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An agar gel technique demonstrates diffusion limitations to Cd uptake by higher plants

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Environmental Context. Uptake of metals by biota is usually predicted with equilibrium models, which assume that transport of the metal from the solution to the biosurface does not limit uptake. In this study, uptake of Cd by higher plants is shown to be limited by the transport of the free ions to the root surface under a range of conditions.

Abstract. Uptake of Cd by spinach and wheat was higher in presence of fast-dissociating complexes than in unbuffered solutions with same free ion concentration. This contribution of metal complexes to metal uptake cannot be explained by equilibrium free-ion-based models, which assume that transport of the free ion to the biosurface is not limiting the uptake. To demonstrate diffusion limitations to metal uptake, we used an agar gel technique in which Cd and Zn concentrations around the roots, after 6 hours of uptake, were compared with bulk concentrations. Obvious metal depletion around the roots was observed in agar where the ion activities were not buffered by complexes, whereas the depletion was less pronounced in buffered agar. Metal uptake by the plants in unbuffered media was greater as the degree of agitation increased (stirred solution > unstirred solution > agar), while no such dependence on hydrodynamic conditions was found in buffered media, which is in agreement with theoretical predictions.

Keywords. cadmium – metals – contaminant uptake – diffusion

Toxic effects of trace metals are often related to the amount of metal that is internalized. It is well-known that the internalization flux does not only depend on the solution concentration, but also on the speciation of the metal in solution.^[1] Models that predict uptake and toxicity of metals, such as the Free Ion Activity Model (FIAM) and the Biotic Ligand Model (BLM), assume that only the free ion interacts directly with the membrane surface sites. However, several studies have reported the contribution of metal complexes to metal uptake, both by aquatic organisms^[2] and plants,^[3-5] which cannot be explained by free-ion-based equilibrium models. Uptake of intact complexes may explain the larger metal uptake in presence of complexes. Some studies have indeed shown uptake of metal-EDTA complexes.^[6,7] It has been speculated that these complexes enter the xylem vessels through an apoplastic pathway, e.g. at the tips of roots where the Casparian bands are not fully developed or at breaks in the endodermis. Indications have also been found that uptake of the metal complexes is enhanced by damage of the root membranes at high concentrations of the ligand.^[6]

Direct uptake of complexes is, however, not the only possible explanation for deviations from the FIAM. In a previous study,^[8] we found that the contribution of complexes to Cd uptake by spinach from solution could be explained by transport limitations of the free ion to the root surface, as was indirectly shown from the relation between the rate of dissociation of complexes and plant uptake in solutions with same free ion activity and same total Cd concentration. In this work, we further investigated the diffusional limitations in metal uptake, including also a monocotyledonous plant and using a method to detect concentration gradients. By means of an agar gel technique, we were able to demonstrate depletions of Cd and Zn around the plant roots in unbuffered solutions.

In a first experiment, the 4-hour uptake of Cd by seedlings of wheat (*Triticum aestivum* L.) and spinach (*Spinacia oleracea* L.) was measured in aerated solutions. The free ion activity of Cd was either unbuffered, or buffered with Cd-ligand complexes (CdL) of varying dissociation rate (Table 1), quantified by the dissociation rate constant, k_d :

Uptake was measured for plants that were either pregrown in Cd-free nutrient solution or at 30 nM Cd^{2+} . In the unbuffered solutions, a 30-fold increase in Cd concentration, from 1 to 30 nM Cd^{2+} , resulted in a similar increase in Cd uptake. This linear response is expected at low Cd^{2+} concentrations. At higher Cd^{2+} concentration, the increase in uptake by spinach was less than proportional (Table 1), indicating saturation of the uptake process. The Cd uptake by spinach was well-described by Michaelis-Menten kinetics ($v_{\text{max}}=40 \text{ nmol g}^{-1} \text{ root fresh weight (RFW) h}^{-1}$; $K_m = 0.15 \text{ }\mu\text{M}$). In the ligand-buffered solutions, uptake was greater than in a ligand-free solution with the same free Cd^{2+} concentration, and followed the same order as the dissociation rate constant (k_d) of the complexes (NTA>HEDTA>CDTA). The relative contribution of the complex, ξ , was calculated from the uptake in the ligand-buffered solution (Cd_{pl}) and the uptake in the control solution with same Cd^{2+} concentration ($\text{Cd}_{\text{pl}}^{\text{contr}}$):

$$\xi = \left(\frac{\text{Cd}_{\text{pl}}}{\text{Cd}_{\text{pl}}^{\text{contr}}} - 1 \right) \cdot \frac{[\text{Cd}^{2+}]}{[\text{CdL}]} \quad (2)$$

where $[\text{CdL}]$ and $[\text{Cd}^{2+}]$ are the bulk concentrations of the Cd complex and free ion, respectively. A ξ value of 0 indicates that the complex is fully inert with respect to plant uptake, while a value of 1 indicates that the complex contributes as much as the free ion. The value of ξ is larger for fast dissociating complexes and at lower degree

of buffering (Table 1), which is in agreement with theoretical predictions (see below). For instance, at $[CdL]/[Cd^{2+}]=100$, the NTA complexes contribute ca. 10%, but at $[CdL]/[Cd^{2+}]=10^4$, the NTA complexes contribute only about 1% (or less when uptake becomes saturated, e.g. at 10 nM Cd^{2+} ; see Table 1). The effect of complexes on Cd uptake was similar for both plant species and for both pretreatments (with or without Cd), indicating that diffusion limitations prevail even for plants with higher Cd status. These findings confirm our previous results^[8] and indicate a general validity, the effect of complexes being similar for monocotyledonous and dicotyledonous plant species, and for different pre-exposures.

From the above, it follows that Cd uptake in ligand-buffered solutions depends on the dissociation rate of the complexes and on the degree of buffering, at least for the conditions used in these experiments (e.g. low Cd^{2+} concentration). We have previously explained these deviations from the FIAM based on diffusive limitations of the free ion to the root surface.^[8] An alternative explanation is that labile complexes contribute because the transporter is under kinetic control, i.e. the rate of internalization is relatively fast and metal binding to the transport site is rate-limiting.^[9] However, in view of the relatively fast water exchange kinetics of Cd^{2+} ($k_w=3\times 10^8\text{ s}^{-1}$)^[10], this seems rather unlikely.

To obtain direct evidence for the diffusion-limitation hypothesis, we used an agar medium to demonstrate depletions around the root (Experiment 2). Spinach and wheat plants, grown on nutrient solutions, were embedded with their roots in a ^{109}Cd - and ^{65}Zn -labeled agar medium and, after 5–6 hours, the agar was sampled for several plants at different positions along the root axes. Obvious depletions around the roots of both Cd (at concentrations of 1 nM or 1 μ M) and Zn (at trace level) were observed in the unbuffered media (Fig. 1). The Cd concentration of agar disks (with a diameter

of 0.8 cm) sampled around the roots was, on average, only 47% (range 8–89%) for wheat or 35% (range 8–87%) for spinach of the bulk concentration in the medium with 1 nM Cd^{2+} . These ratios were only slightly higher in the medium with 1 μM Cd^{2+} . The smallest depletions were observed for the smallest root densities (only 1 or 2 roots crossing the agar disk) and the largest depletions where more than 10 roots crossed the agar disk. In contrast, there was almost no depletion around the plant roots in agar where Cd was buffered (1 nM Cd^{2+} , 1 μM Cd-CDTA), irrespective of the root density. For Zn, there was also some depletion in the buffered medium, which may be explained by the weaker buffering of Zn (free ion fraction of 0.35% compared to 0.1% for Cd) and the relatively higher uptake of Zn in this medium. Overall, there was a clear relation between the depletion around the roots in the agar medium and the root absorbing power α , i.e. the ratio of the surface-based uptake flux and the total concentration in solution (Fig. 1). The uptake flux was calculated from the measured metal uptake, F_{upt} [$\text{nmol g}^{-1} \text{ RFW h}^{-1}$], in the agar medium assuming a specific root area (SRA) of $0.02 \text{ m}^2 \text{ g}^{-1} \text{ RFW}$. The larger metal depletion around the roots at higher root absorbing power is also expected based on theoretical considerations.^[11]

In the unbuffered media, the short-term uptake of Cd and Zn was much greater in the solutions than in an agar medium with same composition, and also greater in the stirred solution than in the unstirred solution (Fig. 2). In contrast, the uptake in the buffered media did not depend on the hydrodynamic conditions, and Cd uptake was higher than in the unbuffered solutions with same free Cd^{2+} concentrations. We also measured the diffusion flux in these media with the DGT (diffusive gradients in thin films) technique,^[12] in which a diffusion flux in a hydrogel layer is created through the infinite sink action of an adjacent resin layer. The DGT measured concentration

(c_{DGT}), which is derived from the amount of metal accumulated on the resin, is proportional to the diffusion flux and corresponds to the concentration at the hydrogel–medium interface that would result in the same diffusion flux in an unbuffered medium. As expected, c_{DGT} was nearly equal to the solution concentration for the stirred unbuffered solution, but 37% lower than the solution concentration for the unstirred solution, indicating that a diffusive boundary layer (DBL) at the surface of the device increased the effective diffusive layer thickness by 0.6 mm (from 0.94 to ca. 1.5 mm). In the unbuffered agar medium, c_{DGT} was even seven-fold lower, pointing to an effective increase in the diffusive layer thickness of 5.6 mm, which is in good agreement with theoretical calculations (as shown immediately below). While the diffusion layer thickness, δ , is constant (0.94 mm) in the stirred solution, it increases with time in absence of flow according to $\delta = \sqrt{\pi D t}$,^[10] resulting in a decrease of the diffusion flux ($= Dc/\delta$). The effective diffusion layer thickness during the deployment can be calculated from the estimated mass of metal (per unit area) accumulated on the resin layer, M , obtained by integrating the diffusion flux:

$$M = \int_t \frac{D \cdot c}{\delta} dt \Rightarrow \text{constant } \delta \text{ (stirred solution)} : M = \frac{D \cdot c}{\delta} t \quad (3a)$$

$$\delta = \sqrt{\pi D t} \text{ (agar)} : M = 2 \frac{D \cdot c}{\sqrt{\pi D t}} t \quad (3b)$$

where D is the diffusion coefficient (for which a value of $4.3 \times 10^{-5} \text{ m}^2 \text{ d}^{-1}$ or $5.0 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$ is assumed below^[8]). Comparison of Eqns 3a and 3b shows that the effective diffusive layer thickness in the agar medium theoretically corresponds to $\frac{1}{2} \sqrt{\pi D t}$ or 5.8 mm (since $t=1$ day). In the buffered media with same free Cd^{2+} concentration (1 nM) as in the unbuffered media, but 1000 times higher total Cd concentration, c_{DGT} did not depend on the degree of agitation, as was also observed for the plant uptake. The DGT-measured concentration was about 30-fold higher than

the free-ion concentration in the buffered media, indicating a (3%) contribution of the Cd-CDTA complexes. Overall, the DGT-measured concentrations correlated well with plant uptake (Fig. 2).

These results clearly indicate diffusive limitations to Cd uptake. We therefore compared the uptake fluxes with estimated diffusion fluxes.^[2] The limiting diffusion flux (i.e. for zero concentration at the surface), F [nmol g⁻¹ RFW h⁻¹], in an unbuffered medium was calculated as:

$$F = \frac{D}{\delta} \cdot \text{SRA} \cdot [\text{Cd}^{2+}] \quad (4)$$

where δ is the diffusion layer thickness, D is the diffusion coefficient, and SRA is the specific root area (0.02 m² g⁻¹ RFW). The equation for a well-buffered medium is similar, except that the diffusion layer thickness is replaced by the thickness of the reaction layer, μ :

$$F = \frac{D}{\mu} \cdot \text{SRA} \cdot [\text{Cd}^{2+}] \quad (5)$$

The reaction layer is the zone near the surface where the rate of dissociation of the metal complex is not fast enough to keep up with the depletion of the free metal ion. The reaction layer thickness depends on the rate of dissociation of the complex and degree of buffering $\left(\mu = \sqrt{D/k_d \cdot [\text{Cd}^{2+}]/[\text{CdL}]} \right)$, and is physically only meaningful if μ is smaller than δ .^[13,14] This expression for μ indicates that an increase in the degree of buffering, $[\text{CdL}]/[\text{Cd}^{2+}]$, by a factor of 100 decreases μ by a factor of 10, and therefore only increases the flux 10-fold at a constant free ion concentration (Eqn 5). This predicted decrease in ‘lability’ is in agreement with the observed decrease in relative contribution of complexes to the metal uptake (ξ) at higher degree of buffering (Table 1). In contrast to δ , the reaction layer thickness does not depend on

time or on hydrodynamic conditions. In order to match the observed uptake flux with the estimated diffusion flux (Eqn 5), the reaction layer thickness in the buffered media should be ca. 0.03 mm (Fig. 2), which is smaller than the value (0.2 mm) predicted from the previously determined dissociation rate constant of Cd-CDTA complexes ($k_d = 10^{-5} \text{ s}^{-1}$)^[8]. For the unbuffered media, a diffusion layer thickness of 6, 0.6 and 0.35 mm was estimated in the agar, unstirred and stirred solution, respectively. The values for the agar and unstirred solution agree well with those estimated from the DGT measurements (5.6 and 0.55 mm respectively), but the DGT measurement suggested that there was no appreciable DBL in the stirred solution, since c_{DGT} corresponded to the solution concentration. However, recent findings^[15] have shown that such a 100% recovery does not preclude the presence of a DBL. Even in well-stirred solutions, the DBL was found to be around 0.2 mm. The same study showed that the effective sampling area of the DGT device was 20% greater than previously assumed, which explains that a 100% recovery is obtained for an unbuffered, stirred solution, in spite of the presence of a DBL, as both errors cancel one another.

A possible criticism of our calculations is that the diffusion layer is too large to assume semi-planar diffusion (Eqn 4), since the root radius of the plants was only 0.11 mm (spinach) to 0.15 mm (wheat). The equation for radial diffusion towards a cylinder,^[16] which gives unrealistically high estimates for δ when applied to our results, may seem more appropriate. However, the roots in the solution and the agar were close together and the depletion zones around the roots were likely overlapping, also invalidating the cylindrical diffusion equation. Other uncertainties for the diffusion equations are the value of D in the root apoplast, and whether the whole root surface is contributing to the uptake.^[17] As a result, it is hard to draw conclusions about possible diffusion limitations based solely on a comparison between the uptake

flux and the estimated diffusion flux, which led us to the use of other approaches. The larger contribution of fast dissociating complexes to Cd uptake (Table 1), the measurable depletion of Cd (and Zn) around roots in an unbuffered agar medium (Fig. 1), and the good agreement between DGT measured diffusion fluxes and Cd uptake (Fig. 2) all point to diffusion-limited uptake of Cd, even in nutrient solutions.

Comparisons of plant uptake from chelator-buffered versus chelator-free solutions have indicated that uptake of Cu and Zn may also be limited by diffusion.^[4,18] Diffusion limitations for metal uptake by higher plants may thus be more important than generally believed. In stirred, unbuffered solution, we found a value for the root absorbing power, α , for Cd uptake of ca. $1.4 \times 10^{-6} \text{ m s}^{-1}$, resulting in an estimate of the diffusion layer thickness, δ , of 0.35 mm (Fig. 2) assuming that diffusion is limiting (in which case α corresponds to D/δ , since $\alpha = F_{\text{upt}}/c/\text{SRA}$). It is striking that values of α for higher plants are seldom higher than $2 \times 10^{-5} \text{ m s}^{-1}$,^[19] suggesting a lower limit for δ of 25 μm (assuming that $D = 5.0 \times 10^{-10} \text{ m}^2 \text{ s}^{-1}$). If diffusion does limit uptake, which is likely if α is $\sim 10^{-6} \text{ m s}^{-1}$ or higher, the uptake parameters (Michaelis-Menten parameters or α) do not reflect the affinity of the plant roots, but indicate how fast the element can be transported to the roots. Ion depletions near the root surface have also been reported for K^+ . Using a vibrating microelectrode, it was shown that the K^+ concentration at the root surface of maize was considerably lower than in the bulk solution.^[20] Also for microorganisms, several cases (mostly for Cd and Zn) have been reported where the observed transport conductance or permeability, defined in the same way as α , was of the same magnitude as the calculated limiting value if diffusion controls the uptake.^[9,10] This limiting value for microorganisms is usually $\sim 2 \times 10^{-4} \text{ m s}^{-1}$, i.e. higher than for plants since radial diffusion towards an organism with a diameter of a few μm exceeds planar (or cylindrical) diffusion. Caution is

therefore warranted when using equilibrium models such as the BLM to predict metal uptake by biota, since the inherent assumption that transport is not limiting uptake may be violated more frequently than previously believed. While the concepts of the BLM model (competitive binding at the biotic ligand described by equilibrium constants) may still hold, free ion activities at the root surface rather than in the bulk solution are expected to control metal uptake.

Experimental

Experiment 1 (uptake in solution). Seeds of spinach, *Spinacia oleracea* L. (cv. ‘Bloomsdale’) and wheat, *Triticum aestivum* L. (cv. ‘Yecora rojo’), were surface sterilized for 15 minutes in 0.04 M NaOCl, rinsed with deionized water, and germinated for four (wheat) or six (spinach) days at 25 °C between sheets of germination paper continuously wetted with metal-free nutrient solution. Seedlings were transferred to a Cd-free 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, 0.25 CuSO₄, 0.07 (NH₄)₆Mo₇O₂₄, 1 ZnSO₄, 10 FeNaEDTA) or a solution with identical composition that also contained 30 nM CdCl₂. The solutions were buffered with 2 mM MES at pH 6. Plants were grown in a growth chamber with 14-10 h day/night cycle, day-night temperature of 22-16 °C, and photosynthetic photon flux density of 400 μmol m⁻² s⁻¹. Plants were taken from the nutrient solutions at 8 (wheat) or 18 (spinach) days after transplanting (DAT). Two plants from each pre-treatment solution were analyzed for total metal content after hot-acid digestion. Cadmium-exposed wheat had a shoot Cd concentration of 1.9 mg kg⁻¹ and a root concentration of 12 mg kg⁻¹; spinach had Cd levels of 10 and 15 mg kg⁻¹ in shoot and root, respectively. Roots were rinsed with distilled water, and two plants from each pre-treatment were transferred to 1 L of aerated treatment solution, that was radiolabeled with ¹⁰⁹Cd (~10 kBq L⁻¹) and contained 2 mM Ca(NO₃)₂, 2 mM MES (pH 6), 15 μM

H₃BO₃, and concentrations of Cd and ligand to obtain the free Cd²⁺ concentrations shown in Table 1. The solution speciation was calculated using the GEOCHEM-PC program.^[21] Plants were removed from the treatment solutions after 4 hours, and transferred to a 10 mM Ca(NO₃)₂ solution for 10 minutes, to remove cell-wall bound ¹⁰⁹Cd²⁺. The roots were blotted, and plants were divided in shoots and roots. Shoots and roots were weighed and the ¹⁰⁹Cd activity was determined with a Packard Cobra II auto-gamma counter.

Experiment 2 (agar versus solution). Seeds of spinach, *Spinacia oleracea* L. (cv. ‘Géant d’hiver’) and wheat, *Triticum aestivum* L. (cv. ‘Ordeal’), were germinated and grown in the same way as described above in a Cd-free nutrient solution. Plants were removed from the nutrient solutions at 8 (wheat) or 19 (spinach) DAT. The roots were rinsed with distilled water, and two plants were transferred to 1 L of either a stirred or unstirred treatment solution or to an agar medium with same composition. The basal solution composition was 2 mM Ca(NO₃)₂, 2 mM MES (pH 6), and 15 µM H₃BO₃. The unbuffered solutions contained 1 nM or 1 µM CdCl₂. The buffered solution contained 1 µM CdCl₂ and 1.36 µM CDTA (trans-1,2-cyclohexylene-diamine-tetraäcetate) and had a calculated^[21] free Cd²⁺ concentration of 1 nM. The solutions were radiolabeled with ¹⁰⁹Cd (1–20 kBq L⁻¹) and ⁶⁵Zn (20–200 kBq L⁻¹). To a subsample of the treatment solutions, 0.75% noble agar was added. The plants were embedded with their roots in a 0.4-cm thick agar layer by putting the plants in a tray and pouring the agar solution (at 36 °C) into the tray.^[22] After between 5 and 7 hours, agar was sampled at several (up to 10) positions along the root axes and in the bulk medium, by punching out agar disks with a pipette tip (diameter 0.8 cm), taking care not to damage the roots. The agar samples were weighed and transferred in counting vials with 4.5 mL of water. The plants were removed from the agar, and transferred to a 10 mM Ca(NO₃)₂ solution for 10 minutes. The roots were blotted, and shoots and

roots were separated and weighed. Simultaneously, the uptake was also measured under identical conditions in the stirred and non-stirred solutions with identical composition, and plants were sampled in the same way. The ^{109}Cd and ^{65}Zn activity of the agar and plant samples and of the solutions was determined with a Minaxi 5530 auto-gamma counter. The diffusion flux was measured by placing a DGT device^[12] (with a diffusion layer thickness of 0.94 mm) in the stirred or non-stirred solution, or by gently pushing a DGT device in the agar medium. After 24 hours, the DGT devices were removed, the resin gel was retrieved and transferred to a counting vial to which 1 mL of 1 M HNO_3 was added. The ^{109}Cd and ^{65}Zn activities were measured, which allowed us to calculate c_{DGT} .^[12]

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Table 1. Effect of pre-exposure to Cd and type and degree of buffering on Cd uptake by wheat or spinach. The plants were grown for 8 (wheat) or 18 (spinach) days on nutrient solution without Cd ('Pre -Cd') or with 30 nM Cd²⁺ ('Pre +Cd'). The 4-hour uptake was measured in solutions with 2 mM Ca(NO₃)₂, 2 mM MES (pH=6), 15 µM H₃BO₃, and total Cd concentrations (Cd_{tot}) as given in the table, in absence or presence of ligands. The values in parentheses indicate the relative contribution of the complex to the metal uptake (ξ, Eqn 2).

Cd ²⁺ [nM] ^A	Cd _{tot} [nM]	Ligand ^B	Uptake by wheat [nmol g ⁻¹ RFW h ⁻¹]		Uptake by spinach [nmol g ⁻¹ RFW h ⁻¹]	
			Pre -Cd	Pre +Cd	Pre -Cd	Pre +Cd
1	1	na ^C	0.056	0.035	0.26	0.23
30	30	na	2.79	1.21	10.1	4.51
1000	1000	na	–	–	39.6	37.0
1	100	CDTA	–	–	0.21 (0%)	–
1	100	HEDTA	–	–	0.62 (1.4%)	–
1	100	NTA	–	–	3.71 (13%)	–
10	1000	NTA	–	–	26.8 (9.7%)	–
1	10 000	CDTA	1.15 (0.19%)	1.58 (0.45%)	3.09 (0.11%)	1.24 (0.04%)
1	10 000	HEDTA	2.37 (0.41%)	2.52 (0.72%)	11.7 (0.44%)	9.67 (0.41%)
1	10 000	NTA	7.22 (1.3%)	5.35 (1.5%)	18.8 (0.71%)	11.1 (0.47%)
10	100 000	NTA	–	–	32.6 (0.12%)	–

^A Calculated with GEOCHEM-PC^[21]; ^B k_d of Cd-ligand complex: 10⁻⁵, 10^{-2.8}, and >10⁻² s⁻¹ for CDTA, HEDTA and NTA respectively^[8];

^C na=not applicable.

Fig. 1. The average ratio of the concentration of Cd (circles) or Zn (triangles) in agar disks (0.4 cm radius) sampled along plant roots in an agar medium ('rhizosphere concentration') relative to the bulk concentration, as a function of the root absorbing power α for that element (calculated as the ratio of the uptake flux by wheat or spinach and the metal concentration in the agar medium). The agar had a basal composition of 2 mM $\text{Ca}(\text{NO}_3)_2$, 2 mM MES (pH=6) and 15 μM H_3BO_3 , and was radiolabeled with ^{109}Cd and ^{65}Zn (trace level). The unbuffered agar media contained 1 nM Cd^{2+} (indicated by the letter L) or 1 μM Cd^{2+} (H). The buffered agar contained 1 nM Cd^{2+} and 1 μM Cd-CDTA. The agar was sampled after the plants had been in the agar for ~6 hours. The smallest depletion (<30%) of metals in the 'rhizosphere' was observed in the buffered agar, and the largest depletion in the unbuffered agar with the lowest Cd concentration.

Fig. 2. Uptake flux of Cd by spinach or wheat as a function of the DGT-measured concentration (which is a measure of the diffusion flux) in media with 1 nM Cd^{2+} in absence (unbuffered) or presence of complexes (buffered; 1 μM Cd-CDTA). The basal composition was 2 mM $\text{Ca}(\text{NO}_3)_2$, 2 mM MES (pH=6) and 15 μM H_3BO_3 . The letters next to the symbols indicate whether the (~6-hour) uptake was measured in stirred (S) or non-stirred (NS) solutions, or in an agar medium (A). The horizontal lines show the calculated diffusion flux to the roots (Eqn 4 or 5) for given values of the thickness of the diffusion layer (δ) or of the reaction layer (μ).