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RESEARCH ARTICLE

Can ligand addition to soil enhance Cd phytoextraction? A mechanistic model study

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Abstract Phytoextraction is a potential method for cleaning Cd-polluted soils. Ligand addition to soil is expected to enhance Cd phytoextraction. However, experimental results show that this addition has contradictory effects on plant Cd uptake. A mechanistic model simulating the reaction kinetics (adsorption on solid phase, complexation in solution), transport (convection, diffusion) and root absorption (symplastic, apoplastic) of Cd and its complexes in soil was developed. This was used to calculate plant Cd uptake with and without ligand addition in a great number of combinations of soil, ligand and plant characteristics, varying the parameters within defined domains. Ligand addition generally strongly reduced hydrated Cd (Cd^{2+}) concentration in soil solution through Cd complexation. Dissociation of Cd complex (CdL) could not compensate for this reduction, which greatly lowered Cd^{2+} symplastic uptake by roots. The apoplastic uptake of CdL was not sufficient to compensate for the decrease in symplastic uptake. This explained why in the majority of the cases, ligand addition resulted in the reduction of the simulated Cd phytoextraction. A few results showed an enhanced phytoextraction in very particular conditions (strong plant transpiration with high apoplastic Cd uptake capacity), but this enhancement was very limited, making chelant-enhanced phytoextraction poorly efficient for Cd.

Keywords Phytoextraction · Ligand addition · Cadmium · Mechanistic model · Plant root uptake · Complexation

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Introduction

Soil pollution by heavy metals threatens soil functioning, agricultural production, groundwater quality, ecosystems and human health. This pollution due to various human activities occurs in mining, industrial and urban areas as well as in agricultural soils (Alloway 2013). Cadmium (Cd), one of these toxic metals, is receiving much attention from researchers and policy makers since exposure of the world populations to this metal through food intake, i.e. through Cd accumulation by cultivated plants, can reach or even exceed the recommended provisional safe intake limits (Clemens et al. 2013).

Few methods are available to clean up Cd-polluted soil. Among them, phytoextraction appears as the most promising for moderately contaminated soils (Koopmans et al. 2008; McGrath et al. 2006; Schwartz et al. 2003; Zhao et al. 2003). This consists in extracting Cd from soil by cultivated plants in situ through their root absorption of the metal and its subsequent translocation to the harvestable plant parts. However, one of the factors limiting the efficiency of phytoextraction is the low availability of soil trace metals for root uptake, particularly as most metallic ions are sorbed onto the solid phase. To increase the availability of metals to root absorption, i.e. to increase the concentration of metal in the soil solution, researchers have first experimented with the addition of a ligand with a high affinity for the target metals. Ligands so far considered include aminopolycarboxylate acids (EDTA, DTPA, NTA) or organic acids (citrate, malate, oxalate) (Blaylock et al. 1997; Ebbs and Kochian 1998; Evangelou et al. 2006; Huang et al. 1997; Huang and Cunningham 1996; Quartacci et al. 2007; Souza et al. 2013; Wallace et al. 1974). This addition could extract the metallic ions bound to the solid phase (Collins et al. 2002; do Nascimento 2006; Jiang et al. 2003; Qin et al. 2004; Wu et al. 2003). However, if the ligand addition led in some cases to increased Cd concentration in plants, this increase was often moderate (Blaylock et al. 1997; Kayser et al. 2000; Kulli et al. 1999; Lombi et al. 2001). The ligand could also have negative (McGrath et al. 2006) or no effect on Cd phytoextraction (Jiang et al. 2003; Meers et al. 2004; Shen et al. 2002). These contrasting results could be the consequence of numerous combined soil and plant processes.

In the case of cations, plant roots are considered to preferentially absorb the free hydrated forms of Cd (Cd^{2+}) through the symplastic pathway (Welch and Norvell 1999). Therefore, adding ligand (L) to the soil, which then forms a Cd complex (CdL), should reduce the Cd^{2+} activity in the soil solution and consequently the phytoextraction of the metal (Custos et al. 2014). On the other hand, it has been shown that in hydroponics, sufficiently labile CdL (i.e. whose dissociation and association kinetics were sufficiently great) could enhance the diffusion flux of Cd^{2+} towards the root surface (Degryse et al. 2006; Panfili et al. 2009). This would suggest that ligand addition to soil with a low Cd^{2+} concentration in solution could favour the plant's Cd uptake.

In non-hyperaccumulating plants, Cd mainly accumulates in the root system with only a minor proportion being transported to the above-ground parts (Clemens 2006). However, the contrary occurs in hyperaccumulators (Verbruggen et al. 2013). Plant roots have no known transport system for metal complexes, such as CdL , and these complexes have generally been considered unavailable for root absorption. However, Pb-EDTA complexes have been identified in the xylem sap of the common bean (*Phaseolous vulgaris*) (Sarret et al. 2001); EDTA complexes with Pb, Cd and Fe were also found in the xylem sap of Indian mustard (*Brassica juncea*) (Schaider et al. 2006) and Zn-EDTA complexes in that of barley (*Hordeum vulgare*) and potatoes (*Solanum tuberosum*) (Collins et al. 2002). An apoplastic pathway has therefore been suggested for the uptake of these metal complexes (Nowack et al. 2006), which were supposed to avoid the apoplastic barriers (Enstone et al. 2003) to enter the stele and the xylem at the root tip or along the root axes, where the barriers were disrupted, i.e. where lateral roots emerged. It has also been hypothesised that ligands, such as EDTA, could alter the root tissues and increase the root permeability, thereby causing uncontrolled metal complex influx (Huang and Cunningham 1996; Niu et al. 2012; Vassil et al. 1998).

Various soil ions, including Ca^{2+}, Mg^{2+} and Fe^{3+}, can compete with Cd^{2+} for complexation with the exogenous ligand (Custos et al. 2014), thus affecting the amount and the lability of the Cd complex, and consequently the amount of Cd phytoextracted. Moreover, desorption from the solid phase as well as transport through mass flow and diffusion determine the soil supply of Cd^{2+} and complexes to plant roots (Sterckeman et al. 2004). Therefore, the uptake of Cd by plants will depend on other parameters, including the soil water content, tortuosity and sorption capacity (or buffer power) for Cd^{2+} and complexes. The uptake will also depend on plant characteristics, such as the Michaelis-Menten parameters K_m and I_{max} governing the symplastic influx, as well as on plant and climate characteristics, through the water uptake rate and the transpiration stream concentration factor, which describes the apoplastic uptake of solutes (Collins et al. 2006; Nowack et al. 2006). It must be noted that pH has an influence on most of the above-mentioned processes, as protons compete with Cd^{2+} during sorption on solid phase, complexation in solution and absorption by root transport systems.

Calculating the relative weight of each of these processes in the resulting Cd plant uptake is far from being straightforward, and it is necessary to assess the effects on the individual processes and their interactions in order to explain the variability of the experimental results for Cd phytoextraction. In addition, in situ chelate-enhanced phytoextraction is considered as dangerous for groundwater resources because of the potential leaching of toxic chelants (such as EDTA) and the chelated metals present in the soil solution at high concentrations (Nowack et al. 2006). It is therefore necessary to identify the situations where ligand addition could cause an excessive increase in Cd concentration in the soil solution.

The objective of this study was to evaluate the usefulness of ligand addition in the phytoextraction process and disclose those important factors and kinetic processes controlling Cd phytoextraction. A mechanistic model was developed to formalise most of the mechanisms presented above, including the reaction kinetics (adsorption on solid phase, complexation in solution), transport in the porous media (by advection and diffusion) and root absorption (symplastic and apoplastic) of Cd and its complexes in soil. The model was used to virtually experiment plant Cd uptake in contaminated soils with and without ligand addition in a great number of combinations of soil, ligand and plant characteristics, varying each of the model parameters at random within a realistic domain.

Materials and method

Cadmium root uptake models

In the soil solution, the exogenous ligand (L) added to enhance phytoextraction is assumed to react with cations (M) to form a 1:1 complex (ML):

$$\mathrm{M}+\mathrm{L}\underset{k_{\mathrm{d}}^{\mathrm{ML}}}{\overset{k_{\mathrm{a}}^{\mathrm{M,L}}}{\rightleftarrows}} \tag{1}$$

where $k_{\mathrm{a}}^{\mathrm{M,L}}$ is the second-order association rate constant of M and L in soil solution, in L mol^{-1} s^{-1}, and k_d^{ML} is the first-order dissociation rate constant of ML in soil solution, in s^{-1}. The main cations considered to react with the ligand in the soil solution are Cd^{2+}, Ca^{2+}, Mg^{2+} and Fe^{3+}. However, compared with the concentration of the calcium complex (CaL), that of magnesium complex (MgL) is much lower because of the lower Mg^{2+} content in soil and of its lower affinity for the ligand in general (see below). In the soil solution, the concentration of iron complex (Fe(III)L) may be higher than that of CaL, but its dissociation rate constant is much lower (Morel and Hering 1993). For all these reasons, the dissociation of MgL and Fe(III) are ignored in order to simplify the formalism. The reactions of Cd and Ca with L in soil solution can be written as follows (Custos 2012; Custos et al. 2014):

$$\left\{\begin{array}{c} \frac{\partial}{\partial t}[Cd]=k_{\mathrm{d}}^{\mathrm{CdL}}[CdL]-k_{\mathrm{a}}^{\mathrm{Cd,L}}[Cd][L]=-\frac{\partial}{\partial t}[CdL] \\ \frac{\partial}{\partial t}[Ca]=k_{\mathrm{d}}^{\mathrm{CaL}}[CaL]-k_{\mathrm{a}}^{\mathrm{Ca,L}}[Ca][L]=-\frac{\partial}{\partial t}[CaL] \\ \frac{\partial}{\partial t}[L]=\frac{\partial}{\partial t}[Cd]+\frac{\partial}{\partial t}[Ca] \end{array}\right\} \tag{2}$$

In Eq. (2), [*Cd*] [*Ca*] [*L*] [*CdL*] and [*CaL*] represent the concentrations of Cd^{2+}, Ca^{2+}, ligand and their complexes in soil solution, respectively (mol L^{-1} solution); t is time (s).

The Cd^{2+} adsorption on the soil solid phase is formalised as a kinetic process (Roose et al. 2001):

$$\theta\frac{\partial}{\partial t}[Cd]=k_{des}\{CdS\}-\theta k_{ads}[Cd]=-\frac{\partial}{\partial t}\{CdS\} \tag{3}$$

In Eq. (3), θ is the volumetric soil water content (L solution L^{-1} soil); $\{CdS\}$ is the concentration of sorbed Cd^{2+} in soil (mol L^{-1} soil); k_{des} is the first-order desorption rate constant of sorbed Cd^{2+} (s^{-1}); k_{ads} is the first-order adsorption rate constant of Cd^{2+} onto the soil surface (s^{-1}).

The sorption of Ca^{2+}, CdL, CaL and L is not as high as that of Cd^{2+} (Custos et al. 2014). Therefore, it is assumed to be an equilibrium process and formalised as a buffer power b_x (L solution L^{-1} soil) (Roose et al. 2001):

$$b_x=\frac{\partial\{xS\}}{\partial[x]} \tag{4}$$

In Eq. (4), x represents Ca^{2+}, CdL, CaL or L, respectively, $[x]$ is the concentration in soil solution (mol L^{-1} solution), $\{xS\}$ is the concentration of sorbed x in soil (mol L^{-1} soil).

The diffusion coefficients and buffer powers of CdL and CaL and are assumed to be the same as that of L. Based on Barber's model (Barber 1995), equations governing the Cd root uptake model including adsorption, diffusion, convection and complexation in solution can be written as follows (Custos et al. 2014):

$$\begin{array}{l} \theta\frac{\partial}{\partial t}[Cd]=\frac{1}{r}\frac{\partial}{\partial r}\left(rf\theta D_{\mathrm{Cd}}\frac{\partial}{\partial r}[Cd]+r_0v_0[Cd]\right)+\theta\left(k_{\mathrm{d}}^{\mathrm{CdL}}[CdL]-k_{\mathrm{a}}^{\mathrm{Cd,L}}[Cd][L]\right)+\left(k_{\mathrm{des}}\{CdS\}-\theta k_{\mathrm{ads}}[Cd]\right) \\ (\theta+b_{\mathrm{L}})\frac{\partial}{\partial t}[CdL]=\frac{1}{r}\frac{\partial}{\partial r}\left(rf\theta D_{\mathrm{L}}\frac{\partial}{\partial r}[CdL]+r_0v_0[CdL]\right)+\theta\left(k_{\mathrm{a}}^{\mathrm{Cd,L}}[Cd][L]-k_{\mathrm{d}}^{\mathrm{CdL}}[CdL]\right) \\ (\theta+b_{\mathrm{ca}})\frac{\partial}{\partial t}[Ca]=\frac{1}{r}\frac{\partial}{\partial r}\left(rf\theta D_{ca}\frac{\partial}{\partial r}[Ca]+r_0v_0[Ca]\right)+\theta\left(k_{\mathrm{d}}^{\mathrm{CaL}}[CaL]-k_{\mathrm{a}}^{\mathrm{Ca,L}}[Ca][L]\right) \\ (\theta+b_{\mathrm{L}})\frac{\partial}{\partial t}[CaL]=\frac{1}{r}\frac{\partial}{\partial r}\left(rf\theta D_{\mathrm{L}}\frac{\partial}{\partial r}[CaL]+r_0v_0[CaL]\right)+\theta\left(k_{\mathrm{a}}^{\mathrm{Ca,L}}[Ca][L]-k_{\mathrm{d}}^{\mathrm{CaL}}[CaL]\right) \\ (\theta+b_{\mathrm{L}})\frac{\partial}{\partial t}[L]=\frac{1}{r}\frac{\partial}{\partial r}\left(rf\theta D_{\mathrm{L}}\frac{\partial}{\partial r}[L]+r_0v_0[L]\right)+\theta\left(k_{\mathrm{d}}^{\mathrm{CdL}}[CdL]-k_{\mathrm{a}}^{\mathrm{Cd,L}}[L]\right)+\theta\left(k_{\mathrm{d}}^{\mathrm{CaL}}[CaL]-k_{\mathrm{a}}^{\mathrm{Ca,L}}[Ca][L]\right) \\ \frac{\partial}{\partial t}\{CdS\}=\theta\,k_{\mathrm{ads}}[Cd]-k_{\mathrm{des}}\{CdS\} \end{array} \tag{5}$$

In Eqs. (5), f is the impedance factor (dm^2 soil dm^{-2} solution); D_{Cd}, D_{Ca} and D_{L} are the diffusion coefficients of Cd^{2+}, Ca^{2+} and L in water, respectively (dm^2 s^{-1}); r is the radial distance from the centre of the root (dm); r_0 is the root radius (dm); v_0 is water uptake rate at the root surface (dm^3 solution dm^{-2} soil s^{-1}). Solving the six governing equations gives the concentrations of the six chemical species with time and distance to the root surface.

These governing equations were solved in a 1-D cylinder domain extending from the root surface to the half distance between roots, taking into consideration competition between roots for the uptake (Barber 1995). The initial concentration conditions of the model could be written as follows:

$$\left\{\begin{array}{c}[Cd]=[Cd]_0\\ [CdL]=[CdL]_0\\ [Ca]=[Ca]_0\\ [CaL]=[CaL]_0\\ [L]=[L]_0\\ \{CdS\}=\{CdS\}_0\end{array}\right\}\quad r_0\leq r\leq r_1\quad t=0 \tag{6}$$

In Eq. (6), all square brackets with subscripted zero represent initial concentrations (mol L^{-1} solution); $\{CdS\}_0$ is the initial concentration of sorbed Cd^{2+} (mol L^{-1} soil); r_1 is the half distance between roots (dm).

Root growth was not considered in the model since differences were avoided in assessing the effect of ligand addition on phytoextraction by unit surface root segment or for the whole root system. Assuming that plant roots only absorb Cd^{2+} at the root surface in a symplastic pathway (plant S) and that all fluxes at half distance between roots were nil, the boundary conditions of the model for plant S can be written as follows:

$$J[Cd,CdL,Ca,CaL,L,CdS]=\left\{\begin{array}{ccc}\left[-\frac{I_{\max}[Cd]}{K_{\mathrm{m}}+[Cd]},\ 0,\ 0,\ 0,\ 0,\ 0\right] & r=r_0 & t\geq 0\\ [0,\ 0,\ 0,\ 0,\ 0,\ 0] & r=r_1 & t\geq 0\end{array}\right\} \tag{7}$$

For simplification, Ca^{2+} and CaL are considered not to be absorbed. This is permitted because the Ca influx would not change the relatively high Ca concentrations in soil solution at the root surface (Barber 1962; Custos et al. 2014) and consequently would not affect the plant Cd uptake. In Eq. (7), $I_{\max}$ is the maximum Cd^{2+} influx by plant root (mol dm^{-2} s^{-1}); K_{m} is the affinity coefficient of the Michaelis-Menten absorption kinetics (mol L^{-1} solution).

The cumulative Cd uptake for plant S can be calculated as follows:

$$U_{\mathrm{cum}}=\int_0^T\left(\frac{I_{\max}[Cd]}{K_{\mathrm{m}}+[Cd]}\right)dt \tag{8}$$

where U_{cum} is the cumulative Cd uptake per dm^2 of root surface over the uptake duration (mol dm^{-2} $month^{-1}$); T is the uptake duration (s).

Another type of plant was also considered for which roots absorbed both Cd^{2+} and CdL in symplastic and apoplastic pathways, respectively, (plant SA). Its boundary conditions can be written as follows:

$$J[Cd,CdL,Ca,CaL,L,CdS]=\left\{\begin{array}{ccc}\left[-\frac{I_{\max}[Cd]}{K_{\mathrm{m}}+[Cd]},\ -TSCFv_0[CdL],0,\ 0,-TSCFv_0[L],\ 0\right] & r=r_0 & t\geq 0\\ [0,\ 0,\ 0,\ 0,\ 0,\ 0] & r=r_1 & t\geq 0\end{array}\right\}$$

For the same reason as above, Ca species are considered not to be absorbed. In Eq. (9), *TSCF* is the transpiration stream concentration factor (dimensionless) considered to be the same for L and CdL (Barber 1962; Custos et al. 2014). The cumulative total Cd uptake of plant SA can be calculated as follows:

$$U_{\mathrm{cum}}=\int_0^T\left(\frac{I_{\max}[Cd]}{K_{\mathrm{m}}+[Cd]}+TSCFv_0[CdL]\right)dt \tag{10}$$

Cadmium root uptake without exogenous ligand (and omitting the complexation effect of endogenous ligand, such as dissolved organic matter) was also simulated as a control scenario; the equations governing the control scenario can be written as:

$$\left\{\begin{array}{c}\theta\frac{\partial}{\partial t}[Cd]=\frac{1}{r}\frac{\partial}{\partial r}\left(rf\theta D_{\mathrm{Cd}}\frac{\partial}{\partial r}[Cd]+r_0v_0[Cd]\right)+\left(k_{\mathrm{des}}\{CdS\}-\theta k_{\mathrm{ads}}[Cd]\right)\\ \frac{\partial}{\partial t}\{CdS\}=\theta k_{\mathrm{ads}}[Cd]-k_{\mathrm{des}}\{CdS\}\end{array}\right\} \tag{11}$$

The initial concentration conditions of the control scenario are given by

$$\begin{Bmatrix} [Cd] = [Cd]_{0\_C} \\ \{CdS\} = \{CdS\}_{0\_C} \end{Bmatrix} \quad r_0 \le r \le r_1 \quad t = 0 \tag{12}$$

In Eq. (12), $[Cd]_{0\_C}$ is the initial concentration of Cd^{2+} in soil solution without ligand addition (mol L^{-1} solution); $\{CdS\}_{0\_C}$ is the initial concentration of sorbed Cd^{2+} in soil without ligand addition (mol L^{-1} soil). The flux boundary conditions of the control scenario can be written as follows:

$$J[Cd, CdS] = \begin{Bmatrix} \left[-\frac{I_{\max}[Cd]}{K_m + [Cd]}, 0 \right] & r = r_0 & t \ge 0 \\ [0, 0] & r = r_1 & t \ge 0 \end{Bmatrix}, \tag{13}$$

and the cumulative Cd uptake by the plant root of the control scenario could be calculated as for plant S (Eq. 8).

Initialization of the models

When $t=0$, sorption and complexation kinetic processes are assumed to be at equilibrium (partial derivatives of all concentrations with respect to time in Eqs. (2) and (3) are equal to 0). According to this equilibrium assumption, the stability constant of a complex in soil solution can be written as

$$K_S^{ML} = \frac{k_a^{M,L}}{k_d^{ML}} = \frac{[ML]_0}{[M]_0[L]_0} \quad M = Cd,\ Ca, Mg,\ Fe(III) \tag{14}$$

In Eq. (14), K_S^{ML} is the stability constant of ML in soil solution (L mol^{-1}); $[ML]_0$ and $[M]_0$ are the initial concentrations of ML and M (mol^{-1} L solution). Stability constants for CaL, MgL and Fe(III)L can be calculated from the stability constant of CdL using the following equations:

$$\begin{Bmatrix} \log_{10}\left(K_S^{CaL}\right) = \alpha_1 \log_{10}\left(K_S^{CdL}\right) \\ \log_{10}\left(K_S^{MgL}\right) = \alpha_2 \log_{10}\left(K_S^{CdL}\right) \\ \log_{10}\left(K_S^{Fe(III)L}\right) = \alpha_3 \log_{10}\left(K_S^{CdL}\right) \end{Bmatrix} \tag{15}$$

In Eq. (15), α_1, α_2 and α_3 are empirical coefficients determined from the K_S^{ML} of characterised complexes (dimensionless).

According to the equilibrium assumption, the buffer power of Cd^{2+} (b_{Cd}) can be written as

$$b_{Cd} = \frac{\theta k_{ads}}{k_{des}} = \frac{\{CdS\}_0}{[Cd]_0} \tag{16}$$

where b_{Cd} is in L solution L^{-1} soil. It is calculated using the following equation:

$$b_{Cd} = \rho K_{d\_Cd} \tag{17}$$

in which ρ is the soil bulk density, taken as 1.3 kg soil L^{-1} soil; K_{d_Cd} is the soil/solution partitioning coefficient of Cd^{2+} (L solution kg^{-1} soil), which is estimated using the following empirical equation (Degryse et al. 2009):

$$\log_{10}(K_{d\_Cd}) = -1.7 + 0.62pH + 0.61\log_{10}(SOC) \quad (R^2 = 0.71, n = 86) \tag{18}$$

When $t=0$, the concentration of total M in soil ($\{M\}_0^T$, mol L^{-1} soil) can be divided into concentrations of soluble and sorbed metal ions and complexes:

$$\{M\}_0^T = (\theta + b_M)[M]_0 + (\theta + b_L)[ML]_0 \quad M = Cd,\ Ca, Mg \tag{19}$$

Combining Eqs. (14) and (19), the initial concentrations of M and ML in soil solution can be calculated as follows:

$$\begin{Bmatrix} [M]_0 = \frac{\{M\}_0^T}{(\theta + b_M) + (\theta + b_L)K_S^{ML}[L]_0} \\ [ML]_0 = \frac{K_S^{ML}\{M\}_0^T}{(\theta + b_M) + (\theta + b_L)K_S^{ML}[L]_0}[L]_0 \end{Bmatrix} \quad M = Cd,\ Ca, Mg \tag{20}$$

The concentration of Fe(III) in soil solution depends on soil pH, according to the solubility product (K_{SP}, $mol^4\ L^{-4}$) (Schwertmann 1991):

$$K_{SP} = [Fe(III)]_0[OH^-]^3 \tag{21}$$

In Eq. (21), $[OH^-]$ is OH^- concentration in soil solution (mol L^{-1} solution). Assuming that ligand addition would not change the soil pH value and combining Eqs. (14) and (21), the initial concentration of Fe(III)L ($[Fe(III)L]_0$, mol^{-1} L solution) can be calculated as follows:

$$[Fe(III)L]_0 = \frac{K_{SP}K_S^{Fe(III)L}}{10^{3(pH-14)}}[L]_0 \tag{22}$$

When $t=0$, the concentration of total ligand in soil can be expressed as

$$\{L\}_0^T = (\theta + b_L)\{[L]_0 + [CdL]_0 + [CaL]_0 + [MgL]_0 + [Fe(III)L]_0\} \tag{23}$$

In Eq. (23), $\{L\}_0^T$ is the initial concentration of total ligand added into soil (mol L^{-1} soil). Substituting Eqs. (20) and (22) into Eq. (23) gives

$$\{L\}_0^{\mathrm{T}} = (\theta + b_{\mathrm{L}})[L]_0 \left\{ 1 + \sum_{\mathrm{M=Cd,\ Ca,Mg}} \left[\frac{K_{\mathrm{S}}^{\mathrm{ML}}\{M\}_0^{\mathrm{T}}}{(\theta + b_{\mathrm{M}}) + (\theta + b_{\mathrm{L}})K_{\mathrm{S}}^{\mathrm{ML}}[L]_0} \right] + \frac{K_{\mathrm{SP}}K_{\mathrm{S}}^{\mathrm{Fe(III)L}}}{10^{3(pH-14)}} \right\} \quad (24)$$

Solving Eq. (24) using an iterative method to obtain $[L]_0$ and replacing this $[L]_0$ into Eqs. (20) and (16) gives all initial concentrations of Eq. (6).

For the control scenario, the initial conditions are calculated using the following:

$$\left\{ \begin{array}{c} [Cd]_{0\_\mathrm{C}} = \dfrac{\{Cd\}_0^{\mathrm{T}}}{\theta + b_{\mathrm{Cd}}} \\ \{CdS\}_{0\_\mathrm{C}} = b_{\mathrm{Cd}}[Cd]_{0\_\mathrm{C}} \end{array} \right\} \quad (25)$$

Variation domains of the model parameters

The constant parameters used in the Cd uptake models are listed in Table 1. Diffusion coefficients of metal ions in water at standard pressure and 20 °C are well-documented (Lide 2004). The diffusion coefficients of complexes were 14–20 % lower than those of the free hydrated ions (Degryse et al. 2006). Therefore, ligand and complex diffusion coefficients in water were set as 6.0×10^{-8} $\mathrm{dm}^2\ \mathrm{s}^{-1}$. The uptake duration T was 2,592,000 s (1 month). Empirical parameters in Eq. (15) were determined using linear regressions (α_1=0.5969, n=12, R^2=0.92; α_2=0.5088, n=9, R^2=0.92; α_3=1.3747, n=11, R^2=0.90) on available stability constants (Degryse et al. 2006; Smith and Martell 2004).

Varying parameters are listed in Table 2. The maximum Cd uptake rate of plant root ($I_{\max}$) and the affinity coefficient of Michaelis-Menten kinetics (K_{m}) were derived from the works of Hart et al. (2002; 2006) and Lux et al. (2011) and also from that of Degryse et al. (2012) who reported that K_{m} was generally overestimated, and proposed a value of 2.1×10^{-9} mol L^{-1}. Synthesising these studies, K_{m} ranged from 2.1×10^{-9} to 10^{-6} mol L^{-1}. The transpiration stream concentration factor (*TSCF*) for CdL and L varied from 0.1 to 1 (Collins et al. 2006; Nowack et al. 2006). The ranges of the water uptake rate at the root surface v_0 and of the root radius r_0 were obtained from the data produced by Adhikari and Rattan (2000), Barber (1995) and Williams and Yanai (1996). The ranges of half distance between two roots (r_1) was calculated from the root length density for agriculture crops (R_{d}: 0.3–20 cm root cm^{-3} soil) (Metselaar and De Jong van Lier 2011; Williams and Yanai 1996), using the following formula:

$$r_1 = \frac{1}{\sqrt{\pi R_{\mathrm{d}}}} \quad (26)$$

Table 1 Constants in the mechanistic model of phytoextraction with ligand addition

Constant	Descriptions	Values	Units
D_{Cd}	Diffusion coefficient of Cd^{2+} in water	7.07×10^{-8}	$\mathrm{dm}^2\ \mathrm{s}^{-1}$
D_{Ca}	Diffusion coefficient of Ca^{2+} in water	7.00×10^{-8}	$\mathrm{dm}^2\ \mathrm{s}^{-1}$
D_{L}	Diffusion coefficient of ligand in water	6.00×10^{-8}	$\mathrm{dm}^2\ \mathrm{s}^{-1}$
T	Simulation duration	2,592,000	s
α_1	Ratio of $\log_{10}$-stability constants between CaL and CdL	0.5969	
α_2	Ratio of $\log_{10}$-stability constants between MgL and CdL	0.5088	
α_3	Ratio of $\log_{10}$-stability constants between Fe(III)L and CdL	1.3747	
ρ	Soil bulk density	1.3	kg L^{-1}

The concentration of total ligand added into the soil ranged from 1 to 26.7 mmol ligand kg^{-1} soil, i.e. 1.3×10^{-3} to 3.5×10^{-2} mol ligand L^{-1} soil (Blaylock et al. 1997; Kulli et al. 1999; Shen et al. 2002). The range of total labile Cd concentration in polluted soil was set as 1 to 100 mg Cd kg^{-1} soil, i.e. 1.157×10^{-5} to 1.157×10^{-3} mol L^{-1}, which corresponds to situations where Cd phytoextraction can be envisaged (Blaylock et al. 1997; Jiang et al. 2003).

Soil pH value varied from 5 to 8.5 and soil organic carbon content (SOC) from 0.1 to 3 g 100 g^{-1} soil (http://bdat.gissol.fr). The first-order desorption rate constant of sorbed Cd^{2+} ranged from 10^{-6} to 5×10^{-4} s^{-1} (Chaturvedi et al. 2006; Chen et al. 2006; Cornu et al. 2007; Ernstberger et al. 2002; Tsang et al. 2007).

The concentrations of total labile Ca and Mg in soil were calculated as follows. Considering that the cation exchange capacity (CEC) can vary between 3 and 70 cmol+kg^{-1} soil and that Ca^{2+}/CEC varies between 50 to 100 % while Mg^{2+}/CEC ranges between 1 and 20 % (Ciesielski and Sterckeman 1997), the initial concentration of total labile Ca in soil could be 7.5×10^{-3} to 3.5×10^{-1} mol kg^{-1}, i.e. 6×10^{-3} to 2.7×10^{-1} mol L^{-1}; and the initial concentration of total labile Mg in soil could be 1.5×10^{-4} to 7×10^{-2} mol kg^{-1}, i.e. 1.15×10^{-4} to 5.4×10^{-2} mol L^{-1} soil.

Considering the above total labile concentration ranges of cations, if Ca^{2+} concentration in soil solution is 1 to 10 mM, the buffer power of this ion should vary from 0.6 to 270 L solution L^{-1} soil. From a similar reasoning, the buffer power for Mg^{2+} should range from 0.6 to 225 L solution L^{-1} soil. The buffer power of ligand and complexes could be calculated from the soil density multiplied by the soil/solution partitioning coefficient of ligand whose range is 1–158 L solution kg^{-1} soil

Table 2 Parameters in the mechanistic model of phytoextraction with ligand addition

Parameters	Descriptions	Min	Max	$\log_{10}$ (min)	$\log_{10}$ (max)	Units
$I_{\max}$	Maximum Cd uptake rate of plant root	10^{-12}	10^{-10}	−12	−10	mol dm^{-2} s^{-1}
K_m	Affinity coefficient	2.1×10^{-9}	10^{-6}	−8.678	−6	mol L^{-1}
$TSCF$	Transpiration stream concentration factor of CdL and ligand	0.1	1			
v_0	Water uptake rate at the root surface	10^{-10}	5×10^{-7}	−10	−6.301	dm s^{-1}
r_0	Plant root radius	0.0005	0.005			dm
r_1	Half root distance between two roots	0.01	0.1			dm
$\{L\}_0^T$	Initial concentration of total ligand in soil	1.3×10^{-3}	3.5×10^{-2}			mol L^{-1}
$\{Cd\}_0^T$	Initial concentration of total Cd in soil	1.157×10^{-5}	1.157×10^{-3}			mol L^{-1}
$\{Ca\}_0^T$	Initial concentration of total Ca in soil	6×10^{-3}	0.27			mol L^{-1}
$\{Mg\}_0^T$	Initial concentration of total Mg in soil	1.15×10^{-4}	5.38×10^{-2}			mol L^{-1}
pH	Soil pH value	5	8.5			
SOC	Soil organic carbon content	0.1	3			g 100 g^{-1}
k_{des}	First-order desorption rate constant of sorbed Cd^{2+}	10^{-6}	5×10^{-4}	−6	−3.301	s^{-1}
b_{Ca}	Buffer power of Ca^{2+} in soil	0.6	270			L L^{-1}
b_{Mg}	Buffer power of Mg^{2+} in soil	0.6	225			L L^{-1}
b_{L}	Buffer power of ligand in soil	1.3	205			L L^{-1}
K_S^{CdL}	Stability constant for CdL			1	22	L mol^{-1}
k_d^{CdL}	First-order dissociation rate constant of CdL	10^{-6}	10^{-1}	−6	−1	s^{-1}
k_d^{CaL}	First-order dissociation rate constant of CaL	10^{-5}	1	−5	0	s^{-1}
K_{SP}	Solubility product of iron			−44	−37	mol^4 L^{-4}
θ	Soil water content	0.05	0.45			L L^{-1}
f	Impedance factor	max (0.01, 0.9943θ−0.1722)	1.3268θ+0.0022			dm^2 dm^{-2}

(Ponizovsky et al. 2006; Yin et al. 2002; You et al. 1999), providing values of 1.3 to 205 L solution L^{-1} soil.

The $\log_{10}$-stability constant of CdL (L mol^{-1}) varied from 1 to 22 (Degryse et al. 2006; Smith and Martell 2004). Considering the wide difference in affinity for Cd between aminopolycarboxylate chelating agents and organic acids, this range was divided into two sub-ranges, 10–22 (high-affinity ligand) and 1–10 L mol^{-1} (low-affinity ligand).

The first-order dissociation rate constant of CdL ranged from 10^{-5} to 1 s^{-1} (Degryse et al. 2006; Schneider and Nguyen 2011; Schneider et al. 2009). According to Carr and Swartzfager (1975), the first-order dissociation rate constants for CaL could be taken as between 10^{-5} and 1 s^{-1}. The solubility product of iron (K_{SP}) varied from 10^{-44} to 10^{-37} mol^4 L^{-4} (Schwertmann 1991).

The soil water content ranged from 0.05 to 0.45 L solution L^{-1} soil; the impedance factor was partly correlated to soil water content using the following formula (Tinker and Nye 2000):

$$\max(0.01,\mathrm{kern3pt}0.9943\theta–0.1722) < f < 1.3268\theta + 0.0022 \qquad (27)$$

Scenario setting and random sampling of the model parameters and variables

Seven Cd uptake scenarios were simulated. There were four scenarios with ligand addition (two plant types × two ligand types according to the affinity range), including scenario S + H; symplastic uptake plant (S) with high-affinity ligand addition (H), scenario SA + H; symplastic and apoplastic uptake plant (SA) with high-affinity ligand addition (H), scenario S + L; symplastic uptake plant (S) with low-affinity ligand addition (L) and scenario SA + L; symplastic and apoplastic uptake plant (SA) with low-affinity ligand addition (L). In scenario SA + H, [L] was very low because of the high affinity of the ligand to the various cations. Consequently, the influence of apoplastic uptake of L on Cd complexation kinetics could be significant, and this uptake was kept in the model. In contrast, in scenario SA + L, [L] was very high, rendering insignificant the influence of apoplastic uptake of free ligand on Cd complexation kinetics. This is the reason why apoplastic L uptake was omitted from Scenario SA + L.

The control scenario had the same initial concentration of total labile Cd in soil as the four scenarios above, but no ligand addition (Eq. 25). Besides, to analyse the contribution of complex dissociation on cumulative Cd uptake, scenario S + Hb and S + Lb were also set, respectively, corresponding to scenarios S + H and S + L but without complex dissociation. These scenarios used the governing equations (Eq. 11) and boundary conditions (Eq. 13) of the control scenario together with the initial concentrations ($[Cd]_0$ and $\{CdS\}_0$) of scenarios S + H and S + L, respectively.

The 22 parameters listed in Table 2 were sampled using quasi-random sampling with low discrepancy sequences of uniform distribution (Saltelli et al. 2004); the sample size was 100,000. Some parameters were sampled after a $\log_{10}$ transformation, as their ranges covered two or more orders of magnitude, thereby ensuring a uniform distribution of the sampling values over different orders of magnitude.

After the random sampling, all the initial concentrations for the seven scenarios were calculated. Considering that Ca^{2+} concentration in soil solution should be greater than 0.1 mM, about 7.7 % of the simulations, whose Ca^{2+} concentration was too low, were disregarded.

The models for the seven scenarios were solved numerically using FlexPDE Professional 6.32 (PDE Solutions Inc. 2012). About 0.7 % of the simulations were unqualified due to numerical computation error. The number of final simulation replicates was 91,902 for each of the seven scenarios. Data processing and analyses (including ANOVA) were performed with R (R Core Team 2013) together with three R packages: the quasi-random sampling using low discrepancy Sobol's sequences was performed using the fOptions package (Wuertz 2013), the kernel density functions and box plots were produced using the lattice package (Sarkar 2008) and empirical cumulative frequency functions were produced using the latticeExtra package (Sarkar and Andrews 2013).

Calculation of indicators

The ratio (β_1) between the initial concentration of soluble Cd^{2+} in soil solution after ligand addition and that of soluble Cd^{2+} in the control scenario (no ligand but the same total Cd soil content), the ratio (β_2) between total soluble Cd (Cd^{2+} and CdL) in soil solution after ligand addition and that of soluble Cd^{2+} in the control scenario and the ratio (β_3) between total CdL (soluble and sorbed) concentration and sorbed Cd^{2+} concentration in soil after ligand addition were calculated as follows:

$$\left\{\begin{array}{c} \beta_1 = \dfrac{[Cd]_0}{[Cd]_{0_\mathrm{C}}} = \dfrac{\theta + b_\mathrm{Cd}}{(\theta + b_\mathrm{Cd}) + (\theta + b_\mathrm{L})K_\mathrm{S}^\mathrm{CdL}[L]_0} \\ \beta_2 = \dfrac{[Cd]_0 + [CdL]_0}{[Cd]_{0_\mathrm{C}}} = \dfrac{(\theta + b_\mathrm{Cd})\left(1 + K_\mathrm{S}^\mathrm{CdL}[L]_0\right)}{(\theta + b_\mathrm{Cd}) + (\theta + b_\mathrm{L})K_\mathrm{S}^\mathrm{CdL}[L]_0} \\ \beta_3 = \dfrac{(\theta + b_\mathrm{L})[CdL]_0}{\{CdS\}_0} \end{array}\right\} \tag{28}$$

The mean concentrations of Cd^{2+} ($\overline{[Cd]_\mathrm{ro}}$) and CdL ($\overline{[CdL]_\mathrm{ro}}$) in solution during the simulation at the root surface were calculated as shown:

$$\left\{\begin{array}{ll} \overline{[Cd]}_\mathrm{ro} = \dfrac{1}{T}\displaystyle\int_0^T [Cd]\mathrm{d}t & \\ & r = r_0 \\ \overline{[Cd]}_\mathrm{ro} = \dfrac{1}{T}\displaystyle\int_0^T [CdL]\mathrm{d}t & \end{array}\right\} \tag{29}$$

The mean volume dissociation rate of the complex (Δ_CdL, mol L^{-1} s^{-1}) in the soil solution at the root surface during the simulation was calculated from

$$\Delta_\mathrm{CdL} = k_\mathrm{d}^\mathrm{CdL}\overline{[CdL]_\mathrm{ro}} \tag{30}$$

The dimensionless equilibrium indicators of complexation and sorption kinetics at root surface could be defined as follows:

$$\left\{\begin{array}{ll} E_\mathrm{CdL} = \dfrac{1}{K_\mathrm{S}^\mathrm{CdL}}\dfrac{1}{T}\displaystyle\int_0^T \dfrac{[CdL]}{[Cd][L]}\mathrm{d}t & \\ & \dfrac{\{CdS\}}{[Cd]}\mathrm{d}t r = r_0 \\ E_\mathrm{CdS} = \dfrac{1}{b_\mathrm{Cd}}\dfrac{1}{T}\displaystyle\int_0^T & \end{array}\right\} \tag{31}$$

If E_CdL is equal to 1, then the complexation kinetics is always in a balanced status during the uptake; at the root surface, $[CdL]$ is coupled with $[Cd]$ and $[L]$ for diffusion. If E_CdS is equal to 1, then the exchange of Cd^{2+} between the soil solution and the solid phase is not limited by the adsorption kinetics.

The contribution of complex dissociation to U_cum in scenarios S + H and S + L (ϕ, %) could be estimated as follows:

$$\phi = \frac{U_\mathrm{cum} - U_\mathrm{cum\_no\_dis}}{U_\mathrm{cum}} \times 100 \tag{32}$$

In Eq. (32), $U_\mathrm{cum\_no\_dis}$ is the cumulative Cd uptake without complex dissociation (mol dm^{-2} $month^{-1}$) and is calculated according to Eq. (11) (scenarios S + Hb and S + Lb, respectively).

For plant (SA) able to absorb CdL, the cumulative apoplastic CdL uptake per dm^2 of root surface over the uptake duration (U_CdL, mol dm^{-2} $month^{-1}$) was calculated as follows:

$$U_\mathrm{CdL} = \int_0^T (TSCFv_0[CdL])dt \tag{33}$$

The contribution of complex uptake to total uptake (λ, %) was calculated from U_{CdL} as a percentage of U_{cum}.

Results and discussions

Speciation of Cd in soil after ligand addition

Figure 1 shows the distributions of the ratios β_1, β_2 and β_3 for low- and high-affinity ligand addition. Ligand addition always reduced Cd^{2+} concentration by up to eight orders of magnitude for high-affinity ligand (Fig. 1a) and by up to four orders of magnitude for low-affinity ligand (Fig. 1b). For low-affinity ligand addition, about 64 % of the simulations showed a less than tenfold reduction of the Cd^{2+} concentration in the soil solution, while for high-affinity ligand, this percentage was only 1.8 %. Additions of both kinds of ligand increased the total soluble Cd concentration in soil solution for most (81.4 %) of the simulations (Fig. 1c, d). When adding high-affinity ligand, the total soluble Cd concentration in the initial soil solution was more than 10 times that of the control scenario in 38.4 % of the cases and more than 100 times that of the control scenario in 5.1 % of the cases (Fig. 1c). For low-affinity ligand addition, these percentages were smaller, respectively 18.5 % and 1.9 % of the cases (Fig. 1d). About 99.6 % of the simulations with high-affinity ligand addition showed a total CdL concentration greater than the sorbed Cd^{2+} concentration in soil (Fig. 1e). This situation represented 54.4 % for low-affinity ligand addition (Fig. 1f).

The effect of ligand addition is to form CdL to the detriment of Cd^{2+}, and thus to reduce the initial amount of Cd^{2+} in soil (Fig. 1e, f), and thus the soil solution Cd^{2+} concentration (Fig. 1a, b). These reductions augment with increasing K_S^{CdL} or $[L]_0$ (Eqs. (1) and (14)). Considering the total Cd concentration in soil solution, two cases can be distinguished. When the soil buffer power for Cd^{2+} (b_{Cd}) is greater than the soil buffer power for the ligand (b_L), the total Cd concentration in soil solution after ligand addition is greater than the Cd^{2+} concentration before the ligand addition ($\beta_2>1$) and increases with K_S^{CdL} or $[L]_0$. Conversely, when $b_{\mathrm{Cd}}<b_L$, the total Cd concentration in soil solution after ligand addition is lower than the Cd^{2+} concentration before the addition ($\beta_2<1$), and it is lower the higher is K_S^{CdL} or $[L]_0$. The ratio β_2 increases when b_{Cd} increases or b_L decreases.

Because the soil buffer power for Cd^{2+} is generally much greater than that for CdL (Table 2), the total Cd concentration in soil solution resulting from ligand addition is generally greater than the Cd^{2+} concentration in soil solution before ligand addition (Fig. 1c, d). This increase in Cd solubility by ligand addition is all the more important that the ligand affinity is high. The increase can reach up to two orders of magnitude even for low-affinity ligands.

This clearly shows the risk of Cd transfer to groundwater if plant absorption capacity is insufficient or in case of low root activity (at low temperature for example). Besides, for the same dose of ligand supplied to the soil, the complex

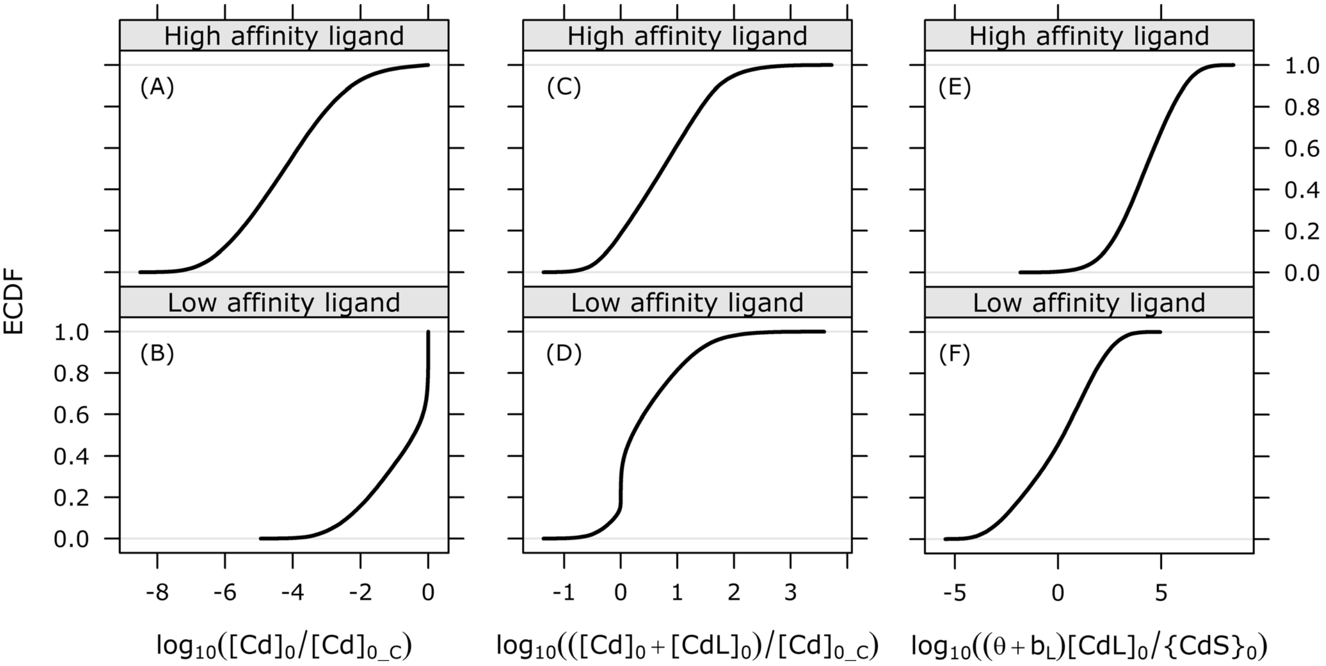


Fig. 1 Empirical cumulative distribution functions (ECDF) of $\log_{10}$-Cd^{2+} concentration in soil solution ($\log_{10}([Cd]_0/[Cd]_{0\_C})$), $\log_{10}$-total soluble Cd concentration in soil solution ($\log_{10}(([Cd]_0+[CdL]_0)/[Cd]_{0\_C})$), and $\log_{10}$-ratios between total CdL concentration and sorbed Cd^{2+} concentration in soil ($\log_{10}((\theta+b_L)[CdL]_0/\{CdS\}_0)$) under high- and low-affinity ligand additions

concentration in soil solution can be much higher for high-affinity than for low-affinity ligand (more than 10 and 100 times in 30.9 % and 17.5 % of the simulations, respectively). Thus, the use of high-affinity ligands such as EDTA could be much more harmful for the environment than that of low-affinity ligands.

Plant Cd uptake after ligand addition

The distributions of cumulative Cd uptake per dm^2 root surface per month (U_{cum}) in four ligand addition scenarios and the control scenario are shown in Fig. 2. All $\log_{10}(U_{\text{cum}})$ ($\mathrm{mol\ dm}^{-2}\ \mathrm{month}^{-1}$) were within the range of −14 to −3.39. The uptake generally corresponded to a small fraction of the initial total Cd present in the soil. The percentages of simulations for which the uptake was less than 10 % of the total Cd were 86.9 % for the control scenario and greater than 91.7 % for the four others. The $\log_{10}(U_{\text{cum}})$ in the four ligand addition scenarios were significantly lower than that in the control scenario (p value of paired t test <0.001).

Figure 3 shows the frequency at which U_{cum} with ligand addition was 1.05 (considering an increase of 5 % to be significant) to 10 times greater than U_{cum} without ligand addition (control scenario). Cases where the predicted U_{cum} with ligand addition was greater than 1.05 times U_{cum} in control scenario were more frequent when the affinity of the ligand was low than when it was high (10.2 and 16.2 % for S + L and SA + L versus 0.3 and 9.4 % for S +H and SA + H, respectively) and when the complex was assumed to be taken up as compared to the case where only Cd^{2+} was absorbed (9.4 and 16.2 % for SA + H and SA + L versus 0.3 and 10.2 % for S + H and S + L, respectively). However, strong increases of U_{cum} as compared to the control scenario were rare. The frequencies of these cases sharply decline with the magnitude of the increase (m in Fig. 3). There were only 5.6 % of the simulations for which the cumulative uptake was doubled by the ligand addition, and this was for the high-affinity ligand scenario with the complex allowed to be absorbed (scenario SA + H, Fig. 3b). This frequency was lower if the complex was not absorbed or if the ligand had a lower affinity. Thus, the situations where a ligand addition might be really beneficial are rare and hypothetical, as these situations assumed that the complex can be taken up (Fig. 3b, d), which is still subject to debate in the literature (Nowack et al. 2006).

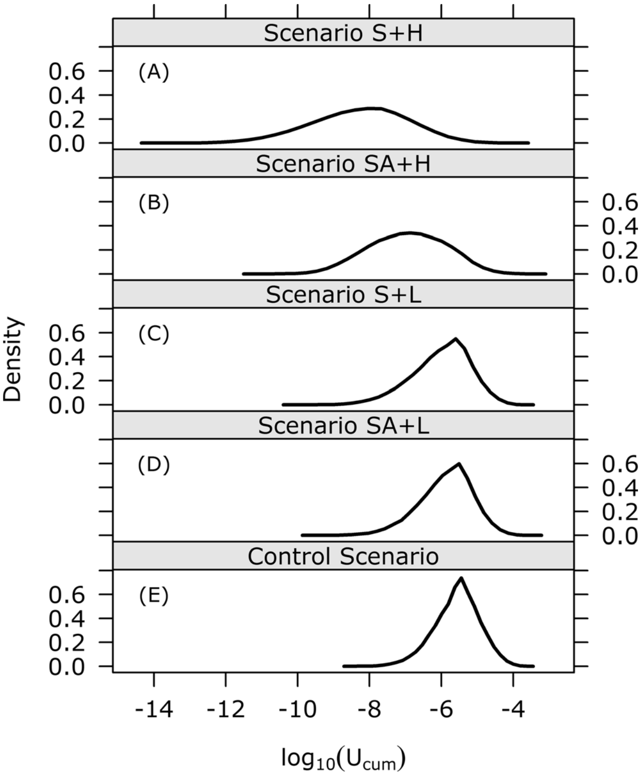


Fig. 2 Kernel density functions of cumulative Cd uptake (U_{cum}, mol) per dm^2 root surface per month in scenarios S + H, SA + H, S + L, SA + L and control scenario

Influential factors on the cumulative Cd uptake with ligand addition

To understand the factors most affecting the cumulative Cd uptake when ligand was added, a full model including variables with their mean effect was fitted to $\log_{10}\left(\frac{U_{\text{cum}}}{U_{\text{cum\_C}}}\right)$, where U_{cum} is the cumulative Cd uptake with ligand and $U_{\text{cum\_C}}$ that without ligand (Control Scenario). The F values from the ANOVA of the model were used to rank the factors according to their mean effect. Figure 4 shows that only two or three factors could explain 64 to 84 % of $\log_{10}\left(\frac{U_{\text{cum}}}{U_{\text{cum\_C}}}\right)$ variability. For scenarios S + H, S + L and SA + L, the initial Cd^{2+} concentration ($\frac{[Cd]_0}{[Cd]_{0\_C}}$) and the complex dissociation rate constant (k_d^{CdL}) were the most influential factors affecting Cd uptake, while plant root water uptake rate (v_0) and complex concentration were the most influential factors for scenario SA + H (Fig. 4b). Explanations of the above-mentioned results are given in the next section.

Processes affecting cumulative Cd uptake after ligand addition

The distributions of the two equilibrium indicators (E_{CdL} and E_{CdS}) are shown in Fig. 5, for S + H and S + L scenarios only since the corresponding scenarios for plants also absorbing CdL (SA + H and SA + L) showed very similar distributions of both indicators. Most values for both indicators were equal to or greater than 1, that is 99.98 and 99.78 % for E_{CdL}, and 90.14 and 99.34 % for E_{CdS} for high- and low-affinity ligands, respectively. Thus, the complex dissociation kinetics in the soil solution and of Cd^{2+} desorption from soil were most probably generally limiting. Both limitations were significantly greater when low-affinity ligand was added as compared to

Fig. 3 Frequencies and percentage of simulations with different multiples (m) of enhanced cumulative Cd uptake (U_{cum}) in scenarios S + H, SA + H, S + L, SA + L versus control scenario (U_{cum_C})

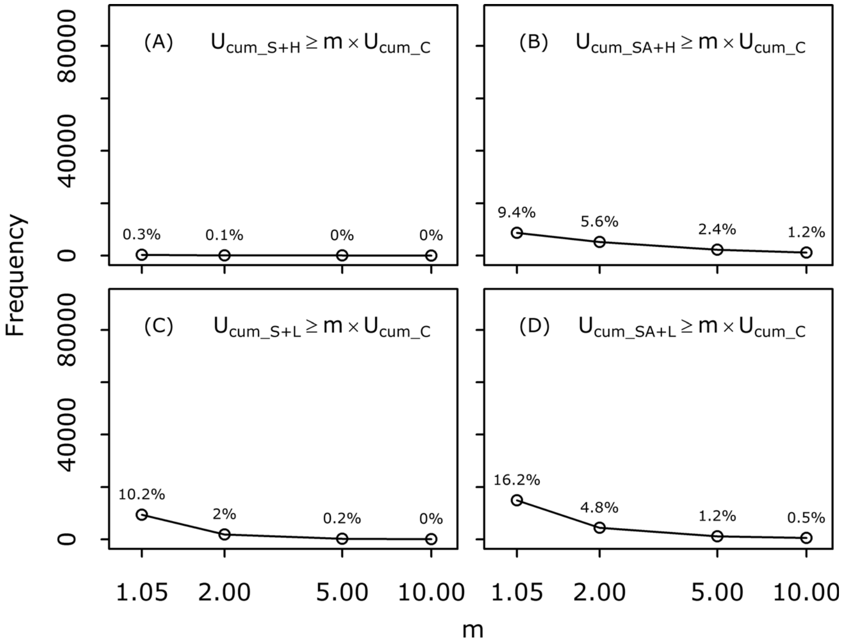


Fig. 4 Influential factors ranked by ANOVA *F* values to explain $\log_{10}$-cumulative Cd uptake with ligand addition versus without ligand addition ($\log_{10}(U_{cum}/U_{cum\_C})$); all signs for these factors are positive

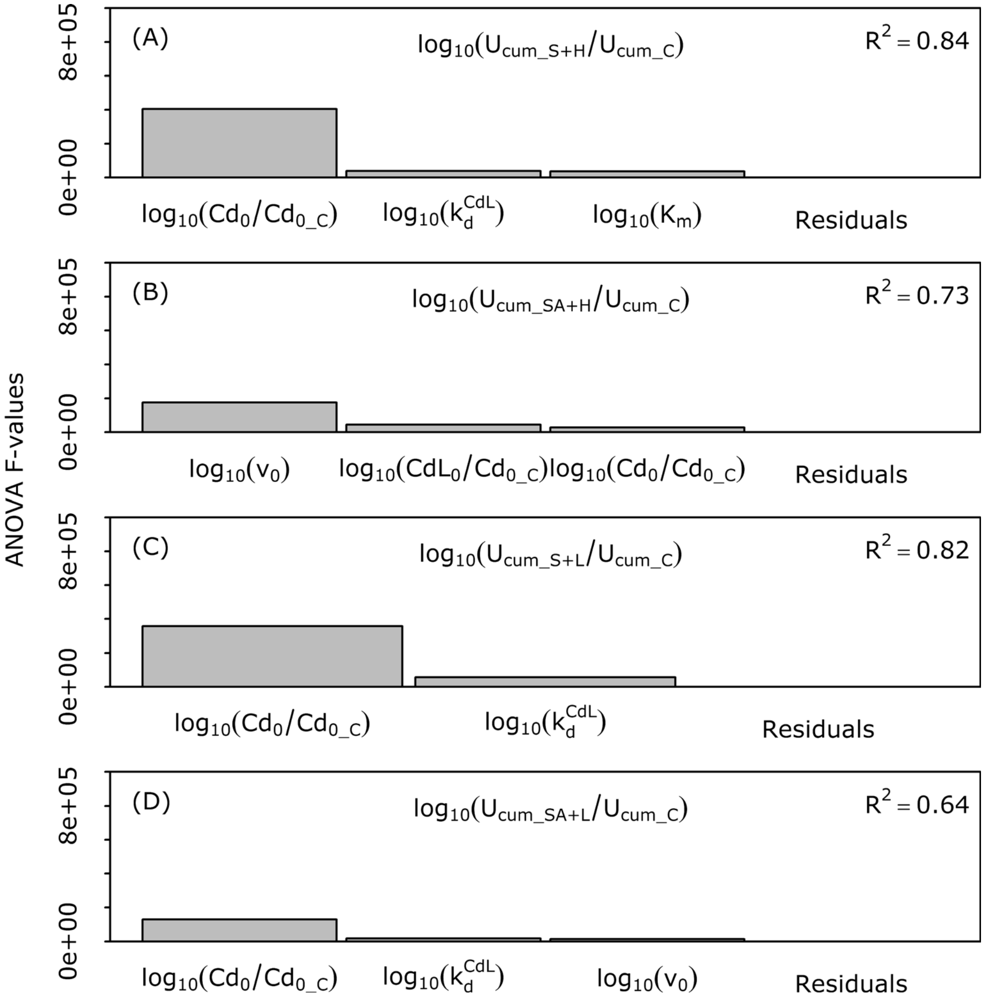
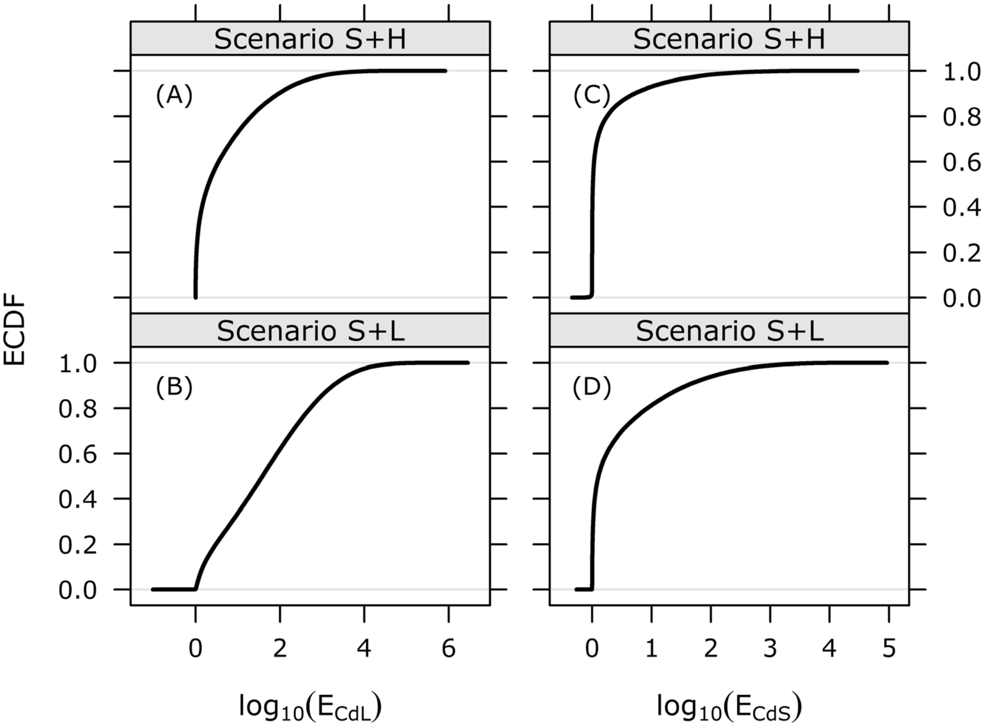


Fig. 5 Empirical cumulative distribution function (ECDF) of $\log_{10}$-equilibrium complexation indicators ($\log_{10}(E_{CdL})$) and sorption kinetics ($\log_{10}(E_{CdS})$) at root surface in scenarios S + H and S + L



high-affinity ligand (p value of paired t test <0.001, comparing scenarios S + H and S + L). For E_{CdL}, this was probably related to the fact that, the uptake being a small fraction of the Cd present in soil, the impact of a limiting kinetics of CdL dissociation was smaller when CdL concentration was very high, as in the case of high-affinity ligand addition. Additionally, whatever the kind of ligand, E_{CdL} was significantly greater than E_{CdS} (p value of paired t test <0.001 for both scenarios), indicating that the dissociation of the complex was more limiting than the desorption of Cd^{2+} from the solid phase.

In the absence of the plant absorption of the complex, the contribution of the complex dissociation to the total uptake appeared particularly strong for high-affinity ligand (Fig. 6). This is revealed by ϕ (%), which was generally much greater in scenario S + H (median 96.1 %, Fig. 6a) as compared to scenario S + L (median 17.9 %, Fig. 6b).

The mean concentration of Cd^{2+} in solution at the root surface ($\overline{[Cd]_{ro}}$) was smaller than the initial Cd^{2+} concentration in solutions ($[Cd]_0$) in 99.2, 90.6, 96.1, 99.4 and 99.7 % of the simulations for the control, S + H, SA + H, S + L and SA + L scenarios, respectively. This reveals that a depletion profile of Cd^{2+} generally existed at the root surface due to the plant absorption of Cd^{2+}, despite the fluxes of Cd^{2+} and CdL. That means that Cd^{2+} soil supply generally limited the uptake and not the root absorption influx. In a few situations in scenarios S + H and SA + H, $[Cd]_{ro}$ was greater than $[Cd]_0$. In these cases, the main parameters explaining the variability of $[Cd]_{ro}$ were v_0 and θ: $[Cd]_{ro}$ increased with v_0 and decreased when θ increased, showing that Cd^{2+} accumulated at the root surface when the water convection flux was high and the counter-diffusion from the root to the bulk soil was restricted by low soil water content.

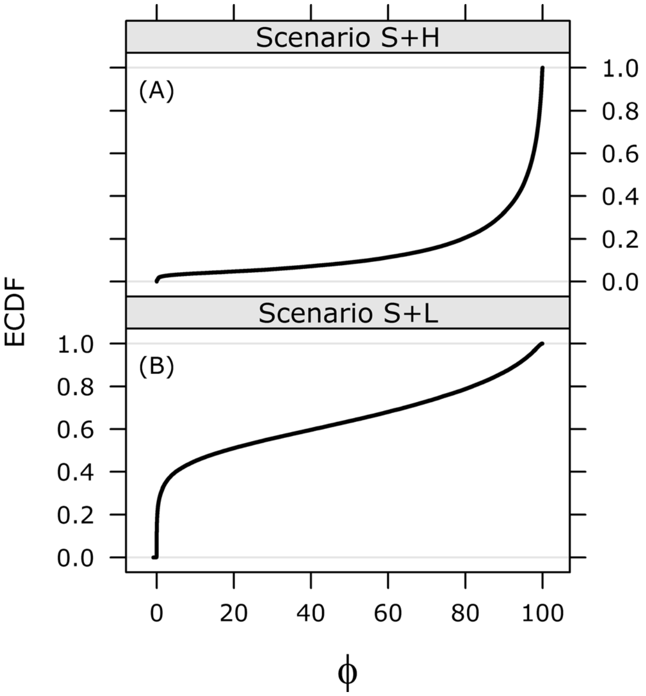


Fig. 6 Empirical cumulative distribution function (ECDF) of contributions (ϕ, %) of complex dissociation to cumulative Cd uptake in scenarios S + H and S +L

When Cd^{2+} soil supply limits the uptake and when there is no CdL absorption, two extreme cases are distinguishable, depending on CdL dissociation. In the first one, the complexation kinetics is very slow and the complex is thus more or less inert. Therefore, the main effect of ligand addition is to decrease plant uptake in proportion to the decrease in soil solution Cd^{2+} concentration. In the second case, there is no restriction in the complexation kinetics so that CdL can be considered as totally labile and the uptake becomes a function of the total Cd concentration in soil solution. The situations simulated here fall between these two extreme cases, and plant uptake after ligand addition compared to that without thus depends not only on the relative decrease in Cd^{2+} concentration but also on CdL concentration in soil solution and on the lability of the complex. Therefore, when no complex absorption is assumed to occur, the effect of ligand addition is generally a decrease in uptake due to the decrease in soil solution Cd^{2+} concentration, which is particularly strong when high-affinity ligands are used. As shown before, the decrease in uptake due to the fall in soil solution Cd^{2+} concentration following ligand addition is generally only partly compensated by complex dissociation in the soil solution at the root surface. This interpretation agrees with the main factors explaining the relative uptake $\log_{10}\left(\frac{U_{\mathrm{cum}}}{U_{\mathrm{cum\_C}}}\right)$, that is $\frac{[Cd]_0}{[Cd]_{0\_C}}$ and $k_{\mathrm{d}}^{\mathrm{CdL}}$, when plant only absorbs Cd^{2+} (Fig. 4a, c).

Ligand use should be clearly avoided when $b_{\mathrm{Cd}}<b_{\mathrm{L}}$, as no benefit can be expected from this use, the total Cd concentration in solution being smaller than the initial Cd^{2+} concentration. This could be the case in acid sandy soils with low organic matter content. Conversely, a potential benefit might be expected for neutral to alkaline, clayey soils rich in organic matter, as the decrease in Cd^{2+} concentration should be limited and total Cd concentration in solution much increased after ligand addition (but with an increased risk of groundwater contamination).

The volume dissociation rate of the complex at the root surface (Δ_{CdL}, mol L^{-1} s^{-1}) should be multiplied by the reaction layer thickness (μ) to approximate the dissociation flux of the complex and thus to enable the comparison of this flux with the uptake flux. However, a model giving μ in the plant-soil-solution system does not as yet exist. Therefore, Δ_{CdL} was used as an indicator of the dissociation flux, comparing it between the different scenarios. Looking at the medians of the distributions for the subset of simulations where ligand supply significantly increased the uptake compared to the control scenario, Δ_{CdL} was 100, 3, 131 and 68 times that of the complementary subset (i.e. simulations for which the uptake was decreased by ligand addition) for scenarios S + H, SA + H, S + L and SA + L, respectively. Except for the SA + H scenario, these data indicate that an increased uptake after ligand addition was essentially related to much higher $k_{\mathrm{d}}^{\mathrm{CdL}}$ values. Thus, except when high-affinity ligand is used and a direct CdL uptake is assumed, the contribution of the complex dissociation to the uptake appears highly significant in explaining those cases where ligand use is beneficial.

Contributions of cumulative apoplastic CdL uptake to U_{cum} (λ, %) are shown in Fig. 7 for plants able to absorb Cd^{2+} together with CdL (scenarios SA + H and SA + L). The uptake of the complex contributed much to the total Cd uptake when the ligand had a high affinity (scenario SA + H): for about 58 % of the simulations, the contribution of CdL uptake ranged between 80 and 100 % of the total uptake (Fig. 7a). In scenario SA + L, the contribution of the complex uptake was much lower with 78 % of simulations where the contribution fell between 0 and 20 % (Fig. 7b).

The cumulative apoplastic uptake of CdL was compared for the two subsets showing a beneficial and detrimental use of the ligand. Looking at the distributions medians, U_{CdL} for the subset with enhanced uptake after ligand addition was 109 and 29 times that of the subset showing a lower uptake than the control scenario, for high-affinity and low-affinity ligand, respectively. This was essentially due to higher mean concentration values of CdL in the solution at the root surface $\overline{[CdL]_{\mathrm{ro}}}$) and of the water convection flux v_0 for the subset benefiting from the ligand addition (for $\overline{[CdL]_{\mathrm{ro}}}$ 2.5 and 1.9 times and for v_0, 43 and 10 times that of the subset showing a decreased uptake, for high-affinity and low-affinity ligand, respectively). Thus, when a plant is assumed to absorb both CdL and Cd^{2+}, the apoplastic uptake of CdL is pre-eminent for

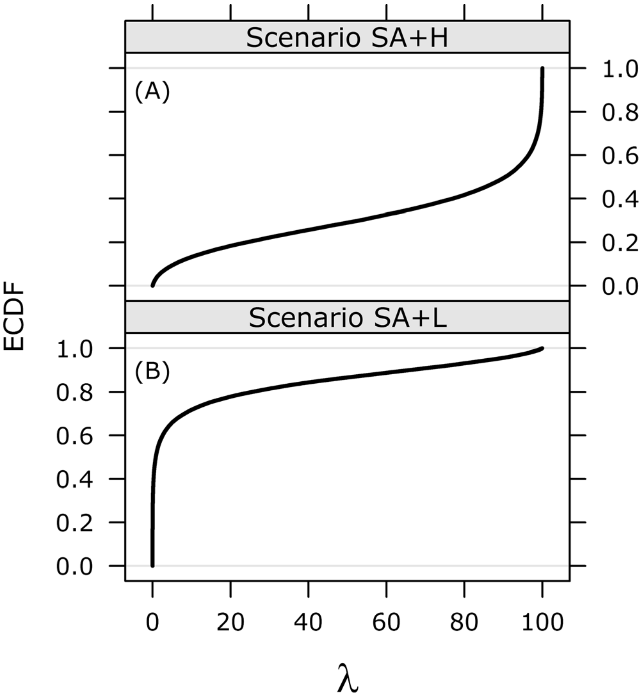


Fig. 7 Empirical cumulative distribution functions (ECDF) for contributions (λ, %) of cumulative apoplastic uptake to cumulative Cd uptake in scenarios SA + H and SA + L

high-affinity ligand addition and relatively less when low-affinity ligand is used.

The main determining factors of the $\overline{[CdL]_{ro}}$ variability are probably $[CdL]_0$ and v_0, which are also those best explaining $\log_{10}\left(\frac{U_{\mathrm{cum}}}{U_{\mathrm{cum\_C}}}\right)$ for the SA + H scenario (Fig. 4b). In the case of the SA + L scenario, the decrease in solution Cd^{2+} following low-affinity ligand addition is less than for high-affinity ligand so that, besides the plant absorption of Cd^{2+}, the three processes, i.e. diffusion of initially sorbed Cd^{2+} towards the root surface, dissociation of the complex near the root, and plant CdL absorption, together contribute to explaining the uptake.

Thus, the risk of groundwater contamination by high-affinity ligand and total soluble Cd associated with the very limited enhancement of the uptake by the plant make any ligand addition of this type useless for Cd phytoextraction. Concerning low-affinity ligands, except if the plant can absorb the complex and if the dissociation constant of the complex is sufficiently high, their use should be avoided.

These conclusions should not be extrapolated to other metals before a specific study. Indeed, in the case of Pb and Cu, with very low solubility in soil, the addition of ligand might significantly increase the potential for root uptake when increasing the concentration in soil solution.

Conclusions

Soil ligand supply aims at increasing the soil solution concentration of toxic trace elements so as to increase their phytoextraction from contaminated soils. From the results of the model developed in this work, this practice should be avoided when the soil buffer power for the trace element (b_{Cd}) is lower than the soil buffer power for the ligand (b_{L}), as the total concentration in solution after ligand addition is then smaller than the initial concentration of the trace element in the soil solution. Thus, ligand addition should not be used in acid sandy soils low in organic matter and reserved to more alkaline, clayey soils rich in organic matter.

For Cd^{2+}, when $b_{\mathrm{Cd}}>b_{\mathrm{L}}$, soil ligand addition leads to a great decrease in sorbed Cd^{2+} concentration together with a dramatic increase in total Cd in solution. This is very pronounced with the addition of high-affinity ligands like aminopolycarboxylate acids (EDTA). The risk of Cd chelate transfer to groundwater by leaching is thus very high. For low-affinity ligands like citrate, these changes and risks are much less pronounced.

Despite the wide range of contaminations studied (1–100 mg Cd kg^{-1} soil), plant Cd uptake is mostly limited by the soil supply and not by the plant absorption capacities. These are conditions where the complex could contribute to the supply of Cd^{2+} towards the root and increase the uptake, if the kinetics of the complexation reaction were sufficiently high. However, the ligand addition generally reduces Cd uptake due to the decrease in solution Cd^{2+} concentration, and this is generally not compensated by complex dissociation, particularly when high-affinity ligands are used. An eventual benefit of the use of high-affinity ligands (about 10 % of cases) relies totally on the assumption that the plant is able to absorb the complex. For low-affinity ligands, the cases where their use is beneficial are 16 % at most, and all processes, i.e. preservation of a high Cd^{2+} concentration, rapid dissociation of the complex near the root, and eventual absorption of CdL by plant,both contribute to the increase in uptake.

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