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DGT-measured fluxes explain the chloride-enhanced cadmium uptake by plants at low but not at high Cd supply

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Abstract

The technique of diffusive gradients in thin films (DGT) has been shown to be a promising tool to assess metal uptake by plants in a wide range of soils. With the DGT technique, diffusion fluxes of trace metals through a diffusion layer towards a resin layer are measured. The DGT technique therefore mimics the metal uptake by plants if uptake is limited by diffusion of the free ion to the plant roots, which may not be the case at high metal supply. This study addresses the capability of DGT to predict cadmium (Cd) uptake by plants at varying Cd supply. To test the performance of DGT in such conditions, we used the chloride (Cl^-) enhancement effect, i.e. the increase in Cd solution concentrations – due to chloride complexation of Cd – and Cd uptake with increasing Cl^- concentrations, as previously characterized in pot, field and solution culture experiments. The uptake of Cd by spinach was assessed in soil amended with Cd (0.4–10.5 mg Cd kg⁻¹) and NaCl (up to 120 mM) in a factorial design. Treatments with NaNO₃ were included as a reference to correct for ionic strengths effects. The effect of Cl^- on the shoot Cd concentrations was significant at background Cd but diminished with increasing soil Cd. Increasing Cl^- concentrations increased the root area based Cd uptake fluxes by more than a factor of 5 at low soil Cd, but had no significant effect at high soil Cd. Short-term uptake of Cd in spinach from nutrient solutions confirmed these trends. In contrast, increasing Cl^- concentrations increased the DGT measured fluxes by a factor of 5 at all Cd levels. As a result, DGT fluxes were able to explain soil Cl^- effects on plant Cd concentrations at low but not at high Cd supply. This example illustrates under which conditions DGT mimics trace metal bioavailability. If biouptake is controlled by diffusive limitations, DGT should be a successful tool for predicting ion uptake across different conditions.

Introduction

To predict the bioavailability of metals to plants, it is necessary to understand solution- and solid-phase supply processes in soils, as well as biotic factors that regulate metal uptake. The technique of diffusive gradients in thin films (DGT) measures trace metal fluxes to a resin sink and has been shown to be a promising tool to assess plant uptake in a wide range of soils. The DGT device contains a resin (Chelex) layer overlain by a hydrogel layer. The resin layer acts as a zero sink for metals, and thus induces a diffusion flux of metals through the diffusion layer. Highly significant correlations have been obtained between the concentration of Cu, Zn and other trace metals in plants and DGT-measured concentrations (Zhang et al. 2001; 2004; Nolan et al. 2005), suggesting that the soil parameters that govern the supply to DGT acted as a major control in uptake of these metals by plants as well (Lehto et al. 2006). The DGT device locally lowers the soil solution concentration, inducing metal resupply from labile metal complexes in solution and the labile metal pool in the solid phase. Plants have a similar effect if the uptake is limited by diffusion, and DGT is therefore considered to mimic diffusion-limited uptake by plants (Zhang et al. 2001).

Diffusive limitations for metal uptake can be alleviated by labile complexes. Several studies have shown that uptake of metals – at same free ion activity – is higher in presence of complexes (e.g. Bell et al. 1991). Increased chloride concentrations at a constant Cd activity, resulting in higher concentration of chloro-complexes, enhanced Cd uptake by Swiss chard in nutrient solutions (Smolders and McLaughlin 1996). Similar effects of chloride-enhanced Cd uptake in crops were reported in the field for potato (McLaughlin et al. 1994), sunflower kernels (Li et al. 1994), durum wheat (Norvell et al. 2000), and in a suite of pot trials under greenhouse conditions (Bingham et al. 1983; 1984; Smolders et al. 1998; Khosgoftar et al. 2004). It was recently shown that the contribution of the complexes to plant uptake, as well as to DGT-measured fluxes, followed the same order as the dissociation rate of the

complexes (Degryse et al. 2006 a; 2006 b). These findings supported the hypothesis that the contribution of complexes to metal uptake by plants is due to diffusive limitations in the uptake. As the free ion is depleted near the root surface, the complexes dissociate and thus enhance the supply of free ion.

Diffusion of metals to plant roots is an important supply mechanism only when the supply through mass flow, which is caused by plant water absorption, is not sufficient to meet the plant's demand. Therefore, the accuracy of DGT measurements as a surrogate for plant uptake may be compromised under conditions where convective transport to the roots surface becomes the most important transport mechanism. This is most likely to occur in contaminated soils where solution concentrations are more likely to exceed the (apparent) Michaelis-Menten constant (K_m) (Lehto et al. 2006). Under these conditions, the uptake by plants is expected to depend on free ion activity only. The DGT-flux, on the other hand, will still be affected by the presence of labile complexes and the extent of solid-phase buffering, since the Chelex resin acts as a zero sink even at elevated metal concentrations unless the resin becomes saturated. As a result, the relation between plant uptake and DGT measurements will likely break down at elevated metal concentrations.

In this study, we assessed whether DGT indeed fails to mimic plant uptake at high Cd^{2+} supply. The chloride enhancement effect was used to test this hypothesis. Uptake of Cd by spinach was assessed in a pot experiment as well as in solution culture at varying Cd concentrations and NaCl salinity in a factorial design. Treatments with NaNO_3 were included as a reference to correct for ionic strengths effects. The equilibrium speciation was determined and diffusion fluxes were measured with the DGT technique in soil solution and solution culture.

Materials and methods

Soil experiment

In this experiment, we tested the response of DGT fluxes and Cd uptake by plants to the NaCl and NaNO₃ salinity in a factorial combination with soil Cd. Plant Cd concentrations and root surface area of plants were measured to calculate the Cd uptake fluxes. The soil solution was analyzed and the soil solution speciation was calculated.

An uncontaminated non-saline soil was sampled from arable land in Belgium (pH 6.7, total Cd concentration 0.4 mg kg⁻¹), air dried and sieved (<2 mm). The dried soil was fertilized with a solution containing 1 mM KH₂PO₄ and 5 mM Ca(NO₃)₂. Treatments were imposed via the same wetting solution by adding NaCl or NaNO₃ to obtain final concentrations of 0, 10, 30, 60, 90 and 120 mM, and CdCl₂ to obtain final Cd addition of 0, 2.3 and 10.5 mg kg⁻¹. Soils were moistened to 23% water content and mixed. All soils were potted (500 g of moist soil per pot, 2 replicates). Seeds of *Spinacia oleracea* L. (Viking) were germinated in moist blotting paper water for 2 days at 20°C. Four germinated seeds were transplanted in each pot and the surface was covered with polyethylene beads. The pots were placed in a growth chamber (Weiss, 18'+5JuPa) programmed in cycles of 16 h day/8 h night at 20°C/16°C, respectively, and 70% humidity. Light regime in the chamber was set at 450 μmol photon m⁻² s⁻¹. Pot weights were recorded daily to estimate evapotranspiration losses, and the soil moisture content was restored daily by adding Milli-Q water. Eight days after planting, plants were thinned to 1 per pot. Final harvest of the plants was made 18 days after planting. Shoots were collected and dried (65°C) and roots were separated from the soil. A subsample of the soil was collected and soil solution and DGT analyses on soils were made immediately after harvest. Roots were rinsed with tap water and dried on blotting paper. Fresh weight of the foliage and the roots was recorded. Root length L_r (cm) was estimated with the line intersection method (Tennant 1975). The root radius R (cm) was determined as the average of

about 50 random root samples per plant, and the root surface area of each sample (cm^2) was calculated as $2\pi RL_r$. Root lengths were measured for 12 individual plants from six treatments (lowest and highest soil Cd at 0 mM salinity, 90 mM NaCl, and 90 mM NaNO_3) and normalized per root dry weight. The relative growth rate (RGR, d^{-1}) was calculated from the difference in shoot dry weights (W) between 8 and 18 days after planting, i.e. $\text{RGR} = \Delta \ln W / \Delta t$, with Δt , the time difference in days. The average flux of Cd from soil to plants on a root surface area basis (F , $\text{nmol Cd cm}^{-2} \text{ root d}^{-1}$) was calculated as (Degryse et al. 2006b):

$$F = M_{\text{pl}} \times \frac{\text{RGR}}{\text{RWR} \times \text{SRA}} \quad (1)$$

where M_{pl} is the concentration of Cd in the plant (nmol g^{-1} dry wt), RWR is the root weight ratio (g root per g plant) on dry weight basis, and SRA is the specific root area ($\text{cm}^2 \text{ g}^{-1}$ root dry wt). The Cd concentration in the roots was not measured but was assumed identical to that in shoots.

Soil solution sampling and characterization

Soil solutions were isolated from saturated pastes, which were prepared following the method suggested by Rhoades (1996). Suitable amounts of Milli-Q water were added to the soil until saturation (i.e. until the paste glistens as it reflects light) and were stored in plastic containers overnight at 20°C . The soil solutions were isolated by centrifugation at 3000 g followed by membrane filtration ($0.45\mu\text{m}$). The first droplets were discarded. Chloride, NO_3^- and SO_4^{2-} were determined in the soil solution by ion chromatography (Dionex ICS-2000). The concentration of dissolved organic carbon (DOC) was determined by thermal oxidation (Thermalox TOC analyser, Analytical Sciences). Metal concentrations in the acidified soil solutions were measured with ICP-OES (Perkin Elmer optima 3300 DV), except for the soils without Cd addition, for which the solutions were analyzed by ICP-MS (Thermo ICP-MS, X series I). Speciation of Cd in the soil solution was calculated with the program GEOCHEM

PC. Input data were the elemental concentrations, and the soil pH. Carbon dioxide was assumed to be in equilibrium with the atmosphere.

Plant analysis

Dried shoot samples were ground, and 50 to 100 mg subsamples were digested in 2 mL of HNO₃ and diluted to 5 mL with Milli Q water. Metal concentrations were determined by ICP-OES. The Cd detection limit of this procedure was 0.05 mg kg⁻¹ (= 0.4 nmol g⁻¹). The digestions were carried out in batches including two blanks and an internal reference plant material. As internal reference material endive leaf from the International Plant-analytical Exchange programme (IPE, University Wageningen) with a consensus value of 1.75 mg Cd kg⁻¹ dry wt was used. In our analyses we found 1.74 ± 0.09 mg Cd kg⁻¹ dry wt.

Cd fluxes measured with DGT

The Cd diffusion flux (F_{DGT}) was measured in soil with the DGT technique (Zhang and Davison, 1995) after plant growth on the treatments with lowest and highest soil Cd in combination with NaCl (all concentrations) or NaNO₃ (30, 60 and 120 mM). The DGT devices had a surface area of 3.14 cm² and a diffusion gel layer thickness of 0.76 mm. The DGT devices were applied to the saturated pastes prepared as described above. The DGT sampling area was pressed onto a portion of saturated paste, ensuring good contact. The deployment time was 97 hours (at 20°C) for all the soils. The metal accumulated on the resin was eluted with 1 M HNO₃ solution and the Cd concentration in the eluate was measured by ICP-OES. The DGT-measured flux was calculated as $F_{DGT} = M/(A.t)$ (Zhang and Davison, 2001), where M is the mass accumulated on the resin gel, measured by ICP-OES, A is the surface area (3.14 cm²), and t is the deployment time.

Solution culture experiment

In this experiment, short term uptake of Cd by plants and DGT-fluxes were assessed in solutions with either 40 mM NaCl or NaNO₃ at 3 different activities of Cd²⁺. Seeds of *Spinacia oleracea* L. (Viking) were germinated in blotting paper moistened with diluted nutrient solution for 7 days at 20°C. The seedlings were transferred to an aerated nutrient solution (composition in mM: 2 Ca(NO₃)₂, 0.5 MgSO₄, 1.2 KNO₃, 0.1 KH₂PO₄; in μM: 25 NaCl, 5 MnSO₄, 15 H₃BO₃, 2 CuSO₄, 0.07 (NH₄)₆Mo₇O₂₄, 1 ZnCl₂, 10 FeNaEDTA). The solution was buffered with 2 mM MES at pH 6. The plants were grown in a growth chamber with a 16/8 h day/night cycle, day/night temperature of 20/16 °C, and photon flux density of 450 μmol m⁻² s⁻¹. The plants were taken from the nutrient solution at 15 days after sowing. Roots were rinsed with 2 mM Ca(NO₃)₂, and two plants were transferred to 300 mL of the treatment solution, that was radiolabeled with ¹⁰⁹Cd (~10 kBq L⁻¹) and ⁶⁵Zn (~50 kBq L⁻¹). The treatment solutions contained 40 mM of either NaCl or NaNO₃, 15 μM H₃BO₃, 2 mM HEPES (pH 7), and Cd(NO₃)₂ at three different concentrations. The (calculated) free Cd²⁺ concentrations were identical for the NaCl and the corresponding NaNO₃ treatment and were 1 nM, 1 μM or 6 μM Cd²⁺. Total Cd concentrations were nearly equal to the free Cd²⁺ concentrations for the NaNO₃ treatments but threefold higher for the NaCl treatments, in which the concentration of CdCl_n²⁻ⁿ complexes was about two-fold higher than the free Cd²⁺ concentration. The solutions were not stirred during plant uptake. Plants were removed from the treatment solutions after 3 hours, and transferred to a 10 mM Ca(NO₃)₂ solution for 10 minutes, to remove ¹⁰⁹Cd bound to the cell wall. The roots were blotted, and plants were divided in shoots and roots and weighed. The ¹⁰⁹Cd activity was determined with a Minaxi 5530 auto-gamma counter and Cd uptake per unit root weight was derived from the specific activity of the solution. The remaining ¹⁰⁹Cd activity in the solutions was determined; depletion of Cd in the solution was always less than 30%. Diffusional fluxes were also

measured on these solutions with the DGT technique. The DGT devices were placed – floating face down – in the radiolabeled solutions, and stirred during deployment with 2-fold replication per treatment. After 1 day, the devices were retrieved and the resin gel was eluted with 1 M HNO₃. The ¹⁰⁹Cd activity of the eluate was measured, and the mass accumulated on the resin was calculated from the specific activity of the solution.

Results

Soil experiment

Dissolved Cd concentrations in soil solutions significantly increased with increasing NaCl or NaNO₃ addition; the relative increase (ratio of solution concentration in NaCl amended soil to that at zero addition) was equal at all soil Cd doses. The relative increase in solution concentration was about a factor of 20 at 120 mM NaCl, and about a factor of 5 at 120 mM NaNO₃. The higher increase for the NaCl treatment can be attributed to the formation of CdCl_n²⁻ⁿ complexes. Increasing salinity slightly increased the Cd²⁺ activity. This increase was not significant for the NaCl treatments, but significant for the NaNO₃ treatments (Table 1). This increase in Cd²⁺ activity in response to increasing salinity has not been detected before (Smolders et al. 1998), but is somewhat uncertain as solution speciation was predicted and not measured. Increasing NaCl (to 120 mM) increased the Cd flux measured by DGT by a factor 5 - 6 with a similar relative effect at low or high Cd (data not shown). This means that CdCl_n²⁻ⁿ complexes equally contributed to DGT flux at low and at high Cd. Increasing NaNO₃ dose increased the DGT flux 2-fold in the same range.

Dry matter yield of spinach grown in the spiked soils was significantly affected by salinity and by the total soil Cd concentration (Table 2). The salinity effects on plant yield were similar for NaCl and NaNO₃ treatments at low Cd but not at high Cd, where increasing NaCl

depressed the yield more than increasing NaNO₃. This additional yield decrease in the NaCl treatments was most likely due to the effect of NaCl on promoting Cd uptake and, consequently, Cd toxicity.

Increasing NaCl addition significantly increased shoot Cd concentration at all soil Cd levels, but the relative effects at the largest soil Cd level were smaller than at the background level (Fig.1). Shoot Cd concentrations also increased with increasing NaNO₃ addition but this increase was significantly smaller than for corresponding NaCl treatments except at the largest Cd dose. This NaNO₃ effect on shoot Cd concentration is most likely a result of the reduced growth rate at increasing ionic strength. Indeed, Cd uptake (shoot yield times Cd concentration in shoot) was not significantly affected by salinity in the NaNO₃ treatments at any soil Cd level ($P>0.05$). In contrast, Cd uptake significantly increased with NaCl at the lowest but not at the middle and highest soil Cd level (details not shown).

As plant yield was affected by the treatments, we calculated the Cd uptake fluxes based on root area (Eq. 1), to take into account the differences in growth rate between treatments. These fluxes were calculated using the average specific root area (SRA) of spinach since the SRA was unaffected by total Cd or ionic strength (SRA= 3900 ± 400 cm² per g root dry weight; mean $\pm$ standard deviation of 10 plants). The Cd uptake fluxes were unaffected by salinity in the NaNO₃ treatments. They increased however significantly with NaCl addition by more than a factor of 5 at the lowest and the middle Cd dose, but not at the highest soil Cd level (Fig.2).

The Cd uptake flux by the plants was increased upon NaCl addition at low but not at high soil Cd, while DGT fluxes increased to the same extent at all Cd levels. Therefore, the DGT flux correlated well with plant uptake flux at low soil Cd, but failed to predict the lack of NaCl effect on Cd uptake at high soil Cd (Fig.3). This analysis suggests that Cd uptake is controlled by diffusion at low but not at large Cd supply. The Cd fluxes measured with DGT

were only 2.5-fold higher than the root area based Cd fluxes to the plants at low Cd (Fig.3). This similarity is striking, given the uncertainty on the fraction of the surface that contributes to ion uptake from soil. Moreover, the DGT deployment was carried out on saturated soils, and the higher moisture content than during plant uptake partly explains the higher DGT fluxes (Davison et al. 2000).

Solution culture experiment

The short-term uptake of Cd by spinach was measured in solutions with 40 mM NaCl or 40 mM NaNO₃. The uptake of Zn did not differ significantly between corresponding treatments, which was expected since the Zn speciation was similar in the NaCl and NaNO₃ solutions (86% of Zn as free ion). At low Cd²⁺ activity, the Cd uptake flux by the plant was 3-fold higher in the NaCl than in the NaNO₃ treatment, differences being statistically significant (Table 3). The free ion activity was the same (pCd, or $-\log(\text{Cd}^{2+})$, 9.3) in both solutions, but the total Cd concentration was 3-fold larger in the NaCl treatments. In other words, the CdCl_n²⁻ⁿ complexes fully contributed to the plant uptake. At pCd 6.3 and 5.5, the uptake flux was not significantly different between NaCl and NaNO₃ treatments, indicating that the CdCl_n²⁻ⁿ complexes did not contribute to the plant uptake. The DGT-measured Cd concentration in solution at the interface with the DGT, calculated according to Zhang and Davison (1995), was nearly identical to the total solution concentration both at low Cd²⁺ (pCd 9.3) and high Cd²⁺ activity (pCd 5.5), illustrating that the CdCl_n²⁻ⁿ complexes are fully labile. Thus, in contrast with the plant uptake, the CdCl_n²⁻ⁿ complexes equally contributed to the DGT-measured flux at low and high Cd²⁺ activity. The DGT-measured diffusion flux was similar to the uptake flux by the plants at the low Cd²⁺ activity, but was 7–15 fold larger at high Cd²⁺ activity (Table 3). Overall, these results show that the plant Cd uptake from

solution was limited by diffusion at low Cd^{2+} activity, but not at high Cd^{2+} activity, corresponding to the observations made in the soil experiment.

Discussion

The experiments in this study were designed to test the hypothesis that DGT measurements do not mimic plant uptake at high Cd supply. The results showed that NaCl salinity affects Cd uptake to a lesser extent as the Cd availability increases, i.e. the enhancement in Cd uptake by chloride complexes is most pronounced at low Cd supply. Two previously reported pot trial studies (Bingham et al. 1983; 1984) did not show a less pronounced effect of chloride on Cd uptake at elevated Cd levels. However, these studies did not include NaNO_3 reference treatments and therefore did not allow evaluating possible effects of increase in ionic strength on growth and ion uptake characteristics.

The effects of salinity on plant growth (Table 2) complicate the interpretation of the data, as differences in plant concentrations may be partly related to the difference in growth rate. The NaNO_3 treatments illustrate this growth dilution effect: shoot Cd concentrations increased (Fig.1) and yield decreased with increasing NaNO_3 rate increases, whereas the shoot Cd uptake (concentration $\times$ yield) or the root area based uptake fluxes (Fig.2) were unaffected. The effect of NaCl on shoot Cd concentrations was significantly larger than that of NaNO_3 at the lowest Cd levels (Fig.1), while growth effects due to salinity were not different between the types of salt (Table 2). This implies that, at low Cd supply, there was a significant net chloride effect on Cd availability beyond the effect of ionic strength, as also becomes apparent when plotting the Cd uptake fluxes (Fig.2). In contrast, no such chloride effect was apparent at the highest Cd level after correction for growth dilution (Fig.2).

The observations for the solution experiment (Table 3) supported these findings and were more clear-cut than for the soil experiment, as the results are not complicated by salinity-

induced differences in growth rate. The solution culture data observed at the low Cd^{2+} activity (pCd 9.3; Table 3) confirm previous data obtained in resin buffered solution, which showed that increasing concentrations of CdCl_n^{2-n} complexes increase Cd uptake at constant Cd^{2+} activity (Smolders and McLaughlin 1996). However, the new data show that the cadmium-chloride complexes do not affect the plant uptake at elevated Cd supply.

The enhanced uptake of Cd in presence of chloride complexes at low Cd levels is in line with the conclusions of previous studies conducted at low Cd concentrations (Smolders and McLaughlin 1996; Smolders et al. 1998). The less pronounced effect of chloride salinity at elevated Cd concentrations supports the findings of a recent field study in a saline mining area in Bolivia with Cd contaminated soils (3–80 mg Cd kg⁻¹), where little effect of salinity on plant uptake was observed (Oporto et al. 2007). The statistical model on these data from Bolivia predicts that tuber Cd increases only 1.6-fold (95% confidence limits: 1.1–2.5) by increasing soil extractable Cl^- between 50–1000 mg Cl^- kg⁻¹, while tuber Cd increased 5-fold for the same salinity range at background Cd in soils from southern Australia (McLaughlin et al. 1997). These findings indicate that the results observed in the pot experiment, where plant uptake was assessed under controlled conditions using artificially Cd contaminated soils, are also valid under field conditions. Cadmium concentrations in field-contaminated soils can reach levels where uptake is not (or less) limited by diffusion, and, as a result, Cd-chloride complexes have little effect on the plant uptake.

The relative effect of chloride on dissolved Cd concentrations or DGT flux was independent of the Cd level, in the soil as well as in the solution experiment. As a result, the uptake fluxes by the plant, for which the effect of chloride decreased with increasing Cd supply, correlated well with DGT at low but not at high Cd supply (Table 3 & Fig.3). At low Cd, the uptake fluxes in solution culture strikingly match diffusive fluxes measured by DGT for the solution culture (Table 3), and were about a factor 3 lower than the DGT fluxes for the soil

experiment (Fig.3). At high Cd levels, however, the plant uptake fluxes levelled off and were similar for the NaCl and NaNO₃ treatments, in contrast with the DGT fluxes. This information strengthens the concept that Cd uptake is limited by diffusive transport of the free ion at low Cd supply. Dissolved labile complexes (such as CdCl_n²⁻ⁿ) enhance the diffusive supply of Cd²⁺ through dissociation of the complex in the diffusion layer, and thus contribute to availability. When uptake is near saturation, diffusive limitations are less pronounced, and the complexes do not – or to a lesser extent – contribute to the plant uptake.

Our study shows that DGT may fail to mimic metal uptake by plants at high metal concentrations at which the plant uptake becomes saturated (Fig.3), i.e. when concentrations in the soil solution exceed the (apparent) K_m value. For Cd, such concentrations are usually only encountered in strongly contaminated soils at which uptake is not limited by diffusion. The DGT may still correlate to metal uptake when diffusion is non-limiting provided that uptake by the plant continues to increase with increasing solution concentrations. The underlying processes are however different under such conditions, and DGT will be limited in its ability to mimic the plant uptake, e.g. it will wrongly predict the contribution of labile complexes.

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Figure Captions

Fig.1. Cadmium concentrations in shoot as a function of salinity for spinach grown in soil with background Cd or in Cd-amended soils (concentrations indicated in figure). The salinity treatments were imposed by adding either NaCl (full symbols, full lines) or NaNO₃ (empty symbols, dotted lines).

Fig.2. Root surface area based uptake fluxes of Cd as a function of salinity (NaCl: full symbols and full lines; or NaNO₃: empty symbols and dotted lines) for spinach plants grown in soil with background Cd or in Cd-amended soils (concentrations indicated).

Fig.3. Relationship between the root area based uptake fluxes of Cd by spinach and the DGT-measured fluxes, for soil with background Cd (circles) or amended with 10 mg Cd kg⁻¹ (triangles). Closed symbols represent the NaCl treatments, and open symbols the NaNO₃ treatments. The diagonal is the 1:1 line. The DGT explains the NaCl enhanced Cd uptake flux at low but not a high soil Cd.

Table 1. Concentrations (square brackets) and activities (round brackets) in the soil solutions (nmol L⁻¹) calculated from the total solution composition using GEOCHEM PC.

Salt rate	NaCl					NaNO ₃				
	(Cd ²⁺)	[Cd ²⁺]	[CdCl ⁺]	[CdCl ₂]	[CdCl ₃ ⁻]	(Cd ²⁺)	[Cd ²⁺]	[CdCl ⁺]	[CdCl ₂]	[CdNO ₃]
	nM									
	Background Cd									
0	1.02	1.16	0.03	0.00	0.00	1.06	1.21	0.03	0.00	0.00
10	1.47	2.06	1.05	0.03	0.00	1.98	2.68	0.08	0.00	0.04
30	1.80	3.08	4.32	0.31	0.00	2.21	3.50	0.22	0.00	0.11
60	1.76	3.65	8.82	1.22	0.04	2.37	4.43	0.21	0.00	0.24
90	1.58	3.73	12.90	2.63	0.14	4.20	9.20	0.54	0.00	0.80
120	1.97	4.89	18.10	4.21	0.25	3.48	7.98	0.36	0.00	0.78
F [†]	n.s	**	***			*	**			
	+ 2 mg Cd kg⁻¹									
0	5.71	7.01	0.32	0.00	0.00	5.72	7.01	0.32	0.00	0.04
10	6.17	9.03	4.84	0.13	0.00	3.80	5.16	0.23	0.00	0.07
30	12.89	22.90	30.60	2.15	0.03	8.07	13.32	0.68	0.00	0.47
60	11.38	23.70	52.20	6.61	0.19	9.73	17.61	0.82	0.00	0.88
90	11.36	26.90	84.70	15.80	0.75	11.02	22.57	1.71	0.01	1.64
120	12.81	32.50	125.00	29.80	1.82	13.60	30.46	1.08	0.00	2.84
F [†]	n.s	**	***			**	***			
	+ 10 mg Cd kg⁻¹									
0	51.42	72.90	4.29	0.01	0.00	51.60	72.78	4.29	0.01	1.24
10	83.85	134.80	77.40	2.28	0.01	55.20	85.70	5.66	0.02	2.36
30	95.22	178.50	244.00	17.90	0.29	72.49	127.35	12.16	0.06	5.69
60	74.60	166.30	471.00	78.70	3.22	78.91	148.59	12.65	0.06	8.35
90	90.11	212.70	656.00	119.00	5.56	98.43	207.49	14.03	0.05	16.50
120	102.50	257.50	951.00	216.00	13.00	131.50	317.69	30.34	0.17	35.70
F [†]	n.s	**	***			***	**			

[†] F = Value significance: *, ** and ***: significant at the 0.05, 0.01, 0.001 probability respectively; n.s. non significant

Table 2. Shoot dry weight (mg per plant) of spinach plants grown in soil at background Cd or amended with 2 or 10 mg Cd kg⁻¹, at 6 levels of salinity (nominal concentrations in soil solution). Statistical analysis by ANOVA for each Cd level is detailed.

Salinity (mM)	background		+2 mg Cd kg ⁻¹		+10 mg Cd kg ⁻¹	
	NaCl	NaNO ₃	NaCl	NaNO ₃	NaCl	NaNO ₃
0	448	448	390	390	150	150
10	445	487	316	364	91	134
30	463	417	266	252	60	106
60	404	373	169	240	69	90
90	293	206	80	106	36	57
120	160	114	69	74	13	41
F-value, significance ^{\$}						
Factor ^a :						
salinity	***	***	***	***	***	***
salt	n.s.		n.s.		**	
salinity×salt	n.s.		n.s.		n.s.	

[†]F= value significance,

*, ** and ***: significant at the 0.05; 0.01 and 0.001 probability level respectively;
n.s. non significant

Table 3. Root area based uptake flux of Cd by spinach plants and DGT-measured flux in solutions with 40 mM NaNO₃ or NaCl. The free Cd²⁺ activity was identical in corresponding treatments, but the total Cd concentration was three-fold higher in the NaCl treatment. Values are mean $\pm$ standard deviation, $n=2$.

pCd	F_{uptake} (pmol cm ⁻² h ⁻¹) [†]		F_{DGT} (pmol cm ⁻² h ⁻¹)	
	NaNO ₃	NaCl	NaNO ₃	NaCl
9.3	0.12 $\pm$ 0.03	0.40 $\pm$ 0.12	0.19 $\pm$ 0.01	0.59 $\pm$ 0.01
6.3	61 $\pm$ 24	64 $\pm$ 12		
5.5	169 $\pm$ 21	258 $\pm$ 76	1122 $\pm$ 31	3760 $\pm$ 90

[†] Cadmium uptake per unit root weight was converted to a root surface based flux using a constant value for root surface area of 200 cm² per g root fresh weight as measured previously for spinach raised in nutrient solution (Degryse et al. 2006 a)

Fig.1

Fig.2

Fig.3