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REGULAR ARTICLE

Cadmium uptake kinetics and plants factors of shoot Cd concentration

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Abstract
Background and aims Accumulation of Cd in the shoots of plants grown on Cd contaminated soils shows considerable variation. A previous preliminary experiment established that one major reason for this variation was the rate of Cd influx into the roots (mol Cd cm^{-2} root s^{-1}). However, this experiment did not distinguish between solubilization of soil Cd on the one hand and difference in Cd uptake kinetics on the other. The main objectives of the present study were thus to characterize Cd uptake kinetics of plants continuously exposed to Cd concentrations similar to those encountered in soils. Furthermore we determined the factors responsible for differences in shoot Cd concentration such as net Cd influx, root area-shoot dry weight ratio, shoot growth rate and proportion of Cd translocated to the shoot.
Materials and methods Maize, sunflower, flax and spinach were grown in nutrient solution with five constant Cd concentrations varying from 0 to 1.0 $\mu molL^{-1}$. Root and shoot parameters as well as Cd uptake were determined at two harvest dates and from these data Cd net influx and shoot growth rates were calculated.
Results and conclusions Cadmium uptake kinetics, i.e. the net Cd influx vs. Cd solution concentration followed a straight line. Its slope is the root absorbing power, α, (α = Cd net influx/Cd solution concentration). The α values of spinach and flax were about double that of maize and sunflower ($5\times10^{-6}cms^{-1}$ vs. $2.5\times10^{-6}cms^{-1}$). Spinach and flax had a 3–5 times higher shoot Cd concentration than maize and sunflower. The difference in shoot Cd concentration was partly due to the higher Cd influx but also to a higher translocation of Cd from root to shoot and also to a slower shoot growth rate.

Keywords Net Cd influx · Shoot Cd concentration · Cd translocation · Root surface area · Root absorbing power · Nutrient solution

Introduction

Cadmium is toxic to plants, animals and humans. Although its concentration in soils is usually very low and therefore does not pose a threat, soils may be contaminated because of geogenic reasons or by treatment as for example, by large amounts of waste water or sewage sludge with a high content of heavy metals. Plant species growing on such soils accumulate varying quantities of Cd in their shoots depending not only on species but also on genotype of the same

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species (Dunbar et al. 2003; Egle 2002; Keller 2000; Römer and Keller 2002) which may affect human health even before affecting plant growth (Guo et al. 2006; Renella et al. 2004). Some possible reasons for this difference in Cd accumulation in plant shoots are investigated in this research.

Stritsis et al. (2012) found that shoot Cd concentration of spinach was 8–30 times higher than that of maize; while sunflower and flax were between these values when grown on a Cd contaminated soil. These workers analyzed the factors responsible for the large differences and found that the size of the root system and the shoot growth rate were of minor significance. By contrast, net Cd influx (mol Cd per cm^2 root surface area per second) varied among species by a factor of 30, being almost parallel to shoot Cd concentrations. Analysis of soil following plant growth revealed that some species decreased Cd concentration in the soil solution whereas others increased it. However, these changes were not enough to explain the observed differences of the Cd influx. Another possible cause may relate to differences in Cd uptake kinetics between species, i.e. for the same Cd solution concentration at the root surface, Cd influx may differ among species as has been found for example for P (Bhadoria et al. 2004).

Uptake kinetics can not usually be studied in soil grown plants because the Cd solution concentration at the root surface is known to be different from the solution concentration of the bulk soil and varies with time. Uptake kinetics can therefore best be studied in nutrient solution as has been done for the major nutrients (Barber 1995) and also for Cd. These latter studies, however, have been mostly of short duration of a few hours, so that these results may not be applicable to long term studies as for plants grown in soil. One reason for this is that in short term experiments Cd uptake may be strongly over estimated as a consequence of a rapid Cd accumulation in the apoplasm which is freely accessible to the outer solution (Redjala et al. 2010) whereas for Cd uptake into the symplasm the ions have to cross the plasmalemma which is a much slower process. Another reason is that in short term studies of a few hours, in contrast to long term studies, no acclimation of the plants to different Cd levels occurs. During exposure to stress, after the initial shock the plants are able to adapt to both internal and external concentration (Küpper et al. 2007). To characterize Cd uptake kinetics, as may be expected for soil grown plants, long term uptake studies in nutrient solution supplied with graded Cd concentrations are needed. The Cd concentrations used in the experiments reported here are in a range up to 1.0 $\mu mol L^{-1}$ as would be expected in low to highly contaminated soils (Keller and Römer 2001). The use of higher concentrations of up to 50–100 $\mu mol L^{-1}$ as reported in some short term studies (He et al. 2007; Lombi et al. 2000, 2001) would not be suitable for use over long periods of time because the plants would probably not survive these concentrations. Moreover such very high concentration is atypical of Cd moderately contaminated soils used for crop production.

Uptake is most often described by a saturation curve of Michaelis-Menten type (Barber 1995) with a maximum influx, I_{max}, and a Michaelis constant, K_m, the concentration at which the net influx, I_n, equals $1/2I_{max}$. Furthermore, a minimum concentration, C_{Lmin}, is included at which I_n equals zero. The saturation curve applies usually when a wide range of concentration is considered, i.e. which exceeds by several fold the K_m value. However, when considering a concentration range below K_m uptake kinetics can well be approximated by a straight line (Sadana et al. 2005; Sayyari-Zahan et al. 2009) as shown below

$$I_n = \alpha \times C_{L0} \tag{1}$$

Where C_{L0} is the concentration at the root surface and α is the root absorbing power, in $cm\,s^{-1}$ (Nye 1973); it is the slope of the uptake isotherm. The α value in the low concentration range can be approximated by the ratio of I_{max}/K_m or measured directly, i.e. I_n/C_{L0}. Having a single parameter, i.e. α value, makes comparisons easier, among nutrients or plants, concerning the effectivity of the uptake system.

Nye and Tinker (1977, p.211) have discussed the factors determining shoot concentration X_S for a particular mineral nutrient which in principle may be applied when considering Cd influx by the root and its translocation to the shoot. Shoot Cd concentration, X_S, should be dependent on the root surface area (RA) per unit of shoot dry weight (SDW), the relative shoot growth rate (RGR_S), the Cd total net influx (I_{n_t}) and the proportion of total absorbed Cd that is translocated to the shoot (p):

$$X_S = \frac{RA}{SDW} \cdot \frac{1}{RGR_S} \cdot I_{nt} \cdot p \tag{2}$$

and net influx into the shoot is

$$I_{n_S} = I_{n_t} \cdot p \tag{3}$$

These equations make the simplifying assumption that X_S and the other parameters do not change very much during the time of measurement (Nye and Tinker 1977).

The main objective of this investigation was to characterize Cd uptake kinetics (root absorbing power) of plants continuously exposed to Cd concentrations between 0 and 1.0 $\mu mol L^{-1}$. Such findings would help to explain differences in Cd net influx observed in plants grown on soils with a Cd concentrations in the micromolar range such as found in an earlier study (Stritsis et al. 2012) and may be of use in Cd uptake modeling of soil grown plants. Further objectives were to determine the factors responsible for differences in shoot Cd concentration among plants (net Cd influx, root area-shoot dry weight ratio, shoot growth rate and proportion of Cd translocated to the shoot) and plant tolerance to external Cd concentration. To achieve this goal, plants were grown in nutrient solution with graded Cd concentrations and root and shoot growth as well as Cd uptake ($mol\ pl^{-1}$) and Cd net influx ($mol\,cm^{-2}s^{-1}$) were determined.

Material and methods

A solution-culture experiment was conducted in a climate chamber under controlled conditions (Temperature day/night 25 °C/18 °C, relative humidity day/night: 31 %/60 %, light intensity: day/night 16 h/8 h, PAR 240 $\mu mol\,m^{-2}s^{-1}$) using four plant species (*Zea mays* L., cv. Cascadas, *Helianthus annuus* L., cv. Ikarus, *Linum usitatissimum* L.ssp. *usitatissimum*, cv. Gold Merchant, *Spinacia oleracea* L., cv. Monnopa) and five Cd levels of 0.0, 0.1, 0.25, 0.5 and 1.0 $\mu mol\ L^{-1}$ supplied as $Cd(NO_3)_2\ 4{\bullet}H_2O$. Cadmium speciation calculations with Visual MINTEQ version 3.0 program (Gustafsson 2012) showed that around 90 % of the supplied Cd remained as Cd^{2+} in the nutrient solution used in this experiment.

Seeds were pre-germinated for 7 days in filter paper rolls and thereafter the seedlings were placed in 3 L plastic pots filled with a complete nutrient solution (Claassen and Barber 1974; Jungk and Barber 1974). The composition of nutrient solution was: 2.0 mM Ca $(NO_3)_2\ 4{\bullet}H_2O$, 0.25 mM NH_4NO_3, 0.25 mM $MgSO_4\ 7{\bullet}H_2O$, 1.5 mM KCl, 250 μM NaH_2PO_4, 46 μM H_3BO_3, 0.5 μM $(NH_4)Mo_7O_6\ 4{\bullet}H_2O$, 17.9 μM Na[FeEDTA], 9.1 μM $MnCl_2$, 0.8 μM $ZnSO_4\ 7{\bullet}H_2O$, 0.3 μM $CuSO_4\ 5{\bullet}H_2O$. The pH was 4.5. The nutrient solutions were aerated continuously by an air pump to induce mixing and supply of oxygen to the roots. The solutions were changed every 5 days up to the first harvest and every 3 days between the first and second harvests. The number of plants per pot was five for the first and three for the second harvest, but the results were later expressed per plant and not per pot.

Two harvests were performed in order to estimate rates (growth rates, and Cd net influx). The first harvest was performed 15, 15, 21 and 27 days and the second harvest 28, 28, 38 and 43 days after transplanting maize, sunflower, spinach and flax respectively into the nutrient solutions. Differences in length of growth periods for the various species prior to harvesting were dependent on differences in growth rates. For the first seven days after transplanting all plants grew in a Cd free solution, thereafter the solutions contained the Cd levels as shown above and were renewed every 5 days until the first harvest. Between the first and second harvest, i.e. the period in which rates were measured, special care was taken to retain the Cd level in solution all time close to the target concentrations of 0.0, 0.1, 0.25, 0.5 and 1.0 $\mu mol L^{-1}$. To achieve this aim, besides renewing the solutions every 3 days, the Cd concentration in the solution of every pot was measured daily and any depletion of Cd due to Cd uptake was replenished immediately. In this way the concentrations were maintained close to the target values. The actual average Cd concentrations which deviated somewhat from the target are shown in the tables and most figures. Cadmium in the solution was analyzed using graphite furnace atomic absorption spectrometer GFAAS. The limits of detection were $0.05\,\mu g L^{-1}$ (i.e. 0.45 $nmol L^{-1}$).

At each harvest, shoots were separated from roots by cutting with scissors and the plant parts dried at 105 °C (24 h). The dried samples were weighed, ground, and a subsample (200 mg) was digested with 4 mL HNO_3 (65 %) at 180 °C under pressure (1.0–1.7 MPa) in an oven for 10 h (Heinrichs et al. 2007). Digests were analyzed for Cd concentration using flame atomic absorption spectroscopy FAAS. The limits of detection were $2\,mg L^{-1}$ (i.e. 18 $\mu mol L^{-1}$).

Root fresh weight was determined after removing the surface water by carefully pressing the roots between filter papers and 0.8 g samples were preserved in 20 % ethanol for later measurement of root length

(RL) by the line intersect method. The line intersects were counted visually (Tennant 1975). Root surface area, RA ($cm^2 pl^{-1}$), was calculated from RL ($cm pl^{-1}$) and the root radius, r_0, using the formula:

$$RA = RL \times 2 \times \pi \times r_0 \quad (4)$$

Since roots are mostly composed of water a specific gravity of 1 $g cm^{-3}$ was assumed and the root considered to be a cylinder an average radius r_0 which was calculated using the following equation:

$$r_0 = \sqrt{\frac{RFW}{\pi \cdot RL}} \quad (5)$$

where RFW is the root fresh weight in g and RL is root length in cm.

The net total influx (I_{n_t}) and the net shoot Cd influx (I_{n_S}) was calculated using the following equations (Williams 1948)

$$I_{n_t} = \frac{(U_{tII} - U_{tI}) \cdot \ln\left(\frac{RA_{II}}{RA_I}\right)}{(t_{II} - t_I) \cdot (RA_{II} - RA_I)} \quad (6)$$

$$I_{n_S} = \frac{(U_{S\,II} - U_{S\,I}) \cdot \ln\left(\frac{RA_{II}}{RA_I}\right)}{(t_{II} - t_I) \cdot (RA_{II} - RA_I)} \quad (7)$$

where U_t is total plant and U_S is shoot Cd content in mol $plant^{-1}$, RA is root-surface area per plant in cm^2, t is time of harvest in s, and subscripts I and II refer to first and second harvest, respectively. The proportion of Cd absorbed between first and second harvest translocated to the shoot, p, was calculated according to Eq. 3.

For young plants with exponential growth, the relative growth rate of the shoot, RGR_S can be calculated as follows (Evans 1972):

$$RGR_S = \frac{\ln\left(\frac{SDW_{II}}{SDW_I}\right)}{t_{II} - t_I} \quad (8)$$

Where *SWD* is the shoot dry weight and t_I and t_{II} are time of the first and second harvest, respectively.

The experimental design was a factorial combination of five concentrations of Cd (0.0, 0.1, 0.25, 0.5 and 1.0 $\mu mol Cd L^{-1}$), four plant species (maize, sunflower, flax and spinach), four replications, two harvests, giving a total of 160 pots. Pots were completely randomized within a plant-growth chamber. After a logarithmic transformation a two-way ANOVA was run on all data sets with the statistic program SigmaStat 5.0 and Tukey test method ($p \leq 0.05$) was used to compare treatments.

Results

Shoot growth and Cd accumulation

The effect of Cd on shoot growth and Cd accumulation is described mostly for the second harvest. But for comparison data of the first harvest are shown in Table 4.

The influence of Cd solution concentration on *SDW* expressed relative to the untreated plants is given in Fig. 1. Up to a Cd concentration of 0.25 $\mu mol L^{-1}$ none of the four species showed any growth shoot reduction. At the concentration of 0.5 $\mu mol Cd L^{-1}$ only flax showed a significant growth reduction of about 30 %, a concentration of 1.0 $\mu mol Cd L^{-1}$ being required before all species, with the exception of maize, showed a significant reduction of *SDW*. The strongest decrease of around 40 % was for sunflower, flax and spinach while the growth reduction of maize was less than 20 %, which was statistically non-significant. Toxicity symptoms were visible at high external Cd concentration in solution as puny growth and yellowing of the intercostal fields to necrosis of tissues and characteristic brown surfaces on the leaves. Flax also showed leaf fall. Symptoms were most severe in sunflower, flax and spinach.

Shoot Cd concentration at the second harvest of all plant species (Fig. 2) increased linearly up to the highest Cd concentration in solution of 0.8–0.9 $\mu mol L^{-1}$ used in the experiment. Even at the highest, already toxic concentration, there was no indication of saturation. Maize and sunflower showed the lowest, and flax and spinach the highest Cd accumulation in their shoots. Shoot Cd concentration was always about four times higher in spinach than in maize.

The toxic effect (shoot growth reduction) of X_S is demonstrated in Fig. 3 in which the relative *SDW* is plotted against the internal Cd concentration in a logarithmic scale. Toxicity thresholds were calculated by correlating the relative shoot dry weight to the internal Cd concentration using a cubic model which gave

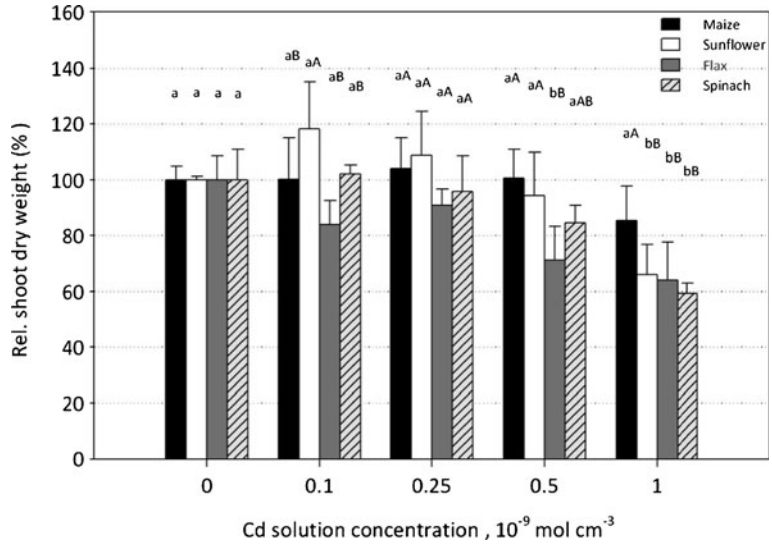

Fig. 1 Relative shoot dry weight (100 % were, in g plant^{-1}, 8.0 for maize, 2.3 for sunflower, 6.9 for flax and 5.0 for spinach) of plants grown at various Cd concentrations in the nutrient solution. Different upper case letters show significant differences among plants at the same Cd concentration in the nutrient solution and different lower case letters show significant differences of the same plant at different Cd concentrations in nutrient solution (Tukey, $P<0.05$, data are means at second harvest of 4 replicates and error bars represent the standard error of the means)

highest R^2 values for all species and thresholds. The results of 15 % shoot reduction show that for maize, sunflower and flax Cd in the shoot became toxic at about 35 mg kg^{-1} while for spinach it was at about 60 mg kg^{-1}. The toxic effect on flax may have started already with 10 mg kg^{-1} but because of the irregular trend of the curve it is possible that this value may be an outlier.

Root growth and Cd accumulation

Table 1 shows various root parameters as affected by Cd concentration in the nutrient solution at the second harvest. Results of the first harvest for comparison and understanding are shown in Table 4. Root dry weight, *RDW*, of maize and sunflower was not affected by Cd solution concentration while that of flax and spinach

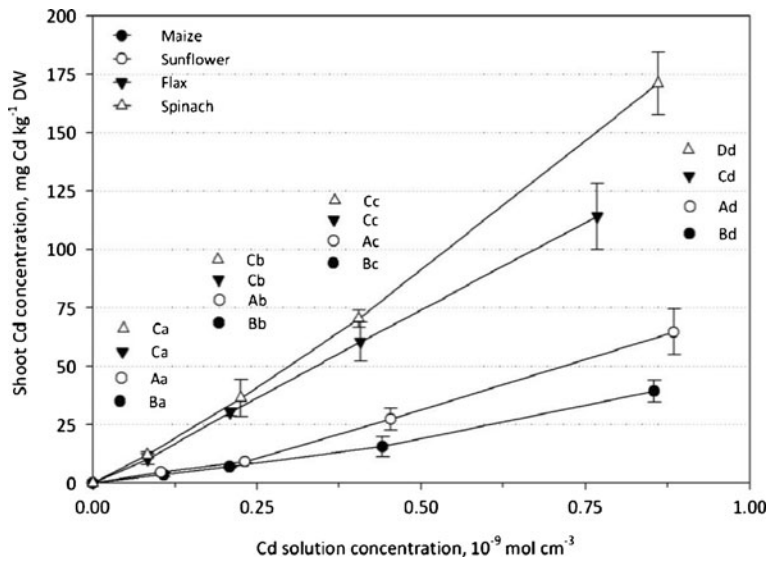

Fig. 2 Shoot Cd concentration of maize, sunflower, flax and spinach as influenced by Cd concentrations in the nutrient solution. Different upper case letters show significant differences among plants at the same Cd concentration in the nutrient solution and different lower case letters show significant differences of the same plant at different Cd concentrations in nutrient solution (Tukey, $P<0.05$, data are means at second harvest of 4 replicates and error bars represent the standard error of the means)

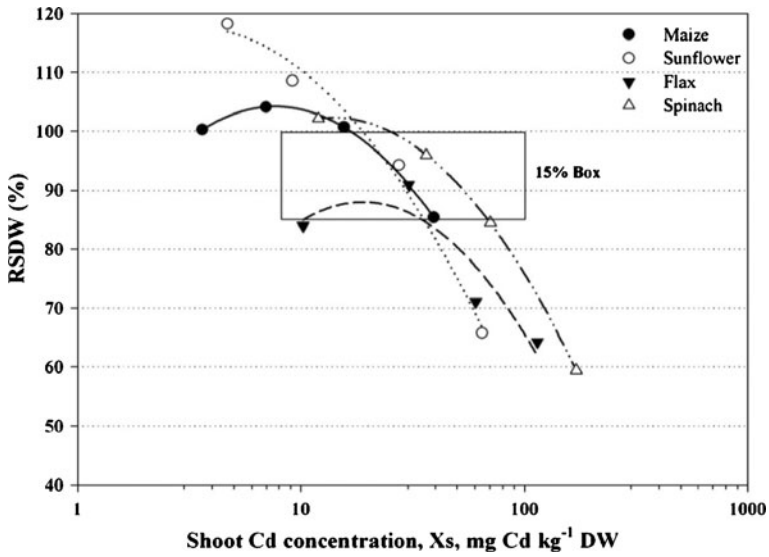

Fig. 3 Shoot growth as affected by Cd concentration in the shoot of four plant species. Relative shoot dry weight was set to 100 % at no Cd in the nutrient solution. Data represent the means at second harvest of n=4 replications. Notice the logarithmic scale of the x-axis. For statistical analysis considerations see Fig. 1

was reduced by 50 %. This root growth reduction occurred already at the lowest Cd level of 0.1 μmol L^{-1}, further increase of Cd in solution being without additional effect. The Table 1 also shows that the root

Table 1 Effect of Cd concentration in nutrient solution on root dry weight (RDW), root Cd concentration (X_R), root area (RA) and root radius (r_0)

Plant	Cd μmol L^{-1}	RDW g pl^{-1}	X_R μg g^{-1}	RA cm^2 pl^{-1}	r_0 cm
Maize	0.0	2.9 (0.3) Abc	0 (0)	3004 (123) Bb	0.029 (0.001) Ab
Sunflower	0.0	0.9 (0.1) Dab	0 (0)	1937 (82) Dab	0.022 (0.001) Bc
Flax	0.0	2.1 (0.3) Ba	0 (0)	4193 (476) Aa	0.017 (0.001) Cac
Spinach	0.0	1.2 (0.2) Ca	0 (0)	2695 (481) Cab	0.017 (0.001) Ca
Maize	0.10	2.7 (0.4) Ab	13.7 (1.6) Aa	2386 (350) Cd	0.033 (0.002) Aa
Sunflower	0.11	1.2 (0.2) Ba	23.8 (2.4) Ca	2427 (271) Ba	0.025 (0.002) Bb
Flax	0.08	1.2 (0.2) Bb	18.0 (5.8) Ba	2723 (323) Ab	0.018 (0.001) Ca
Spinach	0.08	0.8 (0.1) Bab	30.1 (2.8) Da	2363 (265) Dc	0.016 (0.001) Db
Maize	0.21	3.3 (0.4) Aa	32.5 (4.4) Ab	3555 (443) Aa	0.027 (0.001) Ac
Sunflower	0.24	1.0 (0.1) Bab	41.1 (4.0) Bb	1862 (198) Dbc	0.025 (0.001) Bc
Flax	0.21	1.0 (0.1) Bb	68.1 (5.3) Cb	2661 (279) Bbc	0.015 (0.001) Cb
Spinach	0.23	0.9 (0.1) Bab	84.4 (20.7) Cb	2627 (377) Cb	0.015 (0.001) Cb
Maize	0.44	2.9 (0.5) Aabc	64.7 (22.4) Ac	2969 (435) Ac	0.027 (0.001) Ac
Sunflower	0.45	1.0 (0.1) Bab	90.6 (10.3) Bc	1728 (376) Db	0.026 (0.001) Ab
Flax	0.41	0.9 (0.1) Bb	210.2 (34.6) Cc	2343 (369) Cd	0.016 (0.001) Bbc
Spinach	0.41	0.8 (0.2) Bab	202.4 (44.4) Cc	2356 (366) Bbc	0.015 (0.001) Cb
Maize	0.86	2.7 (0.2) Ac	147.4 (19.7) Ad	2811 (223) Ac	0.028 (0.003) Ac
Sunflower	0.88	0.6 (0.1) BCb	242.5 (17.4) Bd	932 (144) Dd	0.028 (0.002) Aa
Flax	0.77	1.0 (0.2) Bb	523.7 (66.7) Cd	2461 (372) Bcd	0.018 (0.001) Ba
Spinach	0.86	0.5 (0.1) Cb	495.9 (61.2) Cd	1381 (266) Cd	0.018 (0.002) Ba

Data represent the means at second harvest of n=4 replications, ± SD in parenthesis (All Pairwise Multiple Comparison occurred with Tukey, α=0.05). Different upper case letters show significant differences among plants at the same Cd concentration in the nutrient solution and different lower case letters show significant differences of the same plant at different Cd concentrations in nutrient solution

surface area, *RA*, followed a similar pattern to *RDW* because the root radius, r_0, is only slightly affected by Cd supply, i.e. there is only a slight tendency for the roots to become thicker at high Cd supply. Table 1 further shows the Cd concentrations in maize and sunflower roots increased almost linearly with increasing concentration of Cd in solution but in flax and spinach the increase was more than proportional. In order to explore whether there may be a barrier for Cd transfer to the shoot Fig. 4 demonstrates the relationships between X_S to X_R for the four crop species. In all cases the relationship was linear and Cd concentrations were three to four times higher in the root than in the shoot indicating possible retention of Cd in the root.

Cadmium uptake kinetics

Total net Cd influx, I_{n_t}, as well as the shoot net influx, I_{n_S}, increased linearly with increasing Cd solution concentration, C_{L0}, over the concentration range studied (0–1.0 $\mu mol L^{-1}$, i.e. $0–1\times10^{-9} mol cm^{-3}$). The concentration in the Fig. 5 was expressed in mol cm^{-3} in order to keep the units according to those used for the influx and to be able to calculate the root absorbing power, α. Even at toxic Cd concentrations I_{n_t} and I_{n_S} increased proportionally to C_{L0} except in sunflower where the increase was more than proportional. The regression lines describing the I_n versus C_{L0} pass through the origin and therefore their slope is equal to I_n/C_{L0}, which is the root absorbing power, α. For a better comparison among species, the α values are shown in Table 2. The root absorbing power, α_t, when considering I_{n_t} of flax and spinach is almost twice that of maize and sunflower. However for the latter, at the highest Cd concentration, the α_t is similar to flax and spinach. For I_{n_S}, α_S is smaller than for I_{n_t} because part of the Cd taken up remains in the root and is not translocated to the shoot. (For proportion of Cd translocated to the shoot, see below). Likewise for I_{n_t} and also for I_{n_S} the α_S values of flax and spinach are higher, but by a factor of about three. The higher difference between the two groups relates to the proportion of Cd translocated to the shoot (see Table 3) which was higher in flax and spinach than in maize and sunflower.

Factors of shoot Cd concentration

Table 3 shows the shoot Cd concentration, X_S, and the plant factors which, according to Eq. 2, are responsible for different X_S values. The plant factors are connected in a multiplicative way. As already shown in Fig. 2 shoot Cd concentration, X_S, was 3–5 times higher in spinach than in maize, the other species were in between. At the lowest Cd supply (0.1 $\mu mol L^{-1}$) the total net influx, I_{n_t}, was the same for all species. The higher X_S of flax and spinach as compared to maize and sunflower was related to the a lower relative shoot growth rate, RGR_S, a higher root to shoot ratio, RA/SDW, and a higher translocation, p. Maize and sunflower had a higher RA/SDW ratio but this was partly compensated by the higher RGR_S. At higher Cd supply spinach and flax remained with the higher X_S, again due to a higher Cd translocation to the shoot and lower RGR_S but also due to a generally somewhat higher I_{n_t} than the other species.

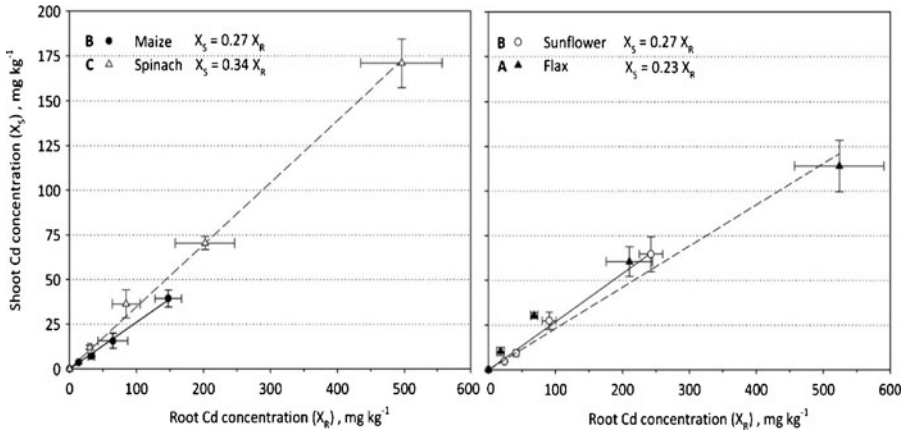

Fig. 4 Relationship between the shoot and the root Cd concentration of four plants species grown at various Cd concentrations in the nutrient solution. Different upper case letters show significant differences between the regressions coefficients (Data are means at second harvest of 4 replicates and error bars represent the standard error of the means)

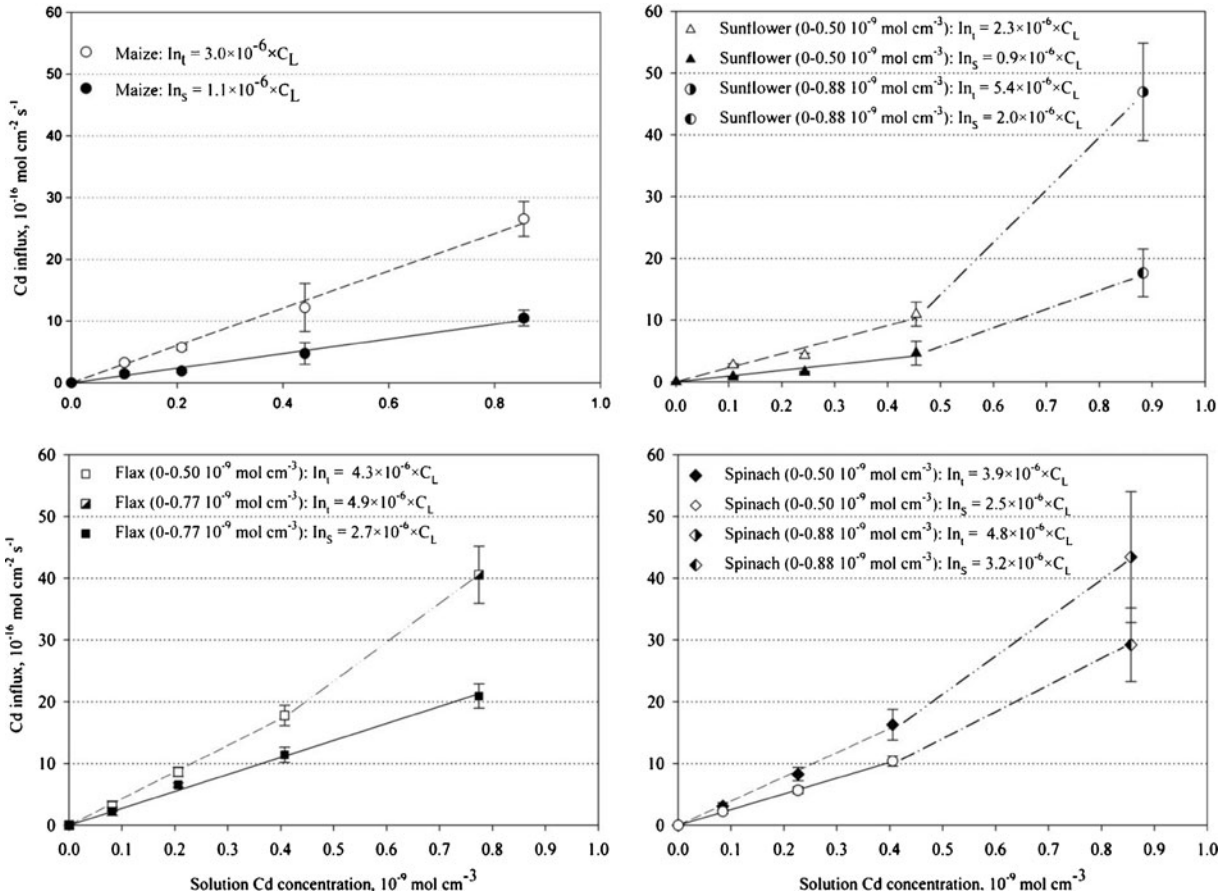

Fig. 5 Cadmium uptake isotherms for four plant species in the low concentration range (0–1.0 μmolL^{-1}). I_{n_t} = Cd total net influx, I_{n_S} = Cd net influx into the shoot (Data are means of 4 replicates and error bars represent the standard error of the means)

Discussion

Tolerance to external Cd concentration

The investigation reported here was a long-term Cd treatment study in nutrient solution in which roots were continuously exposed to defined Cd concentrations simulating soil grown plants for which these values are unknown.

In our study, roots accumulate much greater amounts of Cd than shoots (Tables 1 and 4) and dicotyledonous absorb more Cd from solution than monocotyledon (Inouhe et al. 1994; Kuboi et al. 1986). The root of maize showed an improved tolerance than the other plants. Flax attained only 48 % and spinach 42 % of its maximum RDW under high Cd supply conditions in solution and regarding the root yields were thus as Cd sensitive plants (Table 1). At low Cd solution concentration, up to 0.25 μmolL^{-1}, plants did not show any significant reduction in shoot growth. This began at a Cd solution concentration of 0.5 μmolL^{-1} for flax and spinach (Fig. 1). At 1.0 μmolL^{-1} all plants showed a shoot growth

Table 2 The Cd root absorbing power, α, for four plant species for the Cd concentration range in nutrient solution of 0–1.0 μmolL^{-1}

Plant	α_t 10^{-6}cms^{-1}	α_S
Maize	3.0±0.17	1.1±0.07
Sunflower[a]	2.3±0.19 (5.4)	0.9±0.01 (2.0)
Flax[a]	4.9±0.15 (4.3)	2.7±0.06
Spinach[a]	4.8±0.36 (3.9)	3.2±0.2 (2.5)

The value α_t is for total Cd net influx and α_S is for shoot Cd net influx

[a] The α value is for the concentration of 0–0.5 μmolL^{-1} and the value in parenthesis is at the higher concentration of 0.88 μmolL^{-1}

Table 3 Plant factors that determine the measured shoot Cd concentration (X_S) of plants grown at different Cd concentration in nutrient solution at second harvest; root area/shoot dry weight ratio (RA/SDW) at second harvest, shoot growth rate (RGR_S), Cd total influx (I_{nt}) and proportion of Cd translocated to the shoot (p)

Plant	Cd $\mu mol L^{-1}$	X_S $10^{-8} mol g^{-1}$	RGR_S $10^{-6} g g^{-1}$	RA/SDW $cm^2 g^{-1}$	I_{nt} $10^{-16} mol cm^2 s^{-1}$	*p* %
Maize	0.0	0.0	2.0 (0.1) Aa	357 (25) Dc	0.0	0.0
Sunflower	0.0	0.0	2.1 (0.1) Aa	828 (37) Aa	0.0	0.0
Flax	0.0	0.0	1.3 (0.1) Da	606 (45) Ba	0.0	0.0
Spinach	0.0	0.0	1.0 (0.1) Ca	541 (51) Ca	0.0	0.0
Maize	0.10	3.2 (0.7) Ca	1.9 (0.2) Aa	290 (11) Cb	3.3 (0.4) Aa	44 (6) Ba
Sunflower	0.11	4.2 (0.4) Ba	2.1 (0.1) Aa	880 (45) Aa	2.7 (0.2) Aa	31 (3) Cc
Flax	0.08	9.1 (2.0) Aa	1.5 (0.2) Ba	471 (65) Bb	3.1 (0.8) Aa	74 (8) Aa
Spinach	0.08	10.7 (1.1) Aa	0.8 (0.3) Ca	467 (47) Bb	3.1 (0.6) Aa	72 (3) Aa
Maize	0.21	6.2 (0.2) Cb	1.8 (0.2) Aa	426 (24) Ca	5.7 (0.5) Bb	35 (3) Cb
Sunflower	0.24	8.2 (0.5) Bb	1.8 (0.1) Aa	735 (36) Ab	4.3 (0.3) Cb	37 (3) Cbc
Flax	0.21	27.0 (1.5) Ab	1.2 (0.2) Ba	423 (39) Cc	8.6 (0.8) Ab	74 (3) Aa
Spinach	0.23	32.4 (7.0) Ab	1.0 (0.1) Cb	567 (136) Ba	8.3 (1.1) Ab	69 (5) Ba
Maize	0.44	13.9 (3.8) Cc	1.9 (0.1) Aa	378 (45) Dbc	12.2 (3.9) Bc	40 (5) Ca
Sunflower	0.45	24.4 (4.3) Bb	1.8 (0.2) Aa	775 (74) Ab	10.9 (2.0) Bc	45 (7) Ca
Flax	0.41	53.8 (7.4) Ab	1.2 (0.2) Ba	477 (31) Cb	17.8 (1.6) Ac	62 (3) Ab
Spinach	0.41	62.6 (3.2) Ab	0.9 (0.1) Ca	561 (63) Ba	16.3 (2.5) Ac	65 (5) Ac
Maize	0.86	35.1 (4.2) Dd	1.7 (0.1) Ba	429 (67) Da	26.5 (2.8) Bd	40 (2) Ca
Sunflower	0.88	57.5 (8.7) Cd	2.0 (0.2) Aa	606 (33) Ac	46.9 (7.9) Ad	40 (3) Ca
Flax	0.77	101.5 (12.7) Bd	1.2 (0.2) Ca	558 (29) Ba	40.6 (4.6) Ad	50 (2) Bc
Spinach	0.86	152.1 (12.0) Ab	0.8 (0.1) Da	466 (68) Cb	43.4 (10.6) Ad	68 (6) Aa

Data represent the means at second harvest of $n=4$ replications, ± SD in parenthesis (All Pairwise Multiple Comparison occurred with Tukey, $\alpha=0.05$). Different upper case letters show significant differences among plants at the same Cd concentration in the nutrient solution and different lower case letters show significant differences of the same plant at different Cd concentrations in nutrient solution

reduction which was about 40 % for flax and spinach as well as for sunflower while for maize the less than 20 % decrease was statistically non-significant. These findings demonstrate that these four crop plants differ in tolerance to external Cd solution concentration. Similarly the comparison of *Noccaea caerulescens* (Cd hyperaccumulator), Indian mustard (Cd accumulator), and tobacco (non-accumulator) showed that the metal-accumulator species were more tolerant to Cd toxicity than tobacco growing up to 400 $\mu mol L^{-1}$ $CdCl_2$ for one week (Wang et al. 2008). Not only species, but also genotypes of the same species may differ in tolerance to external Cd concentration as found for 11 barley genotypes (Wu and Zhang 2002). Yield reduction varied among the genotypes from 0 % to 40 % at external Cd concentration of 0.1 $\mu mol L^{-1}$. Differences in Cd tolerance to external Cd supply could be due to both difference in Cd uptake or tolerance to internal Cd concentration.

Tolerance to internal Cd concentration

Growth reduction was shown to be species dependent (Fig. 3) starting at different X_S, thereby indicating differences in tolerance towards internal Cd concentration. Spinach was the most tolerant of the four species studied. Shoot Cd concentrations at 15 % growth reduction measured in this experiment are higher than the phytotoxicity thresholds between 14 and 19 $mg kg^{-1}$ as reported for sunflower (Gopal and Nautiyal 2011). Tolerance to internal Cd concentration is probably related to differences in the detoxification of Cd in the cell brought about by vacuolar compartmentation and binding to cell walls (Vogel-Mikus et al. 2010) or by reaction with endogenous

Table 4 Effect of Cd concentration in nutrient solution on shoot dry weight (SDW), root dry weight (RDW), shoot Cd concentration (X_S), root Cd concentration (X_R) and root radius (r_0)

Plant	Cd $\mu molL^{-1}$	SDW $g\ pl^{-1}$	RDW $g\ pl^{-1}$	X_S μgg^{-1}	X_R μgg^{-1}	r_0 $10^{-2}m$
Maize	0.0	0.94 (0.10) Ba	0.40 (0.01) Ca	0.0 (0.0)	0.0 (0.0)	2.7 (0.1) Ca
Sunflower	0.0	0.23 (0.02) Aa	0.10 (0.01) Ab	0.0 (0.0)	0.0 (0.0)	2.7 (0.1) Cc
Flax	0.0	1.22 (0.15) Bb	0.29 (0.04) Ba	0.0 (0.0)	0.0 (0.0)	2.1 (0.1) Ba
Spinach	0.0	1.20 (0.11) Bc	0.30 (0.04) Bb	0.0 (0.0)	0.0 (0.0)	1.8 (0.1) Aa
Maize	0.10	0.94 (0.10) Ba	0.41 (0.10) Ca	7.3 (0.7) Aa	23.0 (2.4) Ba	3.3 (0.3) Cd
Sunflower	0.11	0.29 (0.03) Ab	0.11 (0.02) Ab	9.3 (0.8) Ba	32.3 (2.0) Ca	2.4 (0.3) Ba
Flax	0.08	0.82 (0.21) Ba	0.26 (0.07) Ba	17.5 (4.6) Ca	21.2 (8.1) Ba	2.0 (0.1) Aa
Spinach	0.08	1.21 (0.57) Cc	0.31 (0.05) Bb	7.2 (0.5) Aa	14.4 (1.5) Aa	1.9 (0..1) Aa
Maize	0.21	1.21 (0.11) Ba	0.54 (0.11) Ca	14.1 (2.1) Ab	45.1 (5.3) ABb	3.2 (0.2) Cd
Sunflower	0.24	0.35 (0.05) Ac	0.14 (0.03) Ac	17.8 (2.4) Bb	73.0 (10.6) Cb	2.5 (0.3) Bb
Flax	0.21	1.21 (0.22) Bb	0.35 (0.05) Ba	31.3 (5.6) Cb	53.8 (9.5) Bb	2.0 (0.1) Aa
Spinach	0.23	1.08 (0.06) Bb	0.27 (0.03) Bb	27.2 (1.7) Cb	40.0 (3.0) Ab	1.9 (0.1) Aa
Maize	0.44	1.02 (0.14) Ba	0.42 (0.14) Ca	39.6 (5.5) Ac	98.7 (4.9) Bc	3.0 (0.4) Cc
Sunflower	0.45	0.31 (0.04) Ac	0.12 (0.02) Ac	74.0 (19.4) Cd	138.1 (20) Cc	2.7 (0.1) Bc
Flax	0.41	1.01 (0.12) Ba	0.32 (0.06) Ba	55.5 (5.3) Bc	160.6 (20.2) Cc	1.9 (0.2) Aa
Spinach	0.41	1.14 (0.04) Bbc	0.35 (0.08) BCb	43.8 (2.3) Ac	71.8 (5.3) Ac	1.9 (0.1) Aa
Maize	0.86	1.08 (0.11) Ba	0.45 (0.11) Ca	64.5 (8.7) Bd	194.6 (11.2) Ad	2.8 (0.2) Bb
Sunflower	0.88	0.17 (0.04) Aa	0.06 (0.02) Aa	52.9 (15.4) Ac	269.2 (15.4) Bd	2.7 (0.2) Bc
Flax	0.77	0.83 (0.14) Ba	0.27 (0.04) Ba	95.9 (12.3) Cd	357.5 (29.6) Cd	2.0 (0.1) Aa
Spinach	0.86	0.95 (0.10) Ba	0.21 (0.04) Ba	90.0 (5.9) Cd	182.3 (9.1) Ad	1.9 (0.1) Aa

Data represent the means at first harvest of n=4 replications, ± SD in parenthesis (All Pairwise Multiple Comparison occurred with Tukey, α=0.05). Different upper case letters show significant differences among plants at the same Cd concentration in the nutrient solution and different lower case letters show significant differences of the same plant at different Cd concentrations in nutrient solution

phytochelatins and GSH (Brunetti et al. 2011; Pomponi et al. 2006).

Cadmium uptake kinetics

The major goal of our experiment was to obtain uptake kinetic data that could also be applied to plants growing in soil, i.e. uptake kinetic data of plants that were, like in soil, continuously exposed to Cd. Furthermore the net Cd influx was determined through the Cd uptake during the entire growing period, i.e. the net Cd influx was an average of plants that had acclimatized to the respective Cd exposure. This approach contrasts markedly to many studies where roots have been exposed to different Cd concentrations for a short time of a few hours only.

For all species the net Cd influx increased linearly with the Cd solution concentration, showing no sign of any saturation, indeed the increase was more than proportional at a Cd concentration greater than 0.5 $\mu molL^{-1}$. This was very clear in sunflower although much less evident in flax and spinach (Fig. 5). This linear increase means that the root absorbing power, α, remained constant for the concentration range studied. However, among the species studied, α varied from about $2.5\times10^{-6}cms^{-1}$ for maize and sunflower to about $5\times10^{-6}cms^{-1}$ for flax and spinach, i. e. among the four species α only varied by a factor of about 2. The same plant species and cv. when grown in soil (Stritsis et al. 2012) also showed a linear relationship between Cd soil solution concentration in the micromolar range and the net Cd influx. These results suggest that for the four species studied and acclimatized to their respective Cd solution concentrations, Cd uptake kinetics can be described by a linear relationship for the concentration range of up to 1.0 $\mu molL^{-1}$.

The linear absorption isotherm reported here only partially agrees with other investigations. This may be due to the method used, i.e. a long term uptake instead of a few hours uptake study using radioactive Cd. Mullins and Sommers (1986) grew maize in a resin buffered solution up to 22 days and found that the Cd influx was between 10×10^{-16} and 25×10^{-16} mol cm^{-2} s^{-1} at Cd concentrations below 0.02 μmol L^{-1} but at increasing Cd concentration the Cd influx rose markedly in a discontinuous manner to around 350×10^{-16} mol cm^{-2} s^{-1} and remained at that level up to a Cd concentration of 0.30 μmol L^{-1}. At that Cd concentration in our experiments the Cd shoot influx of maize was below 10×10^{-16} cm^{-2} s^{-1}, i.e. 30–40 times lower. The Cd influx values obtained by Mullins and Sommers (1986) seem unrealistically high because they approach values of P influx (Bhadoria et al. 2004) and also the shape of the uptake isotherm is not a true saturation curve. The authors did adjust a Michaelis-Menten equation but there was a strong lack of fit to it (Mullins and Sommers 1986). By contrast, for wheat growing under similar conditions to our experiment maintaining a constant Cd solution concentration a Michaelis-Menten uptake kinetic was reported with a K_m value of 1.0 μmol L^{-1} and α of around 6×10^{-6} cm s^{-1} (Burghard 1992). These data are comparable with ours because at Cd concentration below K_m the uptake isotherm may be approximated by a straight line as found in our experiment.

In short term experiments where roots are exposed to Cd for a few hours, and the plant does not acclimatize to possible toxic effects of Cd, the uptake isotherms are a straight line (Perriguey et al. 2008) with α value of 10^{-5} cm s^{-1}, similar to our values. Other studies (Redjala et al. 2009) show a high affinity saturable transport system (HATS) for Cd with a low affinity transport system (LATS). The HATS had a K_m of about 0.3 μmol L^{-1} while LATS was the linear component of the uptake isotherm. The linear component of the Cd uptake system is likely related to that Cd uptake may be passive (Welch and Norvell 1999) through channels of other divalent cations like Ca which are permeable to Cd (Perfus-Barbeoch et al. 2002; White 2000).

For sunflower instead of a saturation of the Cd uptake we observed a more than proportional increase of Cd net influx when the Cd solution concentration was increased from 0.5 μmol L^{-1} to 1.0 μmol L^{-1}, α increased from 2.3×10^{-6} to 5.4×10^{-6} cm s^{-1} (Fig. 5). For flax and spinach there was a small trend in the same direction. Perhaps this trend is due to Cd damage to cell membranes thereby increasing permeability as shown in another study demonstrating increased leakage of K (Gussarsson and Jensén 1992). This effect on Cd uptake kinetics has not been reported by other researchers possibly because the time of exposure to Cd was less than in our experiment.

One of the initial questions of this paper was whether differences in Cd net influx observed in soil grown plants reported in a previous publication (Stritsis et al. 2012) could be explained by inter species differences in uptake kinetics. These workers also found that the Cd shoot net influx, I_{ns}, was linearly related to the Cd solution concentration of the whole soil (bulk and rhizosphere soil). The slope of the lines we may call α′, because the concentration of the whole soil is only an indirect estimation of the actual concentration at the root surface which usually will be lower. We may, however, compare α′ with the α values obtained in the present experiment expecting that they are correlated.

In soil, α′ was similar for maize, sunflower and flax of around 0.25×10^{-6} cm s^{-1}. In nutrient solution, though, α was similar for maize and sunflower only (around 1.0×10^{-6} cm s^{-1}) while for flax it was, with 2.7×10^{-6} cm s^{-1}, almost three times higher. Furthermore, in soil α′ was six times higher for spinach than for flax (1.4×10^{-6} cm s^{-1} vs. 0.24×10^{-6} cm s^{-1}) while in nutrient solution the α values were similar for both species of around 3×10^{-6} cm s^{-1}. This indicates that not only uptake kinetics could determine the Cd uptake from soil solution but that apparently plants may affect the availability of Cd dissolved in the soil solution. For example root exudates may complex Cd in soil solution and make it non accessible to plant uptake (Scheffer and Schachtschabel 2002; Sposito 1989). Furthermore, different availability in soil than in nutrient solution of other ions, e.g. other cations, may have affected the uptake of Cd.

The comparison of the α (nutrient solution) and α′ (soil solution) values also shows that uptake kinetics are not sufficient to fully explain the differences of Cd shoot influx, I_{ns}, for plants grown in soil as observed in our earlier work (Stritsis et al. 2012). Further research is needed to elucidate Cd depletion in soil solution at the root surface and on the effect of root activity on Cd binding in the soil liquid and solid phase.

Factors of shoot Cd concentration

Plant tolerance to Cd supply was found mainly to relate to the X_S obtained by the different plant species. The plant factors determining the X_S are shown in Table 3. According to Eq. (2) the product of the four factors (I_{n_t}, RA/SDW, $1/RGR_S$, p) should be equal to X_S expressed in mol g^{-1}. This is fully given in some cases but in others there is a deviation of 10 %–30 %. These deviations are because the assumption of constancy of the factors and X_S during the period of measurement (between first and second harvest) are not fully given (compare data from Fig. 1 and Table 4). But even so Eq. (2) and the factors of Table 3 allow an assessment of the significance of all factors at once.

At low Cd supply the I_{n_t} for all species is the same and therefore not responsible for the up to 3 times different X_S. Only at higher, already toxic Cd levels, the I_{n_t} differs among species by a factor of about 1.5, but even though related to the X_S it does not explain the actual difference of X_S by a factor of 4–5. The RA/SDW ratio of sunflower is higher than that of the other species. This should lead to a higher shoot Cd concentration but often a lower I_{n_t} and p compensate for it.

The lower X_S of maize and sunflower as compared to flax and spinach is consistently accompanied by a higher RGR_S and a lower p. The higher RGR_S may be seen as a so called dilution effect of the Cd translocated to the shoot. The lower p means that less of the absorbed Cd is translocated to the shoot or in other words, in the case of maize and sunflower, more Cd is retained in the root. This is mainly because they have a larger root/shoot ratio expressed on a dry weight basis (Fig. 1 and Table 1) and not because at the same shoot Cd concentration their root Cd concentration was higher than that of flax and spinach (Fig. 4). Differences in p could also be due to different degree of Cd precipitation as Cd phosphate on the root surface (Kupper et al. 2000). This was significant in Kupper et al. (2000), but they used a 100 times higher Cd concentration in the nutrient solution as in our experiment.

These results obtained in nutrient solution differ strongly from those obtained with the same species grown in soil in the same growth chamber (Stritsis et al. 2012), i.e. in soil it was mainly the Cd influx that explained the differences in Cd accumulation in the shoot. Similar results have been reported in 11 spinach cv. grown in soil where it was the Cd influx that determined differences in Cd uptake (Keller and Römer 2001; Römer and Keller 2002).

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