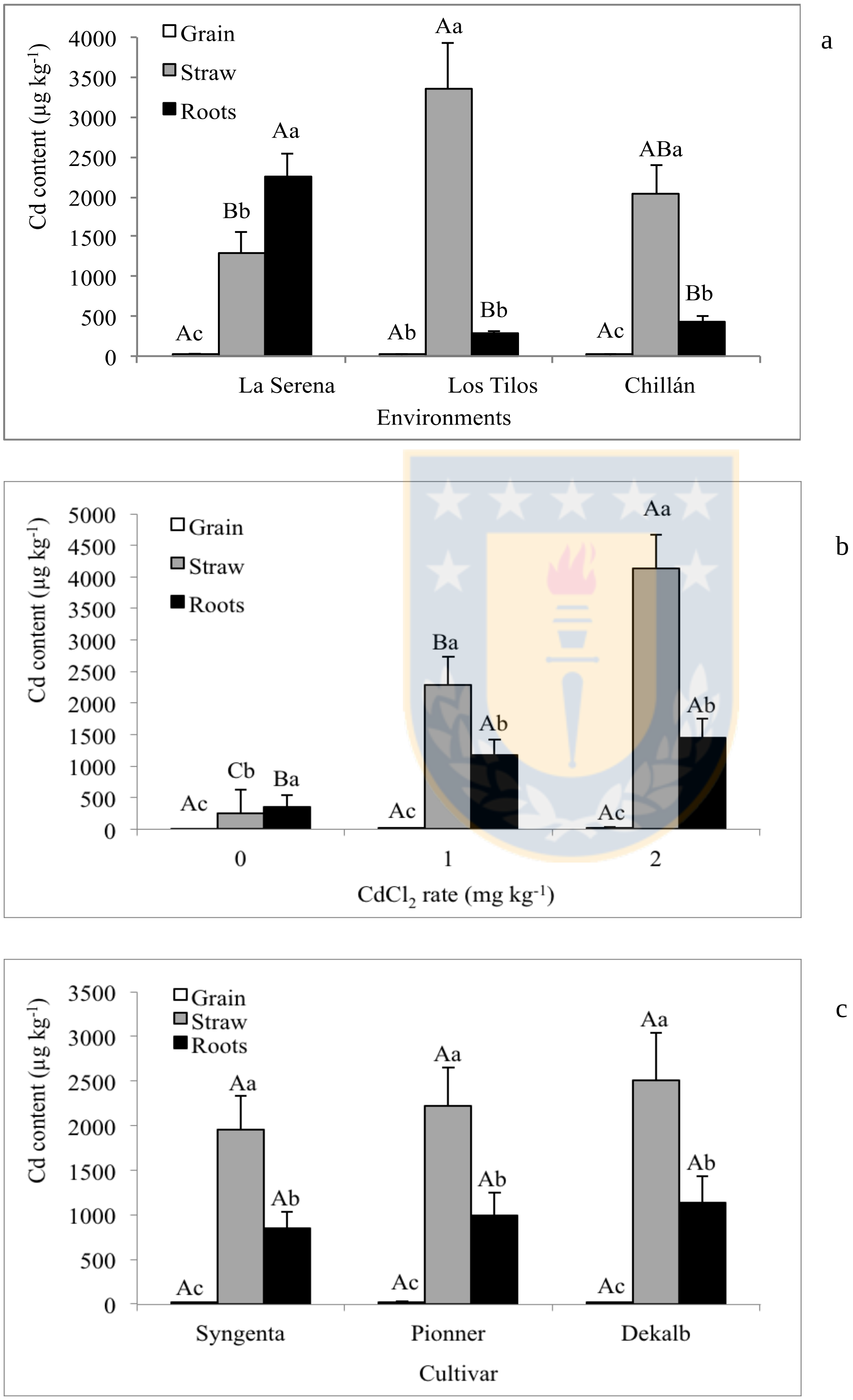
NS, nonsignificant, $*p < 0.05$, $**p < 0.01$.

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 ($p < 0.05$).

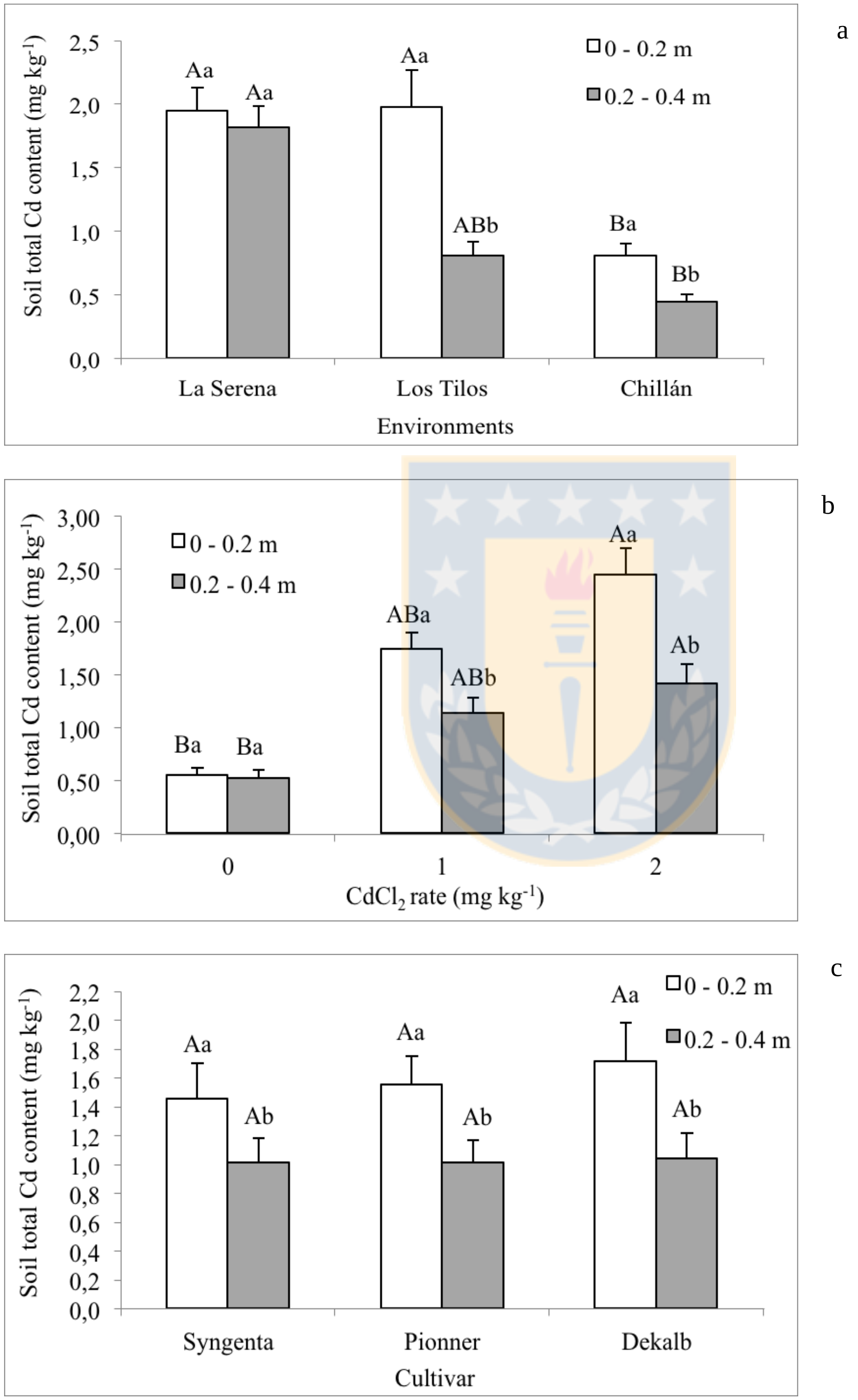
Figure 2. Cadmium content in the maize plant for a) different environments, b) cadmium rates, and c) hybrids.



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 ($p < 0.05$).

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 ($p < 0.05$).

IV. CAPITULO II

BIOABSORPTION AND BIOACCUMULATION OF CADMIUM IN THE STRAW AND GRAIN OF MAIZE (*ZEA MAYS* L.) IN GROWING SOILS CONTAMINATED WITH CADMIUM IN DIFFERENT ENVIRONMENT

Jorge Retamal-Salgado [1,2,3], Juan Hirzel [4,*], Ingrid Walter [5] and Iván Matus [4]

[1] Faculty of Agronomy, Universidad de Concepción, Vicente Méndez 595, Casilla 537, Chillán 3812120, Chile; jorgeretamal@unach.cl
[2] Doctoral Program in Agronomic Sciences, Faculty of Agronomy, Universidad de Concepción, Vicente Méndez 595, Casilla 537, Chillán 3812120, Chile
[3] Faculty of engineering and business, Universidad Adventista de Chile, km 12 camino a Tanilvoro, Chillán 3780000, Chile
[4] Instituto de Investigaciones Agropecuarias INIA, Avenida Vicente Méndez 515, Chillán 3800062, Chile; imatus@inia.cl
[5] Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Apdo. Correos 8111, Madrid 28080, Spain; inwal@hotmail.com

***** Correspondence: jhirzel@inia.cl; Tel.: +56-42-220-9774

Publicado en International Journal of Environmental Research and Public Healt, 2017, Volumen 14, Número 11, paginas 1-15.

Abstract: There is a worldwide increase of heavy metal contamination in agricultural soils caused mainly by human and industrial action, which leads to food contamination, such as in maize. Cadmium (Cd) is a heavy metal often found in soils and it is ingested through food. It is necessary to determine the bioabsorption, distribution, and accumulation levels in maize to reduce or prevent food chain contamination. Cadmium absorption and accumulation in three maize cultivars were evaluated in three agricultural environments in Chile by increasing $CdCl_2$ rates (0, 1, and 2 $mg \cdot kg^{-1}$). Evaluation included Cd accumulation and distribution in different plant tissues, bioaccumulation factor (BAF), bioconcentration factor (BCF), translocation factor (TF), and tolerance index (TI). Cadmium whole-plant uptake was only affected by the $CdCl_2$ rate; the highest uptake was obtained with 2 $mg \cdot kg^{-1}$ $CdCl_2$ (34.4 $g \cdot ha^{-1}$) ($P < 0.05$). Cadmium distribution in the maize plant usually exhibited the highest accumulation in the straw ($P < 0.05$), independently of the environment, Cd rate, and evaluated cultivar. Given the results for

TF (TF > 2) and BAF (BAF > 1), the Los Tilos and Chillán environments were classified as having a high capacity to contaminate the food chain for all evaluated cultivars.

Keywords: bioabsorption; heavy metals; translocation factor, tolerance index, food chain contamination.

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1. Introduction

There is a worldwide increase of heavy metal contamination in agricultural soils caused mainly by human and industrial action, which has generated concern for food contamination by these metals [1]. Heavy metals present in soils cannot be degraded; cadmium (Cd) is one of these heavy metals that is usually found in different soil types [2]. Cadmium is not an essential plant nutrient; however, this metal can be absorbed in greater amounts than other elements without any adverse effects to growth [3]. It interacts with the metabolism of other essential metals, such as calcium, zinc, and iron [4-6] and can be bioaccumulated and/or biotransformed by plants [3].

Cadmium intake through the food chain can become toxic for living organisms and is carcinogenic for human beings [7]. Cadmium can be absorbed in human beings through food, especially leaves and grains; it accumulates in the liver and kidneys for more than 30 years and causes health problems [7,8]. Toxicity of this metal involves kidney and skeletal organs and is largely the result of interactions between Cd and essential metals, such as calcium [8]). A daily intake of 1 mg·kg^{-1} body weight is considered toxic for human beings [9]. On the other hand, the soil Cd concentration considered to pose a risk is 1.0 mg·kg^{-1} [10,11].

Crops and cultivars usually differ in their capacity to absorb, accumulate, and tolerate Cd [12,13]. Among the agricultural crops that are important in the human diet, the main species that absorb and translocate Cd to the grain are hard wheat (*Triticum turgidum* L. var. durum), maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), oat (*Avena sativa* L.), and rice (*Oryza sativa* L.). These have exhibited Cd concentrations over the maximum permitted for human health [14-17]. The amount of Cd in the soil depends mainly on physical and chemical properties, such as fertilization with phosphorus (P) and nitrogen (N), organic amendment applications, and exposure to sources of contamination [1,12,18,19].

Among the important agricultural crops, more specifically cereals, maize is pointed out as the second most important as for cultivated area worldwide. Given an increasing demand for food, a larger land area accessible for agricultural use will be necessary in which the total available Cd concentration is not over the critical 1 mg·kg^{-1} level [20,21]. At the same time, a tendency to high Cd concentrations in the grain has been observed in new cereal crop cultivars worldwide. According to Cd distribution in the different plant organs, more than 40% of Cd is absorbed and translocated to the aerial part of the plant (grain and straw), and it could be directly (grains) or indirectly (animals) ingested and negatively affect humans [22-24]. Therefore, soils with higher Cd concentrations associated with the cultivation of cultivars with higher Cd accumulation capacity in the aerial part will generate higher risk of ingesting this metal. To reduce food chain contamination, the present study evaluated plant Cd absorption, distribution or translocation, and accumulation in different maize cultivars cultivated in different environments and with increasing soil Cd rate treatments.

2. Materials and Methods

2.1. Climatic and soil characteristics of each environment

Three field experiments were conducted during the 2013-2014 season in different agricultural environments in Chile. The three environments and their characteristics were as follows: La Serena (30º3’ S; 71º14’ W) has soil of alluvial-colluvial origin (Typic Haplocambids) [25], an arid climate with a maritime influence, and had 40 mm precipitation concentrated during the winter. Los Tilos (33º34’ S; 70º37’ W) has soil of alluvial origin (Haploxeroll) [25], a semi-arid and temperate Mediterranean climate with hot and dry summers and cold winters, and had 163 mm precipitation. Chillán (36º31’ S; 71º54’ W) has soil of volcanic origin (Melanoxerand) [25], temperate Mediterranean climate with hot and dry summers and cold and humid winters and reached 672 mm precipitation concentrated during the winter and beginning of spring [26].

The physical and chemical properties of each soil are shown in Table 1. Soil samples were collected at two depths (0-0.2 and 0.2-0.4 m) and the physical and chemical properties of each soil analyzed at the start of the experiment are displayed in Table 1; analyses were performed according to the methodology indicated by Sadzawka et al. [27]. Soil pH was measured in a ratio of 1:2.5 soil:water solution with a pH meter. Soil organic matter (OM) was measured by the Walkley-Negro wet digestion method. Available soil N (NO_3-N and NH_4-N) was extracted with

KCl_2 1M and was determined by colorimetry in a Skalar autoanalyzer (segmented flow spectrophotometer). Available P in the soil sample was determined by 0.5M $NaHCO_3$ (Olsen P) using the ascorbic acid molybdate method. Exchangeable calcium (Ca), magnesium (Mg), potassium (K), and sodium (Na) were determined by extraction of 1M Ac. NH_4O followed by flame spectroscopy: absorption (Ca and Mg) and emission (K and Na). The concentration of exchangeable soil aluminum (Al) was determined through extraction of KCl 1M by absorption spectroscopy. Soil concentrations of iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu) were determined in the DTPA extract [28] by atomic absorption spectrometry (AAS). Boron (B) was determined by colorimetry in a solution obtained by acid digestion. Total soil and plant Cd was determined by electro-thermal atomic absorption spectrophotometry (graphite furnace technique) with Thermo elemental solar M5 equipment coupled to a graphite furnace model GF95. Samples were digested in a microwave oven (MARS-Xpress, CEM Corporation, Matthews, North Carolina, USA) before the spectrophotometry readings. For each soil sample, 0.5 g was weighed and 10 mL nitric acid 65% (Suprapur, Merck, Darmstadt, Germany) was added. For the plant tissue samples, 1 g DM maize was placed in a digestion tube and 10 mL Suprapur HNO_3, + 1 mL 30% H_2O_2 was added. Quality control for the analyses was based on certified reference material (ISE 979 for soil and IPE 981 for plant tissue), sample comparison between laboratories, internal control samples, and duplicates [29].

Table 1. Soil physical and chemical properties (0 to 0.2 and 0.2 to 0.4 m depth).

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 ($g \cdot cm^{-3}$)	1.76	1.80	1.20	1.24	1.00	1.05
pH ($_{soil:water\ 1:2.5}$)	6.94	6.87	8.25	8.19	5.74	5.76
Organic matter ($g \cdot kg^{-1}$)	11.6	11.3	19.6	21.7	63.0	56.2
EC * ($dS \cdot m^{-1}$)	0.15	0.23	0.11	0.15	0.11	0.07
Available N ($mg \cdot kg^{-1}$)	18.0	20.0	11.0	14.0	40.0	38.0

P Olsen (mg·kg^{-1})	51.3	44.9	3.9	5.1	35.2	25.3
Exchangeable K (cmol·kg^{-1})	0.85	0.67	0.35	0.41	0.65	0.39
Exchangeable Ca (cmol·kg^{-1})	8.12	8.22	20.70	19.66	6.74	5.89
Exchangeable Mg (cmol·kg^{-1})	2.41	2.61	0.92	0.86	0.95	0.72
Exchangeable Na (cmol·kg^{-1})	0.59	0.69	0.49	0.40	0.16	0.19
Exchangeable Al (cmol ·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 genotipes

Cadmium was applied as $CdCl_2$ (61.3% Cd) and the rate of this salt was 0, 1, and 2 mg kg^{-1} adjusted for a 0-0.2 soil depth taking into account the apparent density of each soil (Table 1). The equivalent amount of Cd applied at the 1 and 2 mg kg^{-1} rates of $CdCl_2$ in each environment was: La Serena 2,157.76 and 4,315.52 g·ha^{-1}, respectively, Los Tilos 1,593.8 and 3,187.6 g·ha^{-1}, respectively, and Chillán 1,226.0 and 2,452.0 g·ha^{-1}, respectively.

The maize cultivars used in the present study were Syngenta NK 703, Pionner P32D12, and Dekalb DK 627 in La Serena and Los Tilos and Syngenta NK exp, Pionner P 37W05, and Dekalb DK 469 in Chillán. These selected Syngenta, Pionner, and Dekalb cultivars have different genetic characteristics because companies have different genetic lines, which allows assessing the cultivar on the parameters to be evaluated in the plant.

2.3. Agronomic management of the experiment

Agronomic management practices were standardized for all the locations. The fertilization rate with N, P, and K were 360, 120, and 120 kg·ha^{-1}, respectively, and the sources of fertilization were urea, triple superphosphate, and potassium chloride. Nitrogen was applied 30% and 70% at sowing and at the 6-leaf stage, respectively. Both P and K were applied 100% at sowing.

Each experimental unit consisted of six rows 3 m long with 0.6 m row spacing (10.8 m^2). The cultivated area in each environment was 291.6 m^2 for three Cd rates, three maize cultivars, and three replicates. Experiments were sown on 8, 17, and 24 October 2013 in La Serena, Los Tilos, and Chillán, respectively, and the sowing rate was eight seeds per linear meter. The seedbed in each environment was prepared with a plow at 0.3 m depth followed by a surface cultivator.

Seven irrigation events were applied to all the environments, the first after sowing until the milk stage to complement accumulated precipitation between July and December 2013 (8.7, 44.5, 334.7 mm in La Serena, Los Tilos, and Chillán, respectively) (Table 2). Each irrigation event was 50-60 mm, which was enough to maintain the soil with adequate moisture during crop development. The pre-emergent herbicide, a mixture of atrazine and S-metolachlor, was applied at a rate of 6.0 $L\cdot ha^{-1}$ at the post-emergence stage to control dicotyledonous weeds. The insecticide chlorpyrifos was applied before sowing at a rate of 5.0 $L\cdot ha^{-1}$ to control larvae in the soil. Diseases and incidence of insects during crop growth were very low in the three environments; therefore, foliar fungicides or insecticides were not used. Plots were harvested on 22, 24, and 29 April 2014 in La Serena, Los Tilos, and Chillán, respectively.

Table 2. Climatic characteristics of each environment during the season 2013 - 2014.

Parameters	**La Serena**			**Los Tilos**			**Chillán**		
	Tm[1]	**pp**[2]	**Ev**[3]	**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

Dicember	17.8	0.0	142.6	19.2	0.0	146.9	19.1	0.0	156.7
Total accumulate	--	83.8	804.3	--	85.2	853.7	--	672.1	845.0

[1] Tm, Temperature average (ºC); [2] pp, Precipitation (mm); [3] Ev, evaporation (mm).

2.4. Plant tissue dry matter, soil analysis, and plant tissue

The crop was harvested when the grains reached 15% moisture content. Plant samples with roots were collected from 1.0 m^2 in each experimental unit and DM production was determined in the grain, straw (stem + leaf), and root. Tissue samples were washed with distilled water and oven-dried at 70 ºC for 72 h to determine DM. At the end of the experiment, 10 soil sub-samples were collected from each plot at two depths (0-0.2 and 0.2-0.4 m); they were air-dried, later ground, and passed through a 2 mm sieve to determine total Cd concentration. The methodologies to determine Cd concentration in plant tissue and soil were mentioned above.

2.5. Data analysis

The following factors were determined.

2.5.1. Bioconcentration and bioaccumulation factor

The soil and plant Cd concentrations were calculated based on dry weight. The bioconcentration factor (BCF) and bioaccumulation factor (BAF) is an index of the plant's capacity to accumulate Cd with respect to the concentration of the soil substrate. It was calculated as follows [30-32]:

$BAF_{grain} = C_{grain} / C_{soil}$

$BAF_{straw} = C_{straw} / C_{soil}$

$BCF_{root} = C_{root} / C_{soil}$

where C_{grain}, C_{straw}, and C_{root} are the Cd concentrations in the grain, straw, and root, respectively, and C_{soil} is the concentration in the soil.

2.5.2. Translocation factor

The translocation factor (TF) or mobilization ratio determines the relative displacement of Cd from the concentration of the plant part in the soil (root) toward the aerial parts of the plant (grain and straw); it is calculated by the following equation [33]:

$TF_{grain} = C_{grain} / C_{root}$

$TF_{straw} = C_{straw} / C_{root}$

where C_{grain}, C_{straw}, and C_{root} are the Cd concentrations in the grain, straw, and root, respectively.

2.5.3. Tolerance index

The tolerance index (TI), or the tolerance of plants to Cd can be measured as the variation of the biomass response of the plant part (grain, straw, or root) by the toxicity of Cd compared with the biomass of the treatment with no added Cd. This index was determined by the following equation [34]:

$TI = MS_{Cd\ treatment} / MS_{control}$

where $MS_{Cd\ treatment}$ and $MS_{control}$ are the DM of each treatment with added Cd and DM of control treatment, respectively.

2.6. Experimental design and statistical analysis

The experimental design was a split sub-sub-plot where the main plot was the environment (3), the split plots were the Cd rates (3), and the split sub-sub-plots were the maize cultivars (3) with three replicates. Results were analyzed by ANOVA and Tukey's test ($P = 0.05$) by the SAS PROC MIXED Model procedure (SAS Institute, Cary, North Carolina, USA) and considering the locations as random effects. For the significant interactions, contrast analysis was used to compare the treatment effect separately.

3. Results

The environment and its interaction with the cultivar significantly affected DM production in the straw (Table 3). Dry matter production in the straw fluctuated between 7.0 and 14.7 $Mg{\cdot}ha^{-1}$ (Table 4). The highest value in all the cultivars was attained in La Serena ($P < 0.05$) then Chillán and Los Tilos; there were no significant differences between them ($P > 0.05$) and values were 14.7, 9.3, and 7.0 $Mg{\cdot}ha^{-1}$, respectively (Table 4). Comparing DM production in the straw between cultivars, and as a mean of the environments and Cd rates, higher production was observed in Syngenta with 10.9 $Mg{\cdot}ha^{-1}$, followed by Pioneer and Dekalb with values of 9.0 and 9.7 $Mg{\cdot}ha^{-1}$, respectively, and no differences between them ($P > 0.05$) (Table 4).

Table 3. Significance levels of the evaluated parameters for the experiments maked in three corn cultivars fertilized with three $CdCl_2$ rates on three environments.

Parameter	**L^{1}**	**R^{2}**	**C^{3}**	**$L^{X}R$**	**$L^{X}C$**	**$R^{X}C$**	**$L^{X}R^{X}C$**
Grain Yield	**	NS	NS	NS	NS	*	NS
Straw DM production	**	NS	NS	NS	**	NS	NS
Roots DM production	**	NS	**	NS	**	NS	NS
Grain Cd uptake	**	**	NS	**	*	NS	NS
Straw Cd uptake	NS	**	NS	*	NS	NS	NS
Roots Cd uptake	**	**	NS	**	*	NS	NS
Total Cd uptake	NS	**	NS	NS	NS	NS	NS
Grain Cd distribution	**	NS	NS	NS	*	NS	NS
Straw Cd distribution	**	NS	NS	NS	NS	NS	NS
Roots Cd distribution	**	NS	NS	NS	NS	NS	NS

^{1} L, Environments (La Serena, Los Tilos and Chillan); ^{2} R, Cd rates (0, 1 and 2 mg·kg^{-1}); ^{3} C, Corn cultivars (Syngenta, Pionner and Dekalb); *, Significant at $P < 0.05$; ** Significant at $P < 0.01$; NS, non significant.

Table 4. Dry matter production (Mg·ha^{-1}) according to maize plant parts for a) different study environments, b) $CdCl_2$ rates, and c) maize cultivars.

	Parameters	**Dry matter production (Mg·ha^{-1})**			
		Grain	**Straw**	**Root**	**Total**
a	La Serena	15.45 a A	14.74 a A	2.04 a B	32.23 a
	Los Tilos	5.90 c A	7.04 c A	0.82 c B	13.76 c
	Chillán	10.67 b A	9.32 b A	1.36 b B	21.34 b
b	Control	8.28 a A	9.78 a A	1.27 a B	19.33 a
	1 mg kg^{-1}	7.68 a B	9.78 a A	1.25 a C	18.71 a
	2 mg kg^{-1}	8.06 a B	10.19 a A	1.29 a C	19.55 a
c	Syngenta	8.19 a B	10.93 a A	1.48 a C	20.61 a
	Pionner	8.12 a A	9.07 a A	1.10 c B	18.29 b
	Dekalb	7.71 a B	9.75 a A	1.23 b C	18.69 b

Different lowercase letters (a, b, c) in each column indicate significant differences compared with a) Environments, b) Cd rates, and c) Maize cultivars according to Tukey's test ($P < 0.05$). Different uppercase letters (A, B, C) between columns indicate significant differences between structures of the same plant for each a) Environment, b) Cd rate, and c) Maize cultivar according to Tukey's test ($P < 0.05$).

El Dry matter production in the roots was also affected by the environment, cultivar, and environment*cultivar interaction (Table 3). When comparing environments, the highest DM production in roots was found in La Serena (mean 2.0 $Mg \cdot ha^{-1}$), significantly higher than in Chillán (mean 1.3 $Mg \cdot ha^{-1}$) ($P < 0.05$), which, in turn, was significantly higher than in Los Tilos (mean 0.8 $Mg \cdot ha^{-1}$) ($P < 0.05$). The comparison between cultivars indicated that the highest DM production in roots was obtained in Syngenta (mean 1.5 $Mg \cdot ha^{-1}$), significantly higher ($P < 0.05$) than in Dekalb and Pioneer (mean 1.2 and 1.1 $Mg \cdot ha^{-1}$, respectively), and with no significant differences between them ($P > 0.05$) (Table 4). The environment*cultivar interaction in the La Serena environment was generally observed as having a greater effect on the variability of DM production associated with cultivar; Syngenta was noted with a mean of 2.5 $Mg \cdot ha^{-1}$ while Dekalb and Pioneer had means of 2.1 and 1.5 $Mg \cdot ha^{-1}$, respectively (data not shown).

Cadmium uptake in the grain was affected by the environment, Cd rate, environment*Cd rate interaction, and environment*cultivar interaction (Table 3). When contrasting environments (Table 5), the highest Cd uptake in the grain was obtained in La Serena with a mean value of 0.45 g ha^{-1}, which was significantly higher ($P < 0.05$) than in Chillán (0.12 $g \cdot ha^{-1}$) and Los Tilos (0.08 $g \cdot ha^{-1}$), and there were no differences between them ($P > 0.05$). The contrast between Cd rates (Table 5) indicated that the highest Cd uptake was attained with 2 $mg \cdot kg^{-1}$ $CdCl_2$ (0.25 $g \cdot ha^{-1}$), which was significantly higher ($P < 0.05$) than for 1 $mg \cdot kg^{-1}$ $CdCl_2$ (0.19 $g \cdot ha^{-1}$); the latter was significantly higher ($P < 0.05$) than the control (0.06 $g \cdot ha^{-1}$) (Table 5). The contrast between cultivars (Table 5) indicated that the highest Cd uptake in the grain was reached in Pioneer (0.19 $g \cdot ha^{-1}$), then in Syngenta (0.16 $g \cdot ha^{-1}$) and Dekalb (0.14 $g \cdot ha^{-1}$) with no significant differences between them ($P > 0.05$) (Table 5). The environment*Cd rate and the environment*cultivar interactions exhibited greater variability associated with the environment and revealed high values obtained in La Serena (data not shown).

Table 5. Cadmium extraction levels ($g \cdot ha^{-1}$) for each maize plant organ for a) different environments, b) $CdCl_2$ rates, and c) maize cultivars.

	Parameters	**Cadmium extraction (g ha^{-1})**			
		Grain	**Straw**	**Root**	**Total**
	La Serena	0.45 a C	18.87 b A	4.45 a B	23.77 a
a	Los Tilos	0.08 c B	23.93 a A	0.24 b B	24.25 a

	Chillán	0.12 bc B	18.54 b A	0.61 b B	19.27 b
b	Control	0.06 b C	2.24 c A	0.57 c B	2.87 c
	1 mg kg^{-1}	0.19 a C	16.01 b A	1.53 b B	17.72 b
	2 mg kg^{-1}	0.25 a C	32.14 a C	2.06 a B	34.44 a
c	Syngenta	0.16 a C	14.80 b A	1.43 a B	16.39 b
	Pionner	0.19 a C	15.89 b A	1.07 a B	17.15 b
	Dekalb	0.14 a C	19.70 a A	1.66 a B	21.50 a

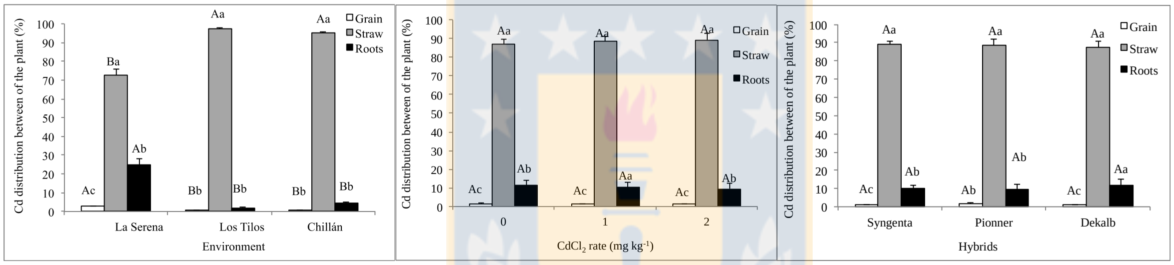
Different lowercase letters (a, b, c) in each column indicate significant differences compared with a) Environments, b) Cd rates, and c) Maize cultivars according to Tukey's test ($P < 0.05$). Different uppercase letters (A, B, C) between columns indicate significant differences between structures of the same plant for each a) Environment, b) Cd rate, and c) Maize cultivar according to Tukey's test ($P < 0.05$).

Cadmium uptake in the straw was only affected by the Cd rate (Table 5). When contrasting the $CdCl_2$ rates (Table 5), the highest Cd uptake in the straw was attained in Los Tilos with a mean of 23.9 g·ha^{-1} ($P < 0.05$), while La Serena and Chillán had 18.8 and 18.5 g·ha^{-1}, respectively, and with no significant difference between them ($P > 0.05$).

As for Cd uptake in the roots, there was an effect of the environment and Cd rate with an interaction between both sources of variation and between environment*cultivar (Table 5). The contrast between environments (Table 5) indicated that the highest Cd uptake in the roots was obtained in La Serena with a mean of 4.5 g·ha^{-1}, which was significantly higher than in Chillán and Los Tilos where uptakes were 0.6 and 0.2 g·ha^{-1}, respectively; there was no significant difference between them ($P > 0.05$). When contrasting the Cd rate (Table 5), higher Cd uptake in the roots was observed when using 2 and 1 mg·kg^{-1} $CdCl_2$; however, they were both similar ($P > 0.05$) and values were 2.1 and 1.5 g·ha^{-1}, respectively. They significantly surpassed the control ($P < 0.05$), which had an uptake of 0.6 g·ha^{-1}.

Whole plant Cd uptake was only affected by the Cd rate (Table 3; Figures 1a, b, c). The highest whole plant Cd uptake was attained with 2 mg·kg^{-1} $CdCl_2$ (34.4 g·ha^{-1}), which was significantly higher ($P < 0.05$) than with 1 mg·kg^{-1} $CdCl_2$ (17.7 g·ha^{-1}); the latter was significantly higher than in the control without Cd (2.9 g·ha^{-1}) ($P < 0.05$) (Figure 1b). Although the cultivars did not show any differences in whole plant Cd extraction (Table 3, Figure 1c), these values were 21.5, 17.1, and 16.4 g·ha^{-1} in Dekalb, Pioneer, and Syngenta, respectively. Cadmium distribution in the maize plant (Table 3; Figures 1a, b, c) revealed that the environment was affected by the distribution to the grain, straw, and roots. There were no interactions between the sources of variation, with the exception of Cd distribution in the grain, which was affected by the environment*cultivar interaction (Table 3).

The highest percentage of Cd distribution to the grain was attained in La Serena (3.0%) ($P < 0.05$), exhibiting significant differences between Chillán (0.8%) and Los Tilos (0.7%); however, there were no significant differences between them ($P > 0.05$) (Figure 1a). The values for Cd distribution to the grain were 1.2%, 0.9%, and 0.9% for Cd rates 2, 1, and 0 mg·kg^{-1} $CdCl_2$, respectively, with no significant differences between them ($P > 0.05$) (Figure 1b). When comparing cultivars (Figure 1c), there was a higher Cd distribution to the grain in Pioneer (1.4%), while Syngenta and Dekalb obtained 0.9% and 0.8% respectively, with no significant differences between the three cultivars ($P > 0.05$).



(a) **(b)** **(c)**

Figure 1. Cadmium distribution in the maize plant for different a) environments, b) cadmium rates, and c) maize cultivars.

Comparing environments for Cd distribution in the straw indicated that Los Tilos and Chillán did not show any significant differences one from the other ($P > 0.05$) and that their values were significantly higher than in La Serena ($P < 0.05$). The values for Cd distribution to the straw were 97.3%, 94.9%, and 72.5% in Los Tilos, Chillán, and La Serena, respectively (Figure 1a). The Cd rate and its effect on Cd distribution to the straw differed from that described for Cd distribution to the grain (Figures 1a, b); no significant differences were detected ($P > 0.05$) with values of 90.8%, 90.2%, and 88.3% for rates of 2, 1, and 0 mg·kg^{-1} $CdCl_2$, respectively (Figure 1b). No significant differences were observed between the Syngenta (90.4%), Pioneer (90.0%), and Dekalb (88.9%) cultivars (Figure 1c) for Cd distribution to the straw.

Cadmium distribution to the roots when comparing environments (Figure 1a) revealed a higher value in La Serena (24.8%) ($P<0.05$) than in the other environments. In turn, there were no significant differences between Chillán and Los Tilos ($P > 0.05$) with values of 4.3% and 2.0%, respectively. As for Cd rates, values for distribution to the roots were 10.5%, 8.9%, and 8.3% for rates of 0, 1, and 2 $mg \cdot kg^{-1}$ $CdCl_2$, respectively (Figure 1b); only the control was significantly higher than the other treatments ($P < 0.05$) with no differences between rates of 1 and 2 $mg \cdot kg^{-1}$ $CdCl_2$ ($P > 0.05$). The comparison of cultivars (Figure 1c) indicated values for distribution to the roots of 10.3%, 8.7%, and 8.6% in Dekalb, Syngenta, and Pioneer, respectively (Figure 1c), with not significant differences between them ($P > 0.05$). In addition, when comparing Cd distribution between the three maize plant structures, the straw usually concentrated the highest accumulation of the metal ($P < 0.05$), independently of the environment, Cd rate, and evaluated cultivar (Figures 1a, b, c). The percentage accumulation of Cd in the grain was lower than in the root ($P < 0.05$) for the different Cd rates and in most of the environments and evaluated cultivars (Figures 1a, b, c).

Table 6 displays the translocation factors (TF) used to evaluate plant capacity to translocate Cd from the roots to the aerial part of the plant (straw + grain), which are the mean values (Table 6; n=27) of the different a) environments, b) Cd rates, and c) maize cultivars. It is noted that there are significant differences in TF between environments ($P < 0.05$), TF in Los Tilos is 20 times higher than in Chillan and 2.6 times higher than in La Serena with values of 11.82, 4.63, 0.57, respectively, for the three environments. Significant differences for TF between the different rates ($P < 0.05$) (Table 6b) were observed for the 0, 1, and 2 $mg \cdot kg^{-1}$ $CdCl_2$ treatments with values of 0.73, 1.96, and 2.84, respectively. The variations of TF, in accordance with the different Syngenta, Pioneer, and Dekalb cultivars (Table 6c), did not indicate any significant differences between treatments ($P > 0.05$) with values of 2.31, 2.23, and 2.21, respectively.

Table 6. Values for cadmium bioaccumulation factor (BAF), bioconcentration factor (BCF), translocation factor (TF), and tolerance index (TI) for grain, straw, and root according to different a) environments, b) $CdCl_2$ rates, and c) maize cultivars.

Parameters	BAF	BCF	TAF	Tolerance index		
				TI grain	TI straw	TI root
La Serena	0.67 c	1.20 a	0.57 c	1.87 a A	1.78 a A	0.15 a B

a	Los Tilos	1.71 b	0.20 c	11.82 a	0.71 c A	0.85 c A	0.10 b B
	Chillán	2.54 a	0.70 b	4.63 b	1.29 b A	1.13 b B	0.16 a C
b	Control	0.48 c	0.66 c	0.73 c	1.33 a A	1.26 a A	0.17 a B
	1 mg kg^{-1}	1.33 b	0.82 a	1.96 b	1.24 a A	1.25 a A	0.17 a B
	2 mg kg^{-1}	1.70 a	0.75 b	2.84 a	1.30 a A	1.25 a A	0.17 a B
c	Syngenta	1.35 a	0.68 c	2.31 a	1.32 a A	1.33 a A	0.20 a B
	Pionner	1.44 a	0.78 b	2.23 a	1.31 a A	1.15 a A	0.14 a B
	Dekalb	1.47 a	0.82 a	2.21 a	1.24 a A	1.28 a A	0.16 a B

Different lowercase letters (a, b, c) in each column indicate significant differences compared with a) Environments, b) Cd rates, and c) Maize cultivars according to Tukey's test ($P < 0.05$). Different uppercase letters (A, B, C) between columns indicate significant differences between structures of the same plant for each a) Environment, b) Cd rate, and c) Maize cultivar according to Tukey's test ($P < 0.05$).

The BCF or enrichment factor is used to quantify plant capacity to accumulate Cd in the root with respect to Cd concentration in the soil. Table 6a indicates that BCF shows significant differences ($P < 0.05$) between environments and highlights the fact that La Serena has a greater capacity to accumulate Cd in the plant root than Chillán or Los Tilos, values are 1.2, 0.7, and 0.2, respectively. When comparing Cd rates, BCF showed no significant differences between 0, 1, and 2 $mg \cdot kg^{-1}$ $CdCl_2$ treatments ($P > 0.05$), and values were 0.66, 0.82, and 0.75, respectively. The same trend was observed when evaluating BCF for the different cultivars (table 6c) where no significant differences were detected ($P > 0.05$) between Syngenta, Pioneer, and Dekalb with values of 0.68, 0.78, and 0.82, respectively.

On the other hand, BAF allows quantifying the capacity to accumulate Cd in the aerial part of the plant with respect to Cd concentration in the soil. Table 6a specifies significant differences between environments for BAF ($P < 0.05$); a higher accumulation rate is observed in the Chillán environment, which is followed by Los Tilos ($P < 0.05$) and a lower bioaccumulation in La Serena ($P < 0.05$) with values of 2.54, 1.71, and 0.67, respectively. The same trend was observed when studying BAF in accordance with the Cd rates (Table 6b); there were significant differences between 0, 1, and 2 $mg \cdot kg^{-1}$ $CdCl_2$ ($P < 0.05$) and values were 1.7, 1.33, and 0.48, respectively. It should be noted that when evaluating BAF for the different cultivars (Table 6c), there were no significant differences between them ($P > 0.05$) and values were 1.35, 1.44, and 1.47 for Syngenta, Pioneer, and Dekalb, respectively.

Root TI (Table 6a) exhibited no significant differences between the environments in La Serena, Los Tilos, and Chillán and TI values were 0.15, 0.10, and 0.16, respectively (Table 6a) ($P > 0.05$). Differences were neither observed for the different $CdCl_2$ rates and values were 0.17, 0.17, and 0.17 for 0, 1, and 2 $g{\cdot}kg^{-1}$ $CdCl_2$, respectively (Table 6b) ($P > 0.05$), nor for the three cultivars with values of 0.20, 0.14, and 0.16 in Syngenta, Pioneer, and Dekalb, respectively (Table 6c) ($P > 0.05$). However, when evaluating TI in the grain, there were significant differences for both the environments and Cd rates ($P < 0.05$). When observing the environments, Los Tilos had the lowest value ($P < 0.05$) and was surpassed by Chillán, whereas La Serena had the highest value ($P < 0.05$). Values were 0.71, 1.29, and 1.87 in Los Tilos, Chillán, and La Serena, respectively (Table 6a). No significant differences were detected between TI at the different Cd rates ($P >0 .05$) and values were 1.33, 1.24, and 1.30 for 0, 1, and 2 $mg{\cdot}kg^{-1}$ $CdCl_2$, respectively (Table 6b). The same trend was observed for TI in the grain for the different cultivars, no significant differences were observed ($P > 0.05$), and values were 1.32, 1.31, and 1.24 for Syngenta, Pioneer, and Dekalb, respectively (Table 6c). Finally, TI of the straw exhibited the same trend as in the grain (Table 6a) with significant differences only between environments ($P < 0.05$) where values were 1.78, 1.13, and 0.85 for La Serena, Chillán, and Los Tilos, respectively (Table 6a). For the different $CdCl_2$ rates (0, 1, and 2 $mg{\cdot}kg^{-1}$), TI values in the straw were 1.26, 1.25, and 1.25, respectively, and with no significant differences ($P > 0.05$) (Table 6b). The same statistical effect was obtained when comparing the different cultivars ($P > 0.05$) which had values of 1.33, 1.15, and 1.28 for Syngenta, Pioneer, and Dekalb, respectively (Table 6c).

4. Discussion

The production of DM in the grain, straw, and root was not affected by the degree of anthropogenic contamination of the soil with Cd (1 and 2 $mg{\cdot}kg^{-1}$ $CdCl_2$) nor by the different evaluated cultivars (Table 4); this is corroborated by TI ($TI > 1$) (Table 6) and concurs with Zhang et al. [34]. Other authors point out that soils contaminated with Cd exhibit decreased crop development that can fluctuate between 55% and 80% [35]; however, soil Cd contamination levels in that study were higher than those generated in the present study. Given that our results were similar to those obtained by other authors under similar Cd concentration conditions applied to the soil [13,36], it can be suggested that Cd concentrations achieved in the soil by applying

$CdCl_2$ are below the danger threshold of 3.5 mg·kg^{-1} [13]. However, the environment significantly affected DM production in Los Tilos (Table 4) for DM in the straw, grain, and root (Table 4). The lack of heat accumulation during the development stage and higher than optimal soil pH could be limiting factors for DM production, which concurs with other authors who point out a significant correlation between DM production and degree-day accumulation [36]. At the same time, the environment*cultivar interaction was affected by DM production, which corroborates what was previously mentioned and the fact that the response to the environmental factor is differentiating in accordance with the thermal and soil requirements of each cultivar [13,37].

Although initial total soil Cd was higher in La Serena compared with Los Tilos and Chillán (Table 1), this order was not maintained at the end of crop development (Table 2); this was probably generated by crop Cd absorption (Tables 1, 5) [29]. For total plant Cd extraction, no significant differences were observed between environments (Table 5), which concurs with Putwattana et al. [21]. However, mean total Cd extraction between different environments, Cd rates, and maize cultivars (22.4, 22.4, and 22.4, respectively) (Table 5) is higher than the mean among cultivars with phytoextraction potential reported by Slycken et al. [38] (18.5 g Cd·ha^{-1}). These extraction levels demonstrate that the maize crop could be used for Cd phytoextraction [20,38] given that Cd extractions up to 42 g·ha^{-1} were reported in our experiment. On the other hand, Cd distribution in the different plant parts was affected by the environment with higher Cd absorption in the grain in La Serena (Table 5); this was 200% more than in Los Tilos, which in turn was 50% higher than in Chillán. This is mainly because DM production was higher in La Serena, which was pointed out by Trejo et al. [29]. These results concur with those obtained by Sarwar et al. [39], who also stated that high soil Zn concentrations, such as those found in the present study (Table 1), form Zn-phytochelatin complexes in the cell cytoplasm to substitute the Cd-phytochelatin complexes. This leaves Cd free inside the root cells and results in increased Cd translocation in the different plant parts, which was observed in the present study. However, low Cd extraction in the maize grain (Table 5) showed values within the range cited by several authors [13,40] with a mean Cd value of 0.18 g·ha^{-1} (mean of 27 values that considered three environments, three Cd rates, and three cultivars). This is an indicator of normal translocation for this species. Wahsha et al. [41] point out that Cd concentration in the maize grain was lower than the detection limit of 0.002 mg·kg^{-1}, leading to low extraction levels for the maize grain, such as observed in the present study. This could be due to several factors, for example, absorption and translocation limitations generated in the root [13], which suggests that maize plants have more efficient defense mechanisms than other crops to regulate Cd toxicity, including the accumulation of this metal at root level [22]. Secondly, the available soil Zn concentration acts as an antagonist for plant Cd absorption [37]. Finally, the low Cd concentration could be attributable to high agronomic efficiency of nutrient use (kg of DM produced per kg of applied nutrient) obtained in the present experiment (Table 5), and which usually implies a Cd dilution effect [24].

It can also be observed in Table 5 that for the environment, Cd rate, and cultivar, total Cd extraction in the straw is always significantly higher than in the roots or grain. These results concur with those reported by Liang et al. [42] and Stritsis et al. [43], who point out that Cd concentrations in maize straw are higher than in the rest of the plant. The same trend was observed in other crops, such as lettuce (*Lactuca sativa*) [44] and bread wheat (*Triticum aestivum* L.) straw [1]. Zhang et al. [45] explain these results by indicating that high S and Zn concentrations in the soil profile, also observed in the present study (Table 1), facilitate Cd translocation from the root to the aerial part of the plant. As previously mentioned, this higher Cd concentration in the straw indicates a higher translocation rate of this metal to the aerial part. Liang et al. [42] reported that it could be influenced by a decrease in sap flow that generates an increase of Cd concentration in the xylem when the crop is exposed to high Cd concentrations. It should be noted that the main factor for the higher translocation rate to the aerial part of the plant in the Los Tilos environment could be the high Ca levels in the soil profile associated with basic pH levels (Table 1). Sarwar et al. [39] pointed out that this generates increased Cd availability because it was substituted by Ca in the soil particles, thus increasing absorption by the plant and its translocation. Given the fact that high Cd extractions in the maize plant straw expose animals to this heavy metal and ultimately contaminate the food chain, which contributes in evaluating Cd levels in these maize cultivars destined for animal feed. Cadmium absorption by the maize plant is associated with the abovementioned factors and is benefited by high organic matter (OM) levels [12]; these levels were observed in the different evaluated environments in the present study and reached values close to 63 $g \cdot kg^{-1}$ in Chillán.

As expected, the Cd concentration in the aerial part of the plant was proportional to the Cd concentration in the soil (Figure 1) where significant differences were noted in Cd extraction by the plant between the control and the 1 and 2 $mg \cdot kg^{-1}$ rates with more than 50% and 100% (Table

5), respectively. This concurs with the literature [46,47], which states that when the $CdCl_2$ rate applied to the soil is higher, absorption rates of this heavy metal are higher. The same trend is observed when evaluating extraction levels in the same plant parts (grain, straw, and root) (Table 5). As regards the studied maize cultivars, no effect on plant total Cd extraction was recorded and no differences existed between cultivars in the same plant part (Table 5); these results concur with Slycken et al [38], who conducted a study with maize and point out that the different evaluated cultivars did not show any significant differences in Cd absorption.

Plant capacity to absorb soil contaminants can be expressed as BCF, which indicates the relationship between the metal content in the plant tissue (root) and the soil [30,32,48]. Most of the BCF results in the present study fluctuated between 0.2 and 0.82, which concurs with results obtained by Usman and Mohamed [31], who pointed out that BCF values fluctuated between 0.38 and 0.9. In the different environment treatments, Cd rates, and cultivars, only the environment in La Serena reached values above the mentioned range (1.2) (Table 6a). Different authors mention that BCF values > 1 are high; this indicates that maize in the La Serena environment, independently of the cultivars and the degree of anthropogenic soil contamination, behaves as a plant with high Cd bioaccumulation efficiency at root level [48]. On the other hand, low root BCF generally found in the de Los Tilos and Chillán environments can be explained by the maize plant's capacity to prevent Cd absorption and probably for the low soil Cd bioavailability in accordance with previously analyzed factors [22,37]. Furthermore, the different evaluated maize cultivars can be classified as excluding Cd because all the BCF values are < 1 [31,48].

However, phytoextraction capacity has been expressed as TF and is defined as the relationship between the Cd concentration in the aerial part and the Cd concentration in the roots [36]. Results for TF obtained in the present study (Table 6) do not coincide with TF values recorded by other authors, who reveal that TF for different fertilization treatments, rotations, and degrees of Cd contamination were < 0.5 [21,36]. Mean TF in the present study (3.26) is higher than the results reported by Liu et al. [36], which could be mainly influenced by soil pH in that study (pH between 8.31 and 9.06) and generate decreased Cd availability to the soil solution; this differs from the pH in the present study that fluctuated between 5.74 and 8.25 (Table 1). The observed TF values in the present study (TF > 1) (Table 6) concur with results mentioned by other authors, who indicate TF values of 2.6 [33]. These results could classify these cultivars as plants with high Cd translocation efficiency from the roots to the aerial part [32]. The TF values obtained in Los Tilos (TF = 11.82) and BAF > 1 (Table 6) classify this environment as having the highest capacity to contaminate the food chain [7]. Therefore, more studies need to be conducted about heavy metal absorption and translocation to the aerial part of the crop in this environment, mainly cultivars used as a food source for animals destined for human consumption. According to values reported by Usman et al. [32], translocation factors for cultivars, Cd rates, and environments in the present study exceeded the efficient plant classification limit of Cd translocation from the root to the aerial part, with the exception of the La Serena environment and the 0 $\text{mg}\cdot\text{kg}^{-1}$ Cd treatment rate where TF < 1. The three maize cultivars (Syngenta, Pioneer, and Dekalb) exhibited high risk of contaminating the food chain with Cd because of the high TF value (TF > 2) [32]. Based on these results, new maize cultivars should be made available or production management strategies be evaluated for this crop to reduce translocation rates of this metal to the aerial part of the plant, as well as more research to decrease both Cd absorption and accumulation in different maize plant tissues destined for animal consumption.

5. Conclusions

According to the results of the present study, it can be concluded that dry matter production is not affected by different degrees of cadmium (Cd) contamination generated in the soil of the different evaluated environments. The highest Cd concentration and extraction levels in the different evaluated maize cultivars were obtained in the aerial straw (stem + leaves), independently of the environment, Cd rates, and maize cultivars.

Among the evaluated environments, the highest Cd concentration in the grain was observed in La Serena, but in no case was it higher than the established limits. According to the translocation factor (TF) (TF > 2) and bioaccumulation factor (BAF) (BAF > 1) values, the environments in Los Tilos and Chillán are classified as having a high capacity to contaminate the food chain for the evaluated cultivars and taking into account the degrees of Cd contamination generated in the soil.

Acknowledgments: The present work benefited from the FONDECYT Project No. 1120992 granted by the National Commission for Scientific and Technological Research of the Chilean Government.

Author Contributions: Juan Hirzel, Ingrid Walter and Ivan Matus, conceived, designed and performed the experiments; Jorge Retamal-Salgado and Juan Hirzel, analyzed the data; Juan Hirzel and Ivan Matus, facilitated access to the experimental sites and soil samples. All authors contributed with valuable discussions and scientific advices in order to improve the quality of the work.

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

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IV. CONCLUSIONES GENERALES

Los resultados obtenidos en este estudio evidencian que la presencia de Cd en el suelo (línea base y la aplicada), no afectó el rendimiento de grano del maíz. Sin embargo, la MS del grano fue superior en el medioambiente La Serena, dado las condiciones climaticas favorables para el desarrollo del cultivo. Conjuntamente, la producción total de materia seca del residuo no se vio afectada por los distintos medioambientes, dosis de Cd y cultivares. Por su parte, la concentración de Cd tanto en el grano como en residuo del maíz fue afectada tanto por el ambiente de cultivo, por la aplicación de Cd al suelo, y por el cultivar. En las raíces, la concentración de Cd sólo fue afectada por el medioambiente y por la aplicación de este metal en el suelo.

Los cultivares evaluados en este estudio, no presentaron diferencias en la concentración de Cd, en niguno de los tejidos analizados, lo que sugiere que incrementos en la concentración de Cd en el suelo no afectan la respuesta de acumulación de Cd entre cultivares. Entre los ambientes evaluados la mayor concentración de Cd obtenida en el grano correspondió a La Serena, pero en ningún caso correspondió al valor considerado como limite (0.2 mg kg^{-1}).

Por otra parte, la concentración total de Cd en el suelo, una vez terminada la cosecha de maíz, fue dependiente del medioambiente y de la dosis empleada para este metal, con una mayor acumulación en la primera capa de suelo.

Los mayores niveles de concentración y extracción de cadmio en los diferentes cultivares de maíz evaluados se obtuvieron en el residuo (tallo + hojas), independiente del medioambiente, dosis de Cd y cultivares de maíz.

Entre los ambientes evaluados, la mayor concentración de Cd en el grano se observó en La Serena, pero en ningún caso fue superior a los límites establecidos.

Según los valores obtenidos de TF (TF > 2) y BAF (BAF > 1) los medioambientes Los Tilos y Chillán se clasificarían de alta capacidad de contaminación de la cadena alimentaria para los cultivares evaluados y considerando los grados de contaminación de cadmio generados en el suelo. Donde la mayor correlacion de transferencia de cadmio a la parte aérea es según la concentración de este metal en el suelo.