



## Modelling sulphate-enhanced cadmium uptake by *Zea mays* from nutrient solution under conditions of constant free $\text{Cd}^{2+}$ ion activity

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### Abstract

A controlled hydroponic experiment was undertaken to investigate Cd uptake in relation to the activity of Cd species in solution other than the free ion ( $\text{Cd}^{2+}$ ) by maintaining a constant  $\text{Cd}^{2+}$  activity under variable  $\text{SO}_4^{2-}$  and  $\text{Cl}^-$  concentrations exposed to maize (*Zea mays* var. *Cameron*) plants. The objectives of these experiments were: (1) to distinguish and quantify the different uptake rates of free and inorganic-complexed Cd from nutrient solution, and (2) to model the uptake of Cd by maize with a Biotic Ligand Model (BLM) in a system which facilitates the close examination of root characteristics. Results of the current experiments suggest that, in addition to the free ion,  $\text{CdSO}_4^0$  complexes are important factors in determining Cd uptake in nutrient solution by maize plants. Higher nominal  $\text{SO}_4^{2-}$  concentrations in solution generally resulted in a greater Cd accumulation by maize plants than predicted by the  $\text{Cd}^{2+}$  activity. A better integration of the complete dataset for the 3 harvest times (6, 9 and 11 days after treatment) was achieved by including consideration of both the duration of Cd exposure and especially the root surface area to express Cd uptake. Similarly, the fit of the BLM was also improved when taking into account exposure time and expressing uptake in terms of root morphological parameters.

**Key words:** free ion activity model; biotic ligand model; root surface area; Cd complexation; metal exposure time; hydroponics; plant uptake

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### Introduction

The occurrence of Cd in edible plants is of special importance since it can be easily transferred into the human food chain. In general, Cd in the free ion  $\text{Cd}^{2+}$  form is widely reported in soil studies to be the determinant for phytoavailability by plants (Chaney, 1988). However, recently, other factors such as salinity have been getting more consideration (McLaughlin et al., 1997; Smolders et al., 1998; Khoshgofar et al., 2004; Weggler et al., 2004; López-Chuken and Young, 2005). Sulphate ( $\text{SO}_4^{2-}$ ) is commonly found in higher concentrations than  $\text{Cl}^-$  in soil solutions and in irrigation water in many salt-affected areas of the world (Rogers et al., 1998). It is therefore likely that a considerable proportion of the inorganic Cd in soil solution is complexed by  $\text{SO}_4^{2-}$  since it has a tendency to form soluble complexes with Cd (García-Mirayaga and Page, 1976), typically at  $\text{SO}_4^{2-}$  concentration  $\geq 30$  mmol/L (McLaughlin et al., 1997). In soil-based experiments it is difficult to determine whether enhanced Cd uptake by plants is due to increased rates of  $\text{Cd}^{2+}$  diffusion to plant roots from local dissociation of Cd-inorganic complexes or the result of direct uptake of complexes (Smolders et al., 1998). Enhanced uptake caused by the offset of  $\text{Cd}^{2+}$

depletion near root surfaces is avoided in stirred nutrient solutions held at constant  $\text{Cd}^{2+}$  ion activity. Hydroponic trials also offer some advantages over soil experiments where there is a particular interest in the role of the root morphology as a control over metal uptake rates.

Given this evidence, it would therefore be of interest to: (1) distinguish and quantify the different uptake rates of free and inorganic-complexed Cd, from nutrient solution, and (2) to model plant uptake of Cd with a free ion activity model FIAM using the model structure commonly employed in the Biotic Ligand Model (BLM) (Hough et al., 2005; Datta and Young, 2005) in a system which facilitates the close examination of root morphology.

### 1 Materials and methods

#### 1.1 Plant growth and treatments application

Seeds of *Zea mays* var. *Cameron* were germinated in perlite and irrigated with a modified complete nutrient solution containing (1) macronutrients:  $\text{KNO}_3$  5 mmol/L,  $\text{KH}_2\text{PO}_4$  1 mmol/L,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  2 mmol/L,  $\text{CaNO}_3 \cdot 4\text{H}_2\text{O}$  6.25 mmol/L; and (2) micronutrients:  $\text{H}_3\text{BO}_3$  46  $\mu\text{mol/L}$ ,  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  9.15  $\mu\text{mol/L}$ ,  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  765 nmol/L,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  320 nmol/L,  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$  15 nmol/L,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$

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and Na<sub>2</sub>EDTA 20 μmol/L. Seven days after sowing, seedlings were strictly selected for homogeneity and transplanted to an aerated complete nutrient solution for another three weeks in a 27.5 L hydroponic growing tray. The complete nutrient solution was replaced with the macronutrient solution one week before transplanting to 500 mL treatment cups filled with aerated macronutrient solution. This intermediate step was thought necessary to avoid possible complicating effects such as competition from micronutrients present in the treatments (Lombi et al., 2001). Cadmium was added as Cd(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O and SO<sub>4</sub><sup>2-</sup> as Na<sub>2</sub>SO<sub>4</sub>. One plant was transplanted per pot, and the treatments were applied at the same time (Table 1).

These treatments were chosen to be within the range of salinity tolerated by the maize plants (López-Chuken and Young, 2005) at concentrations commonly found in salt-affected irrigation water used for the production of semi salt-tolerant crops like wheat (Manchanda, 1976). Treatments were principally chosen to achieve similar Cd<sup>2+</sup> activities in all treatments as predicted from speciation modeling using the program WHAM-VI (Tipping et al., 2002). Sodium concentrations were maintained constant in all treatments by compensating with NaCl. All treatments were replicated ten-fold in a randomized block design. Treatment solutions were replaced after an initial three hours to allow for Cd depletion caused by a (suspected) initial equilibration between the root cation exchange sites and the treatment solutions. Two plants per treatment were harvested and nutrient solution was sampled after the initial three hours to test this hypothesis. In order to maintain constant Cd concentrations in the treated solutions throughout the exposure time of the plants, 10 mL samples were taken from the treatment cups at intervals of 3, 5, 7, 9 and 10 days after treatment (DAT). Following analysis, Cd concentrations in the nutrient solutions were immediately buffered by adding aliquots from a Cd stock solution to return the treatment cups to the original concentrations. During this interval, two plants per treatment were harvested at 6 and 9 DAT, leaving four plants (per treatment) for the final harvest at 11 DAT. Throughout the trial, culture containers were topped up with deionised water on a daily basis. The trial was conducted under glasshouse controlled conditions.

## 1.2 Nutrient solution and plant analyses

Immediately after solution sampling, routine pH measurements were conducted using a combined glass-AgCl electrode (3010 pH Meter, Jenway Ltd., UK). Total organic carbon (TOC) and total inorganic carbon (TIC) were

determined with a Total Organic Carbon Analyser (TOC-V CPH/CPN, Shimadzu Corp., Japan). The earlier assay was intended to check the presence of root exudates, which might compromise the speciation of the Cd. Cadmium was analysed by Flame-AAS (Varian SpectrAA 200FS, Australia). If the concentrations were below detection limits, then Graphite Furnace-AAS (GTA 110, Varian Australia Ltd.) was used. Anion (SO<sub>4</sub><sup>2-</sup>, Cl<sup>-</sup>, NO<sub>3</sub><sup>-</sup> and PO<sub>4</sub><sup>3-</sup>) and other cation (i.e., Mg<sup>2+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Na<sup>+</sup>) concentrations were assumed to be constant throughout the trial due to their high initial concentrations.

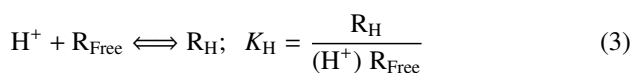
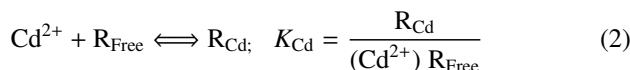
After plant shoots were harvested, intact root systems were washed thoroughly with deionised water. The roots were then kept in a solution of H<sub>2</sub>O<sub>2</sub> (1.5%, 10 mL) with ultra-pure water at < 4°C to avoid biological contamination prior to scanning for morphological characteristics (e.g., root surface area (RSA)) using WinRIZHO™ (Regent Instruments Inc., Quebec, Canada), a scanner-based image analysis system. Fresh and dry biomass was determined. Plant material was finely milled prior to digestion in hot concentrated HNO<sub>3</sub> and analysis metal concentrations by Flame-AAS.

## 1.3 Modelling Cd uptake by maize with a Biotic Ligand Model (BLM)

Cadmium uptake by plants was modelled using a Biotic Ligand Model (Hough et al., 2005; Datta and Young, 2005). This approach assumes sorption of free metal ions (M<sup>2+</sup>), or metal complexes (e.g., MCl<sup>+</sup>) given as activity, from solution onto hypothetical plant root sorption sites with competition between cations and protons for sorption sites (Brown and Markich, 2000). Assuming competition between metals and protons, plant root binding sites (R) can be written as:

$$R_{\text{Tot}} = R_{\text{Free}} + R_{\text{M}} + R_{\text{H}} \quad (1)$$

where, R<sub>Tot</sub>, R<sub>Free</sub>, R<sub>M</sub> and R<sub>H</sub> refer to total, free, metal-bound and protonated (H<sup>+</sup>) sites, respectively. Absorption reactions for a metal ion, such as Cd<sup>2+</sup> (K<sub>Cd</sub>) and protons (K<sub>H</sub>) can be described by Eqs. (2) and (3).



The BLM describes the mass balance in Eq. (4) in terms of the reaction constants (K<sub>Cd</sub> and K<sub>H</sub>) in Eqs. (2) and (3)

**Table 1** Treatments with Cd concentration and speciation as modelled by WHAM-VI in macronutrient solution containing different SO<sub>4</sub><sup>2-</sup> and Cl<sup>-</sup>

Na <sub>2</sub> SO <sub>4</sub> (mmol/L)	Treatment		Activity (nmol/L)			
	Na (mmol/L)	Cd (μmol/L)	Cd <sup>2+</sup>	CdSO <sub>4</sub> <sup>0</sup>	CdCl <sup>+</sup>	CdCl <sub>2</sub> <sup>0</sup>
0	200	4.53	152	23.9	1981	1130
40	160	4.02	152	505	1547	689
80	120	3.60	152	960	1131	368
120	80	3.26	152	1383	738	157
160	40	2.98	152	1779	362	37.7
200	0	2.76	152	2154	0.09	0.00

and then assumes a continuous metal transference (roots to shoots), so that:

$$Cd_{shoots} = \frac{K_{transfer} R_{Tot} K_{Cd}(Cd^{2+})}{1 + K_{Cd}(Cd^{2+}) + K_H(H^+)} \quad (4)$$

where,  $K_{transfer}$  is a proportionality constant which expresses the assumption that there is a constant ratio of metal concentrations in plant shoots and roots over the exposure time. The use of the BLM depends on some assumptions: (1) the rate of Cd translocation (roots to shoots) depends on the density of occupied transport sites at the root surface and reaches a theoretical maximum when all sites are occupied; (2) the root:shoot ratio is constant; (3) the density of sorption sites on the roots is always constant and; (4) free ion activity close to the root surface is constant. The reaction constants ( $K_{Cd}$  and  $K_H$ ) in Eqs. (2)–(4) also ignore possible effects on membrane physiological characteristics. Equation (4) can be adapted to include competition between a range of cations for one or for several hypothetical root adsorption sites. For example, Eq. (5) describes the uptake of Cd and includes the competition between  $Cd^{2+}$ ,  $Zn^{2+}$  and  $H^+$  for one site and  $CdSO_4^0$  and  $H^+$  for a second hypothetical root adsorption site.

$$Cd_{shoots} = \frac{K_{transfer1} R_{Tot(1)} K_{Cd}(Cd^{2+})}{1 + K_{Cd}(Cd^{2+}) + K_{Zn}(Zn^{2+}) + K_{H1}(H^+)} + \frac{K_{transfer2} R_{Tot(2)} K_{CdCl}(CdSO_4^0)}{1 + K_{CdCl}(CdSO_4^0) + K_{H2}(H^+)} \quad (5)$$

Equations (4) and (5) were optimised in Microsoft Excel using the Solver facility.

#### 1.4 Data quality measurements

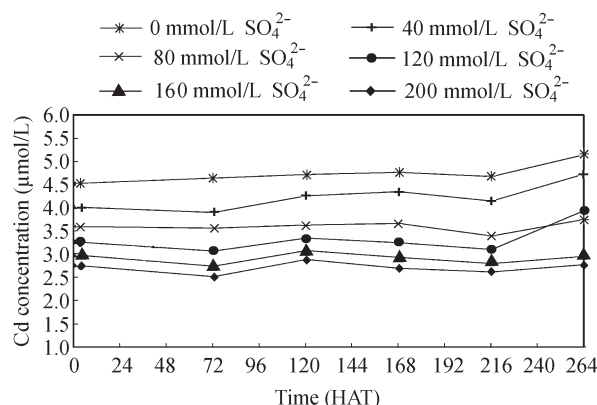
A standard reference material (1573a tomato leaves; NIST, Gaithersburg, USA), containing certified concentrations of Cd ( $1.52 \pm 0.03$  mg/kg) was used to ensure the quality of the data. This quality standard averaged  $1.53 \pm 0.02SE$  ( $n = 10$ ) mg/kg over the whole trial. For all analyses, blanks and known standard samples were analysed to ensure consistency.

## 2 Results and discussion

### 2.1 Cadmium speciation in hydroponic solution

A rapid initial reduction of Cd concentration in solution was observed after an initial three hours. This was thought

to signify a rapid equilibration between the root system and Cd in solution rather than true absorption into roots as suggested by Smolders and McLaughlin (1996). In general, Cd concentrations in the nutrient solution were maintained virtually constant throughout the trial (Fig. 1).



**Fig. 1** Change in cadmium concentration with time in hydroponic test solutions containing different  $SO_4^{2-}$  concentrations. Standard error was  $< 0.01$  for all average samples ( $n: 4-10$ ) and was not included in the graph. HAT means hours after transplanting *Zea mays* var. *Cameron* plants into the  $SO_4^{2-}$  and Cd treated macronutrient test solutions.

Cadmium species in solution (Table 2) were calculated using a time-averaged value of the measured solution characteristics (Cd, pH and TOC) and the original anion ( $Cl^-$ ,  $SO_4^{2-}$ ,  $NO_3^-$  and  $PO_4^{3-}$ ) and cation ( $Mg^{2+}$ ,  $K^+$ ,  $Ca^{2+}$  and  $Na^+$ ) concentrations as input data for WHAM-VI.

The calculated Cd concentrations in solution during the trial showed an average variation of only  $\pm 1.42\%$  compared to the original Cd concentrations selected to keep similar  $Cd^{2+}$  activities according to the speciation model WHAM-VI (Table 1). The Cd speciation showed minimal organic Cd-complexation in the nutrient solution ( $(0.28 \pm 0.21)\%$  of the total Cd) with the inorganic Cd species:  $Cd^{2+}$ ,  $CdSO_4^0$ ,  $CdCl^+$  and  $CdCl_2^0$  were the dominant forms in the nutrient solution ( $> 99\%$  of the inorganic Cd). The calculated average ionic strength (as initially modelled by WHAM-VI) for the lowest  $SO_4^{2-}$  treatment (0 mmol/L) was reduced by 55% compared to the highest  $SO_4^{2-}$  treatment (200 mmol/L) (0.23 and 0.51 mol/L, respectively (data not shown)). The modelled activity of the free  $Cd^{2+}$  in solution remained largely unchanged, although it was significantly low ( $P < 0.05$ ) by increasing  $SO_4^{2-}$  concentration in the nutrient solution (Table 2). The activity of Cd-sulphate and chloride complexes increased

**Table 2** Activities of  $Cd^{2+}$  complexes in the nutrient solution containing different  $SO_4^{2-}$  concentrations as modelled by WHAM-VI

Na <sub>2</sub> SO <sub>4</sub> (mmol/L)	Activity (nmol/L)			
	Cd <sup>2+</sup>	CdSO <sub>4</sub> <sup>0</sup>	CdCl <sup>+</sup>	CdCl <sub>2</sub> <sup>0</sup>
0	155 ± 0.69*	24.4 ± 0.11***	2027 ± 9.05***	1156 ± 5.16***
40	155 ± 1.10*	517 ± 3.68***	1582 ± 11.2***	704 ± 5.00***
80	151 ± 1.31*	957 ± 8.28***	1128 ± 9.76***	367 ± 3.18***
120	151 ± 1.40*	1374 ± 12.8***	733 ± 6.82***	156 ± 1.45***
160	149 ± 1.98*	1744 ± 23.2***	355 ± 4.73***	36.9 ± 0.49***
200	149 ± 1.58*	2116 ± 22.4***	0.09 ± 0.00***	0.00 ± 0.00***

Means followed by \* and \*\*\* are significantly different at 0.05 and 0.001 confidence level respectively. Values are means of the replicates  $\pm$  standard error ( $n = 10$ ).

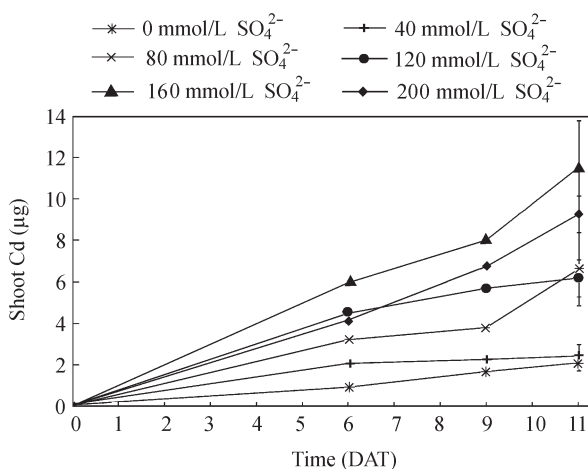
significantly with the increase in  $\text{SO}_4^{2-}$  and  $\text{Cl}^-$  concentration in solution respectively as predicted by WHAM-VI (Table 2).

## 2.2 Plant development and biomass

Maize plants were used for this experiment due to their uniform biomass production during a previous experiment by López-Chuken and Young (2005). *Zea mays* var. *Cameron* plants within the range of  $(2.46 \pm 0.42)$  g were carefully selected and transplanted to the treatment cups for the experiment. There was no statistically significant effect on plant growth during the trial (average fresh plant weight  $(2.53 \pm 0.06)$  g,  $n = 60$ ), except for a negative effect at the lowest  $\text{SO}_4^{2-}$  rates (0 and 40 mmol/L). This could be explained since these treatments contained higher  $\text{Cl}^-$  levels. Chloride is a major osmotically active solute involved in both turgor and osmoregulation (White and Broadley, 2001) and therefore, increased concentrations of salt in solution could create an osmotic stress and interfere with plant physiology and/or membrane function (Shannon and Grieve, 1999), which could slow plant growth. The effect of the treatments on plant development was only significant ( $P < 0.05$ ) for the last harvest, where a 33.2% fresh biomass reduction was observed at the lowest  $\text{SO}_4^{2-}$  treatment (0 mmol/L) compared to the highest treatment (200 mmol/L). RSA was not affected ( $P > 0.5$ ) by the treatments at the different harvest time (total average  $(40.2 \pm 1.49)$  cm<sup>2</sup>,  $n = 60$ ). Time had not statistically effect on RSA, except for the  $\text{SO}_4^{2-}$  (40 mmol/L) treatment ( $P < 0.001$ ) ( $(45.4 \pm 2.05)$  and  $(29.3 \pm 0.36)$  cm<sup>2</sup> at 0 and 11 DAT, respectively).

## 2.3 Plant cadmium concentrations

Cadmium uptake increased over successive harvests of maize shoots (Fig. 2), except for the  $\text{SO}_4^{2-}$  of 40 mmol/L treatment ( $P > 0.05$ ). In contrast, data for all treatments show that Cd uptake by roots after the initial harvest (3 hr),  $(4.78 \pm 1.16)$  µg,  $n = 12$ , was not significantly affected at 6, 9 and 11 DAT ( $P > 0.05$ ) ( $(38.4 \pm 2.66)$  µg,  $n = 48$ ).



**Fig. 2** Cadmium uptake in shoots at different harvest time by *Zea mays* var. *Cameron* grown in nutrient solution at different  $\text{SO}_4^{2-}$  concentrations and constant  $\text{Cd}^{2+}$  activity. Values are means of replicates with standard error bars for the shoots Cd concentration at the final harvest time ( $n: 2-4$ ).

Chloride has been strongly related to enhanced Cd uptake by plants due to increased solubility in the soil solution (McLaughlin et al., 1997; Norvell et al., 2000; López-Chuken and Young, 2005). A recent research reported that irrigation with saline water (0.5% and 3.0% of sea salt) also significantly stimulated Cd translocation from root to shoots in *Tamarix smyrnensis* Bunge cultivated in a growing media containing Cd (16 mg/kg) (Kadukova et al., 2008). Furthermore, recent findings by Ozkutlu et al. (2007) showed that applying NaCl directly to leaves could enhance Cd accumulation and translocation in wheat plants. The authors suggested that mobilization of Cd within the plant tissues could be promoted by the presence of mobile  $\text{CdCl}_n^{2-n}$  complexes.

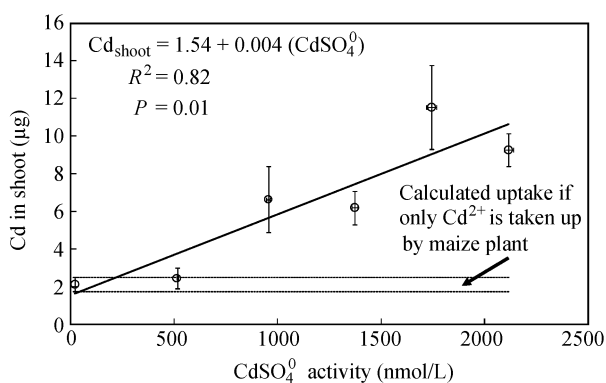
Although effects of  $\text{SO}_4^{2-}$  on Cd uptake by plants in soil are less clear than those for other anions (i.e.,  $\text{Cl}^-$ ) (McLaughlin et al., 1998a; López-Chuken and Young 2005), there is evidence that nutrient solutions that contained increased  $\text{SO}_4^{2-}$  concentrations resulted in a greater accumulation of Cd by Swiss chard (McLaughlin et al., 1998b) and wheat roots (Berkelaar and Hale, 2003) than predicted by the  $\text{Cd}^{2+}$  activity alone. This is in agreement with the results of the present study, in which data for all harvests indicated that the net Cd uptake by maize shoots was negatively affected ( $P < 0.01$ ) by the  $\text{Cl}^-$  concentration in the nutrient solution (Fig. 2). Furthermore, the activity of the Cd-chloride complexes and even the total Cd concentrations in the nutrient solution were negatively correlated to the Cd uptake by maize plants and therefore positively correlated with the concentrations of Cd-sulphate complexes in solution. To give an example, Cd uptake in shoots expressed as µmol Cd/(m<sup>2</sup> root-day), resulted in correlation coefficients ( $R$ ) with their corresponding degrees of freedom ( $df$ ) of  $-0.81$ ,  $df = 22$  and  $-0.82$ ,  $df = 22$  ( $P < 0.01$ ) with the total Cd concentration in solution and the activity of the  $\text{CdCl}^-$  complex respectively. In contrast, a good positive correlation ( $R = 0.80$ ,  $df = 22$ ,  $P < 0.01$ ) was obtained when the same Cd uptake expression was correlated with the activities of the  $\text{CdSO}_4^0$  complex. Several contrasting expressions of Cd uptake by plants (with or without root morphology and time) were used to correlate with the activities of the dominant Cd complexes in solution and showed similar results (data not shown). These results may suggest that  $\text{CdSO}_4^0$  complexes could have alleviated diffusion restrictions by buffering  $\text{Cd}^{2+}$  at the root surface and/or been taken up directly as a complex (McLaughlin et al., 1998b).

It is important to note that the strong correlation between Cd uptake by plants and  $\text{CdSO}_4^0$  activity coincides with a similar correlation between Cd uptake and the ionic strength ( $R = -0.82$ ,  $df = 22$ ). Therefore, Cd uptake by maize plants could also have affect by an osmotic stress caused by the differences in ionic strength for the treatments. The ionic strength could affect the root physiology and/or membrane functions (White and Broadley, 2001) and thereby increase the transfer factor ( $\text{Cd}_{\text{shoots}}:\text{Cd}_{\text{solution}}$ ) in the range of 38.4 to 194 for the 0 and 200 mmol/L  $\text{Na}_2\text{SO}_4$  treatments. The effect observed could therefore have been due to a lower plant availability of  $\text{CdCl}_n^{2-n}$

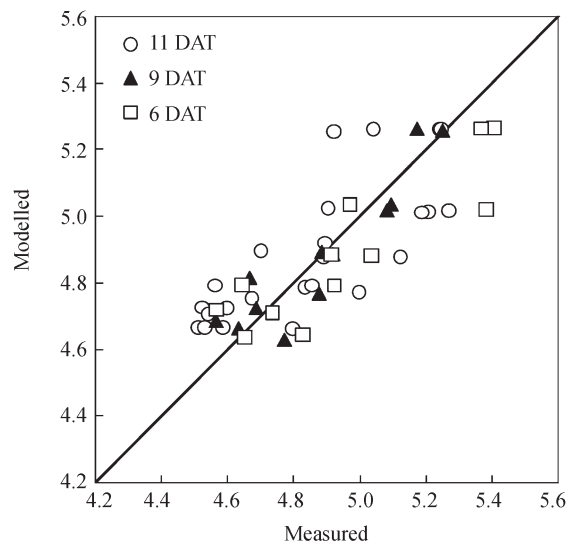
compared with the free  $\text{Cd}^{2+}$ , rather than direct uptake of the  $\text{CdSO}_4^0$  complex (McLaughlin et al., 1998b). However, the variation in ionic strength is quite small in relation to the range of Cd uptake (5.5-fold variation shown in Fig. 2).

McLaughlin et al. (1998b) reported that  $\text{CdSO}_4^0$  complexes were taken up by Swiss chard plants in a constant ionic strength nutrient solution when, despite a reduction of almost 50% in the  $\text{Cd}^{2+}$  activity due to increasing  $\text{SO}_4^{2-}$  concentration in solution, the Cd uptake by plants was unaffected. However, in their study, the ionic strength was compensated using  $\text{NaNO}_3$ , which could have had an effect in the plant growth over the trial, although no significant effect in dry weight was reported. In the present study, the possibility that  $\text{CdSO}_4^0$  complexes could have alleviated a diffusion limitation by buffering  $\text{Cd}^{2+}$  at the root surface seems unlikely since the activity of the free ion  $\text{Cd}^{2+}$  was manually buffered and was maintained virtually constant during the trial and the test solutions were continuously agitated by aeration. Therefore, the differences observed on Cd uptake by maize plants are likely to be entirely caused by the differences in the activity of Cd complexes other than  $\text{Cd}^{2+}$  (i.e., the neutral complex  $\text{CdSO}_4^0$ ) (Fig. 3).

The data in the present trial were also used to test the merit of including root morphology and duration of Cd uptake as a means of integrating the complete dataset for the 3 harvest time (6, 9 and 11 DAT). Cadmium uptake in shoots of maize plants (3 harvests) as a function of the activity of  $\text{CdSO}_4^0$  (which appears to be the principal determinant of uptake rate) was better explained ( $R = 0.80$ ,  $df = 46$ ) when uptake was expressed including both time and root morphology parameters ( $\mu\text{mol}/(\text{m}^2 \text{RSA}\cdot\text{sec})$ ) compared to Cd uptake expressions ignoring root morphology ( $\mu\text{mol}/(\text{kg}\cdot\text{sec})$ ,  $R = 0.73$ ,  $df = 46$ ) and when neither time nor root morphology were included as Cd uptake factors ( $\mu\text{mol}/\text{kg}$ ,  $R = 0.63$ ,  $df = 46$ ). When root morphology and time was included in expressing Cd uptake as a function of solution composition there is both an increase in correlation coefficient and a decrease in the systematic variation between datasets from different exposure time.



**Fig. 3** Relationship between the Cd content in shoots of *Zea mays* var. *Cameron* (final harvest 11 DAT) and the modelled activity of the  $\text{CdSO}_4^0$  complex. Values are means of replicates ( $n = 4$ ) with standard error bars. The space between broken lines show predicted uptake based on of the free metal ion only (i.e., 0 mmol/L  $\text{SO}_4^{2-}$  treatment).



**Fig. 4** Cadmium uptake ( $-\log(\mu\text{mol}/(\text{m}^2 \text{RSA}\cdot\text{sec}))$ ) by *Zea mays* var. *Cameron* shoots modelled by the BLM assuming one common absorption site for  $\text{Cd}^{2+}$  and  $\text{CdSO}_4^0$  ions. The solid line represents a 1:1 relation.

## 2.4 Testing a BLM

The best-fit BLM predicting the uptake of Cd by maize plants (including data from all three harvests) was parameterised including the  $\text{CdSO}_4^0$  activity (as it was shown to be the principal determinant for Cd uptake by maize plants) and expressing Cd uptake as  $-\log(\mu\text{mol}/(\text{m}^2 \text{RSA}\cdot\text{sec}))$ . This formulation assumed a single root sorption site with competition between the activities of ( $\text{Cd}^{2+}$ ) and ( $\text{CdSO}_4^0$ ) ions and a single transfer constant from roots to shoots ( $K_{\text{transfer}}R_{\text{Tot}}$ ) without competition from  $\text{H}^+$  ions (Eq. (6)).

$$-\log(\text{Cd}_{\text{Maize}}) = \frac{K_{\text{transfer}}R_{\text{Tot}} [K_{\text{Cd}^{2+}} (\text{Cd}^{2+}) + K_{\text{CdSO}_4^0} (\text{CdSO}_4^0)]}{1 + K_{\text{Cd}^{2+}} (\text{Cd}^{2+}) + K_{\text{CdSO}_4^0} (\text{CdSO}_4^0)} \quad (6)$$

A coefficient of determination of  $R^2 = 0.67$ ,  $df = 46$  ( $P < 0.001$ ) and constants  $K_{\text{transfer}}R_{\text{Tot}} = 6.51$ ,  $K_{\text{Cd}^{2+}} = 5.22$  and  $K_{\text{CdSO}_4^0} = 1.25$  resulted from the best-fit FIAM (Fig. 4).

The BLM model was not improved by assuming two or three hypothetical root sorption sites for the free ion  $\text{Cd}^{2+}$ ,  $\text{CdCl}^+$  and  $\text{CdSO}_4^0$  or by including proton competition as the pH in solution was constant ( $5.4 \pm 0.3$ ). A good correlation coefficient ( $R = 0.82$ ,  $df = 46$ ) ( $P < 0.001$ ) between the measured vs. modelled Cd in shoot ( $-\log(\mu\text{mol}/(\text{m}^2 \text{RSA}\cdot\text{sec}))$ ) was observed for the best-fit BLM. Although a considerable degree of scatter is shown in Fig. 4, there is no visible sign of systematic variation between datasets.

## 3 Conclusions

Results of the current experiment suggest that, in addition to the free ion  $\text{Cd}^{2+}$ ,  $\text{CdSO}_4^0$  complexes are important factors in determining Cd uptake from nutrient solution cultures by *Z. mays* var. *Cameron* plants. It is also possible that minor differences in the ionic strength between treatments due to the use of  $\text{NaCl}$  and  $\text{Na}_2\text{SO}_4$  salts to maintain a constant  $\text{Na}^+$  concentration, may have had some effect

on root membrane functions. However, no significant differences were observed for root morphological parameters in maize plants that may have suggested root physiological damage in respect to elevated salt concentrations. A better integration of the complete dataset for the 3 harvest times was achieved by including consideration of both the duration of Cd exposure and especially the RSA to express Cd uptake. Similarly, the fit of the BLM was also improved when taking into account exposure time and expressing uptake in terms of root morphological parameters.

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