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Cadmium and zinc influx characteristics by intact corn *(Zea mays* **L.) seedlings**

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Summary Cadmium and zinc uptake parameters were determined for intact corn *(Zea mays* L.) seedlings grown for 15 and 22 d in nutrient solutions containing levels of Cd and Zn that were similar to those found in soil solutions. Uptake of both elements was assumed to follow Michaelis-Menten kinetics. Calculations were based on the concentrations of free ionic Cd (Cd²⁺) and Zn (Zn²⁺) rather than the total solution concentration. Rates of Zn uptake were measured by determining depletion of Zn for periods of up to 30 h from solutions containing initial concentrations of 1.5 and 10 μ mol \mathbb{Z} n 1⁻¹. Depletion curves suggested that Zn uptake characteristics were similar at both levels of Zn in solution. The Imax for Zn uptake decreased from 550 to 400 pmol m^{-2} root surface s⁻¹ between 16 and 22d of growth while Km decreased from 2.2 to 1.5 μ mol Zn²⁺ 1⁻¹. Cadmium uptake parameters were measured by controlling Cd^{2+} activities in nutrient solution between 6.3 to 164 nmol $1⁻¹$ by continuous circulation of nutrient solution through a mixed-resin system. Imax for Cd uptake was 400 pmol m⁻² root surface s⁻¹ at 15 and 22d of growth. The magnitude of Km increased from 30 to 100 nmol $Cd^{2+}1^{-1}$ during this time period. The Km value suggests that corn is efficient for Cd uptake. The results of these uptake studies are consistent with the observed uptake of Zn and Cd by corn seedlings in soils.

Introduction

One of the major factors that controls the accumulation of Cd and Zn in plant tissue is the inherent uptake characteristics of the root system. The rate and degree of Cd and Zn accumulation will depend on the plant species and variety^{4, 5, 7, 9, 17, 18, 19, 20, 26. Both passive and active mechanisms} **have been used to explain Zn and Cd uptake. Passive uptake is implicated at high solution concentrations of Zn and Cd where these ions can** bind to root cell walls and/or diffuse into the interior of cells^{13,27}. Cad**mium and Zn are taken up actively at concentrations normally found in soil solutions 8' 14. Zinc uptake by intact rice** *(Oryza sativa* **L.) seedlings has been shown to be metabolically controlled at concentrations** ϵ 5 μ mols/l⁻¹ while both passive and metabolic uptake occur at greater **concentrations 14. Cadmium uptake by intact soybean** *(Glycine max* **L.) plants was metabolically controlled over a concentration range of 0.01** to $0.40 \mu \text{mol}^{-1}$ (ref.⁸). These Zn and Cd concentrations are similar to the ranges found in soil solutions from normal and sludge treated soil $s^{3,23}$.

Kinetic parameters describing the relationship between solution concentration and plant uptake are needed to predict ion uptake by plants with mechanistic models^{2, 12}. Nutrient uptake kinetics have been shown to follow Michaelis-Menten kinetics²⁵ and described by the equation

$$
\text{In} = \frac{\text{Imax C}}{\text{Km} + \text{C}} \tag{1}
$$

where In is the net nutrient influx rate, Imax is the maximum nutrient influx rate, C is the concentration of nutrient in solution, and Km is an uptake constant. It follows that Km is the nutrient concentration where In $= 1/2$ Imax. Claassen and Barber¹¹ adjusted Equation (1) to account for the efflux (E) of a nutrient from plants roots into the surrounding solution.

The objective of this study was to measure Cd and Zn influx kinetics for intact corn *(Zea mays* L.) seedlings. Uptake parameters were measured at Cd and Zn concentrations that approximate those in the soil solution 23 .

Materials and methods

Cadmium and Zn uptake studies were conducted in a growth chamber maintained at 25° C with a 16 h light exposure (37 W m⁻²). Seeds of *Zea mays L*. cv Becks 65X were wrapped in paper towels and germinated in aerated tap water for 6 d. Roots of seedlings were trimmed to 4 cm per plant before transplanting into 31 pots that contained the appropriate nutrient solution.

Zinc influx kinetics

Zinc uptake was measured with the solution depletion procedure of Claassen and Barber¹¹. Corn seedlings were transplanted into a complete nutrient solution and depletion curves were determined for triplicate pots after the corn seedlings had grown for 16 and 22d. The nutrient solution was adjusted to pH 5.5 with saturated Ca(OH)₂ and contained the following (in μ mol 1⁻¹): Ca(NO₃)₂, 1000; Ca(H_2PO_4), 500; K₂SO₄, 500; MgSO₄, 1000; NH₄NO₃, 1500; H₃BO₃, 46; HCl, 20; $CuSO_4 \cdot 5H_2O$, 0.3; Fe₂(SO₄)₃, 75; DTPA, 75; MnSO₄ \cdot H₂O, 9; Na₂MoO₄ \cdot 2H₂O, 0.8; ZnSO₄, 0.8. Nutrient solution in the pots was continuously aerated and was replaced after the initial 4d of growth and then after every 48 h. There were four corn seedlings per pot in the $1.5 \,\mu \text{mol}^{-1}$ Zn treatment and five corn seedlings per pot in the $10 \mu \text{mol}^{-1}$ Zn treatment.

Since DTPA forms a stable Zn complex that is not readily used by corr^1 , DTPA was removed from nutrient solution 2 d before determining Zn influx. Approximately 12 h prior to determining rates of Zn uptake seedlings were placed in pots with 1.51 of nutrient solution containing no Zn or DTPA. The concentration of Zn in solution was adjusted to either 1.5 or $10 \mu \text{mol}^{-1}$ with ZnSO₄, and starting at 0.5 h after adding Zn, samples were taken every 0.5 or 1.0 h from each pot for periods up to 30 h. The solution volume was maintained at 1.51 with deionized water to adjust for loss by sampling and evapotranspiration. Dilution of Zn in solution by sampling and addition was not important, because < 10% of the total volume was removed during the sampling period and because Zn uptake was rapid. Zinc concentrations were measured by flame atomic absorption.

The corn seedlings were harvested after completing Zn uptake measurements and root length was measured with the line intersect method²⁹. Average root radius was calculated from the relationship

$$
\mathbf{r}_{\mathrm{o}} = (\mathbf{F}_{\mathrm{wr}}/\pi \mathbf{L})^{1/2} \tag{2}
$$

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where r_0 is the average root radius, F_w is the fresh weight of roots and L is the root length. Equation (2) assumes that the roots have a density of 1 g cm^{-3} . The root growth constant (k) was calculated by assuming exponential root growth. Values for k were calculated with the expression

$$
k = \ln(L/L_0)/T \tag{3}
$$

where Lo is the initial root length and L is the root length after a growth period of time T.

Zinc influx rates were calculated from the depletion curve data (Fig. 1) with the Williams³¹ equation

$$
\ln = U/(T_2 - T_1) \cdot ((LnL_2 - LnL_1)/(L_2 - L_1))/2\pi r_0 \tag{4}
$$

where In is the average Zn influx rate (mol Znm⁻² root surface area s⁻¹), U is the amount of Zn taken up during the time period $T₂ - T₁$ *(i.e.* the amount of Zn removed from solution between each sampling time (Fig. 1)), and L_1 and L_2 are the root length at time T_1 and T_2 , respectively. The time between sampling $(T_2 - T_1)$ was either 0.5 or 1.0 h except for an 8 h period between 16 and 24 h. The root length at the beginning and end of each sampling period was calculated with Equation (3) by knowing the root growth constant (k) and total root length at the end of the experiment. Average Zn influx rates for each sampling period were paired with Zn concentrations at the beginning of each sampling period and the data were fitted to Equation (1) by nonlinear regression. Kinetics were based on the concentration of Zn^2 ⁺ computed from the total concentration of metals and ligands by the computer program GEOCHE M^{28} .

Cadmium influx kinetics

Cadmium uptake was measured in nutrient solution culture using a mixed resin system to maintain constant concentrations of nutrients and Cd^{10} . In this system the concentrations of Ca, Mg and K were controlled with Dowex-50-XS, P with aluminum saturated Amberlite-IR-120, Cd, Cu, Fe, Mn, and Zn with Chelex 100 and solution pH with Bio-rex 70. Equililbrium between the mixed resin system and the nutrient solution was aided with the continuous flow of solution over the resin.

Operation of the resin system is based on competitive equilibria between calcium and trace metals^{10,16}. At equilibrium, the selectivity coefficient for Ca-Cd exchange (K_{CA}^{Ca}) is given as ¹⁶

$$
K_{Cd}^{Ca} = \frac{[R\text{-}Ca](Cd^{2+})}{[R\text{-}Cd](Ca^{2+})}
$$
 (5)

where R-Ca and R-Cd^(*) are the concentrations of Ca and Cd on the resin and parentheses indicate activity of Ca^{2+} and Cd^{2+} in solution. Equation (5) can be rearranged to give

$$
pCd = pCa + pK_{Cd}^{Ca} + log(R - Ca/R - Cd) \tag{6}
$$

where p indicates the negative logarithm. Expected pCd values were calculated for the resin mixtures used (Table 1) by assuming that $pCa = 2.80$ and $pK_{Cd}^{Ca} = 4.50^{16}$.

The Ca, Mg, and K saturated resins were prepared by leaching H-Dowex-50-X8 (20-50 mesh) with 1 M metal nitrate solutions. All resins were stored moist in a desicator. Aluminum saturated resin was generated by leaching H-Amberlie-IR-120 (20–50 mesh) with 0.33 M AlCl₃ followed by 0.200 M Na, CO₃ to give an Al:OH ratio of 1:2, and P was then added at a rate of 0.5 meq H₂PO₄ g⁻¹ wet resin.

Calcium saturated resin at pH 6.0 was generated from Na-Chelex 100 (100-200 mesh) according to the method of Hendrickson¹⁶. Cadmium, Cu, Mn, and Zn saturated resins were generated by equilibrating Ca-Chelex 100 with 500 ml of 0.1 M Ca(OAc)₂ containing 63 mmol of the respective metal nitrate in solution. After 4 h, the metal saturated resins* were adjusted to pH 6, filtered under suction and stored moist. Bio-rex 70 (20-50 mesh) at pH 6.0 was generated from the Na⁺ form using the same procedure that was used to prepare the Ca-Chelex I00. Concentrations of metals on the prepared resins were determined by atomic absorption after extracting 0.1 g samples of wet resin with $1 M HNO$, (Table 2).

***** Metal-resins will be designated as R-Ca, *etc.* It should be noted that all resins contain Ca plus a trace metal. In all resin mixtures, R-Ca refers to the weighted concentration of Ca on all resins present and R-Cd refers to the weighted concentration of Cd on all resins present.

Table 1. Composition of the metal-Chelex 100 portion of mixed resin systems (metals in g)

***R**Ca and RCd are the concentrations of Ca and Cd on the Chelex resins (mmol g^{-1} resin).
 ****R**esin mix = sum of Ca-, Cu-, Mn- and Zn-Chelex 100.
 *****Calculated** with Equation (6).

*Mean of samples (Day 0, 7, 15) ta

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* Wet resin weight.

Ten resin mixtures were prepared (Table 1) to provide an expected Cd activity range of 0.39 to 123 nmol 1^{-1} . Initially, Cu-, Zn-, Mn- and Ca-Chelex resins were mixed at a weight ratio of 5:7:2:25. The Cu-Zn-Mn-Ca-Chelex resin mixture was then added to Cd-Chelex at weight ratios ranging from 10 to 160 (Table 1). Each resin mixture also received 75.75 g Ca-Dowex, 6.15 g K-Dowex, 22.35 g Mg-Dowex, 24.00g P-AI-Amberlite and 0.200g Fe-Chelex. Resin mixtures for treatments 1-5 received 37.50 g Bio-rex 70 while resins for treatments 6-10 received 15.5 g Bio-rex 70.

The resin mixtures for the different treatments were slurried into PVC columns (7.6 \times 15.2 cm). Foam-rubber plugs wrapped in glass wool and parafilm wrapped rubber stoppers were used to retain the resin in the columns. March model IE submersible pumps were used to continuously circulate the nutrient solution from the pots through the resin columns. The average flow rate for all systems was 618 ml min⁻¹ (CV 20.4%), resulting in a total exchange of nutrient solution in the 31 pots every 5 min. Some variability in flow rates was caused by bacterial growth on the inner surface of the tubing used. Bacterial growth was profuse on tygon tubing and thus glass was used wherever possible.

Three seedlings were transplanted into each pot after the solution had been circulating through the columns for at least 24 h. For treatments 1-5, two experiments were conducted: the first involved growing corn plants for 22 d while the second was for 15 d. The nutrient solution in the pots was changed and allowed to equilibrate for 24 h before planting the second crop. Only one growth period of 15d was used for treatments 6-10.

The composition of the initial nutrient solution was the same as that used to study Zn uptake except that no Cu, Mn, Zn or Fe-DTPA were added and that the P concentration was $20 \mu \text{mol}^{-1}$. Additional nutrient solution containing 100μ mol NO₃-N l⁻¹, 30μ mol Cal⁻¹, 76μ mol K l⁻¹, $12~\mu$ mol PO₄-P1⁻¹, 7 μ mol SO₄-S1⁻¹, 0.92 μ mol B1⁻¹ and 0.016 μ molMo1⁻¹ was supplied by Marriott bottles to replace water losses by evapotranspiration and to replace part of the macro- and micronutrients taken up by the corn plants. Iron was added to each pot by adding 75 μ mol of FeSO₄ on day one followed by 15 μ mol of FeSO₄ every 48 h. Iron was also supplied in foliar applications as a 1% Fe solution containing 0.02% Dupont WX surfactant and was applied periodically with a small paint brush. After 10 d of corn growth, 3 mmol of $KNO₃$ and 45 μ mol $KH₂PO₄$ were added to each pot.

Samples of nutrient solution were collected periodically from each pot for analysis. Solution pH was measured with a glass electrode, concentrations of Ca, Mg and K by flame atomic absorption, the phosphate by Murphy and Riley²⁴ procedure and sulfate by the BaCl, turbidimetric method¹.

The shoots and roots were harvested at the end of each growth period and roots were rinsed in deionized water before drying. Shoots and roots were separated, dried at 60° C and digested with $HClO₄: HNO₃$. Calcium, Cd, Cu, K, Mg, Mn and Zn in the digests were measured by flame atomic absorption. Root length was measured by the line intersect method $²⁹$ and average root radius was</sup> calculated with Equation (2). Average Cd influx rates were calculated with Equation (4) where U was the seedling content of Cd after the 15 and 22d growth periods $(T_2 - T_1)$, paired with the corresponding average Cd^{2+} concentration measured during the growth period, and fitted to Equation (1) by nonlinear regression. Concentrations of Cd^{2+} were calculated from the nutrient solution data (Figs. 2, 3 and 4) with the computer program $GEOCHEM^{28}$. Cadmium activities (Table 1) were calculated for the average concentration of Cd^{2+} calculated by GEOCHEM, using the Debye-Huckel equation²¹ and ionic strength estimated with GEOCHEM. One cannot assume that $Cd_T = Cd^{2+}$ since GEOCHEM calculated that approximately 20% of Cd_T existed as a soluble complex with sulfate. Other soluble species were $< 0.1\%$ of Cd_T.

Results and discussion

Zinc influx kinetics

The 16d corn plants had an average wet root weight of 13.1 g plant⁻¹ **(CV 21.8%) while the 22d plants had an average wet root weight of 33.7 g plant-1 (CV 8.5%) and, as expected, the older plants depleted Zn from solution faster than the younger seedlings (Fig. 1). Zinc depletion** curves from the 1.5 and $10 \mu \text{mol}^{-1}$ treatments followed the same form **(Fig. 1) which suggests the same uptake kinetics are operating at the two initial Zn concentrations. Depletion curve data for the Zn treatments were therefore combined for each date and fitted to Equation (1) by nonlinear regression.**

The Km values for Zn in Table 3 are lower than the value of 16μ mol $1⁻¹$ previously measured for excised barley roots⁶. One possible **reason for our lower values of Km is that the lowest Zn concentration** in Bowen's⁶ study was $20 \mu \text{mol}^{-1}$ whereas the highest concentration in our study was $10 \mu \text{mol}^{-1}$. The values in Table 3 are in agreement with **Km and Vmax values of 3.18** μ **mol Zn 1⁻¹ and 0.53** μ **molg⁻¹ dry roots h -1, respectively, that were reported for the first phase of Zn uptake by**

Seedling age (d)	Cd ⁹		Zn^*		
	Imax^+ $(pmol\,m^{-2}\,s^{-1})$	Km ¹ $(nmol1^{-1})$	Imax $(pmol\,m^{-2}\,s^{-1})$	Km $(\mu \text{mol})^{-1}$)	
16^{++}	400	30	550	2.2	
22	400	100	400	1.5	

Table 3. Kinetic parameters for Cd and Zn uptake by intact corn seedlings

 $+$ Imax = maximum metal influx rate.

[‡] Km = metal concentration where net influx rate = $\frac{1}{2}$ Imax.

¹ Determined in mixed resin system.

t Determined by depletion of solution Zn.

 $+$ + Does not include the germination period of 6d.

intact barley roots³⁰. Veltrup³⁰ observed three uptake phases for barley roots at Zn concentrations ranging from 0.1 to 1380μ mol 1^{-1} . A phase change in Zn uptake kinetics was not apparent in the present studies since there were no abrupt changes in the slopes of the Zn depletion curves (Fig. 1). In addition, the same uptake kinetics appeared to be operating in the 1.5 and 10 μ moll⁻¹ Zn treatments. Both Imax and Km values for Zn uptake by corn decreased with age (Table 3). Mengel and Barber²² measured Zn influx rates for corn plants grown under field conditions and found that Zn influx decreased from 10.98 to $0.578~\mu$ molm⁻¹ roots d⁻¹ between ages of 20 and 30d.

Cadmium influx kinetics

The ten mixed resin systems resulted in solution Cd activities that ranged from 6.3 to 164 nmol^{1} while the anticipated range was from 0.39 to 123 nmoll⁻¹. It is not known why there was such a large difference between the expected and observed pCd in some of the solutions. This contrasts with the work of Checkai¹⁰ who reported that measured and predicted pCd values were similar with resin systems using Ca-, Cu-, Mn-, and Zn-Chelex/Cd-Chelex* ratios similar to those listed in Table 1. The primary variable in the mixed resin system of this study was the concentration of Cd^{2+} in solution and which, as expected, controlled Cd uptake by corn (Table 4). Cadmium concentrations in the roots were higher than the shoots at 15 and 22 d. Average concentrations of Ca, K, Mg, P, Cu, Mn and Zn in the shoots of the 15 d plants were 128, 728, 82, 114, 0.17, 1.80 and 0.82 mmol kg^{-1} , respectively, while average concentrations of Ca, K, Mg, P, Cu, Mn and Zn after 22 d were 144, 675, 63, 127, 0.12, 1.88 and 0.77 mmol kg^{-1} , respectively. Coefficients of

Treatment	pCd^+	15d		22d	
		Shoots	Roots	Shoots	Roots
	6.79	1.15	4.00	0.71	4.35
	6.92	1.78	6.59	0.74	4.73
	6.87	1.46	4.93	0.98	5.41
4	6.97	1.05	5.87	0.67	4.67
5	8.13	0.03	0.54	0.04	0.55
6	7.60	0.28	5.11		
	7.71	0.21	6.95		
8	7.80	0.07	0.78		
9	7.92	0.05	0.21		
10	8.20	0.05	0.36		

Table 4. Concentrations of Cd in corn seedlings grown in the mixed-resin system for 15 and 22d

+ Mean of samples (Day 0, 7, 15) taken from resin-pot systems used to grow corn seedlings for 15 days.

Fig. 1. Depletion of Zn from solution by intact corn seedlings for initial concentrations of 1.5 and 10μ mol L⁻¹. The seedlings had been grown in complete nutrient solution for either 16 (\Box) or 22 (O) d when Zn uptake was measured.

variation for all nutrient element at both harvest dates ranged from 9 to 38%.

In the presence of corn seedlings the concentration of K and Ca (Fig. 2) and Mn, Zn and P (Fig. 3) in the nutrient solutions decreased with time. Solution pH decreased between 0 d and 7 d (Fig. 2), but increased thereafter due to the addition of 3 mmol KNO_3 per pot at 10d. The concentrations of Cd, Cu, Mn and Zn in the nutrient solutions also decreased with time (Fig. 3 and 4). Growing tomato *(Lycopersicon* e *sculentum* Mill.) plants in a similar mixed resin system¹⁰ for 25 d also resulted in decreases in the concentration of most ions in solution. $Checkai¹⁰$ attributed decreases in solution concentrations to diffusion limitations from the resin to tomato roots. We minimized diffusion limitations in our study by continuously circulating the nutrient solution through columns containing the appropriate resins. Decreasing nutrient concentrations indicate that rates of ion uptake by plants are faster than combined rates of desorption from the resins and distribution through the pot. The resin columns retained the capacity to control solution concentrations (treatments $1-5$) since a 24 h equilibration period between the first and second corn crop was sufficient to adjust trace metal concentrations to the same or higher levels than those observed initially. Decreases in trace metal concentrations with time were also coincident with decreases in macronutrient concentrations and to changes in pH of the nutrient solution. It is anticipated that changes in Cd concentrations with time had minimal affect on the root absorption characteristics for Cd (Table 3) since the ten treatments established a Cd^{2+} range of approximately 26 fold and the range between treatments remained relatively constant during the two growth periods.

Fig. 2. Changes in solution pH and concentration of Ca and K with time in the mixed resin system. Each point is the mean for Treatments $1-5$.

Fig. 3. Changes in solution concentrations of Mn, P and Zn with time in Treatments 1-5 of the mixed resin system.

Fig. 4. Changes in the Cd concentration with time in selected treatments of the mixed resins system.

Fig. 5. Average Cd influx rates for the mixed resin system at 15d plotted as a function of the free Cd concentration in solution. The curve was generated with equation (1) and the Cd influx parameters for the 15d corn seedlings (Table 4).

Cadmium influx rates were a function of Cd concentration in solution and ranged from 20 to 450 pmol m^{-2} root surface s⁻¹ at 15 d (Fig. 5) and from 20 to 260 pmol m⁻² s⁻¹ at 22 d. The calculated Km values (Table 3) are lower than a Km of 220 nmol $Cd_T 1⁻¹$ reported for phase one uptake by intact soybean plants $⁸$. It was not possible to distinguish multiphasic</sup> Cd uptake patterns for corn (Fig. 5) with the data obtained in the current study. The Km values (Table 3) show that corn is very efficient at accumulating Cd at low Cd concentrations. Calculated Imax values for Cd and Zn (Table 3) are similar in magnitude.

The mixed resin system of $Checkai¹⁰$ was used to measure Cd uptake parameters since this sytem can maintain Cd in solution at levels similar to soil solution concentrations of Cd. Cadmium concentrations in soil solutions are usually $< 0.1 \mu$ moll⁻¹⁹⁴⁸, and it would be difficult to maintain this Cd level in normal nutrient solutions. The establishment and operation of the mixed-resin system is time consuming, but it should be an important aid in future studies of trace metal-plant interactions. Macronutrient-plant interactions can also be studied if improvements in the resin system are made to insure that constant ion concentrations can be maintained in the presence of an expanding root system. Decreases in ion concentration with time in the presence of growing roots probably reflect a combination effect of diffusion limitations, resin desorption kinetics and flow rate of nutient solution. It is further suggested that wider resin:plant and solution:plant ratios be used. Having a larger reservoir of resin and nutrient solution should also help in maintaining constant ion concentrations in the presence of a growing root system.

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