

Sorption and pH determine the long-term partitioning of cadmium in natural soils

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Abstract The bioavailability of metals in soil is a dynamic process. For a proper extrapolation to the field of laboratory studies on fate and effects, it is important to understand the dynamics of metal bioavailability and the way it is influenced by soil properties. The aim of this study was to assess the parallel (concurrent) effect of pH and aging time on the partitioning of cadmium in natural LUFA 2.2 soil. Cadmium nitrate-spiked pH-amended LUFA 2.2 soils were incubated under laboratory conditions for up to 30 weeks. Measured pH_{pw} was lower after 3 weeks and decreased only slightly toward the end of the test. Cadmium concentrations in the pore water increased with time for all soil pH levels, while they decreased with increasing pH. Freundlich k_f values ranged between 4.26 and 934 L kg⁻¹ ($n=0.79$ to 1.36) and were highest at the highest pH tested (pH = 6.5). Multiple linear regression analysis, based on a soil ligand modeling approach, resulted in affinity constants of 2.61 for Ca²⁺ (log K_{Ca-SL}) and 5.05 for H⁺ (log K_{H-SL}) for their binding to the active sites on the soil surface. The results showed that pH and aging time are two important factors which together affect cadmium partitioning and mobility in spiked natural soils.

Keywords Soil properties · Bioavailability · Soil solution · Soil ligand model · Aging · Partitioning

Introduction

Trace metals remain among the most investigated soil contaminants because of concerns linked with their potential risk to soil organisms. It has been shown that total concentrations are poor indicators of metal toxicity in soils. The pore water hypothesis assumes that metals are available to soil organisms through the solution phase of the soil (Van Gestel 1997). At equilibrium, the distribution of metals between the soil solid phase and the pore water can be described by partition coefficients. The partitioning of metals is an important issue when assuming pore water is the main uptake route of metals by soil organisms. This has been reported, for example, for collembolans (Fountain and Hopkin 2005) and earthworms (Saxe et al. 2001).

In soil, sorption therefore is an important factor that needs to be taken into account when performing toxicity or bioaccumulation tests. Sorption of metals depends strongly on soil characteristics such as organic matter content, cation exchange capacity, and pH. The bioavailability of metals is considered a dynamic process (Peijnenburg et al. 1999; Van Straalen et al. 2005), with both the partitioning of metals between the soil solid phase and the soil solution and their uptake and internal distribution in the soil organism being dependent on time of exposure (Hamelink et al. 1994). The present study focuses on the sorption of cadmium in spiked soils at different pH, as an important soil property that may influence each of these dynamic processes and therefore affect the bioavailability of metals in soil (Sauvé et al. 2000a, b; Peijnenburg et al. 2000).

Standard toxicity tests with soil organisms usually are relatively short-term and do not assess the long-term fate and

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effects of chemicals in soil. The observed differences in the toxicity of metals in short-term and long-term tests may be attributed to differences in bioavailability, since aging is known to increase the binding of metals to soil (Van Gestel 1997). Aging experiments may help in understanding the long-term changes in metal availability with time, and therefore explain the long-term effects of the metal in soil. Insight into the gradual, ongoing process of sorption and fixation of metals in soil, known as aging, is useful for environmental risk assessment and decision making (e.g., in relation to “safe” metal loading rates).

The competition of major ions in the soil solution with the free metal ions for binding to the soil solid phase may affect metal availability in soil. Metal retention in soils has usually been related to the displacement of H^+ ions from phenolic-type functional groups initially occupied by H^+ , which also have high affinity for binding metal ions (Tipping et al. 2003). Recently, Rachou and Sauv e (2008) proposed an alternate soil ligand model (SLM), integrating metal chemistry and cation competition at the soil–water interface. Based on the proposed SLM model, cations including metal ions show strong and competitive affinity for binding to the negatively charged parts (such as clay particles) of the surface of the soil solid phase.

There are many studies in the literature considering the effect of soil properties on the sorption and availability of metals (e.g., Doelman and Haanstra 1984; Wilke 1990; Smit and van Gestel 1998; Smolders et al. 2004; Oorts et al. 2006; Rooney et al. 2007; Criel et al. 2008). However, these studies focus more on soil properties rather than the importance of aging time. On the other hand, long-term equilibration of metals in soils has been investigated in some studies without paying much attention to the effect of soil properties (e.g., Smit and van Gestel 1998; Lock et al. 2006; Ma et al. 2006; Oorts et al. 2007; Smolders et al. 2009). Therefore, there is a lack of knowledge on the interaction of time and soil properties on the sorption of metals.

This contribution is one of a series of related articles exploring the effect of soil properties on bioavailability of metals to soil organisms. In Ardestani and van Gestel (2013), we reported results of experiments on the effect of pH on the bioaccumulation of Cd in the soil invertebrate *Folsomia candida* after 3 weeks of exposure using a toxicokinetics approach. Here, we further expand our synthesis of patterns between metal availability and sorption in soil considering important environmental parameters. Therefore, the objective of this study was to determine the effect of time and pH on the partitioning of cadmium in natural soils. Soil pH is an important factor influencing the availability of cadmium in soils; however, the interaction of pH and time on metal sorption is less studied. So, the interaction of time vs. soil properties makes the results of this study very interesting. We expect a decrease in cadmium availability with time, but pH may play an

important role in changing the time-related development of cadmium partitioning in soil. We used a soil ligand model to describe the long-term equilibrium of cadmium in metal-amended natural LUFA 2.2 soil with pH adjusted to a range between 4.5 and 6.5. Test soils were kept for 30 weeks under experimental conditions in a climate chamber to investigate changes in pore water cadmium concentrations. We also aimed at determining the affinity constants for two major competing ions, Ca^{2+} and H^+ , for binding to the soil surface.

Materials and methods

LUFA 2.2 soil

Standard LUFA 2.2 natural soil (Speyer, Germany) was used as test soil. This soil is characterized as sandy loam with an organic carbon content of 1.93 %, pH (0.01 M $CaCl_2$) of 5.6 ± 0.4 , cation exchange capacity (CEC) of 10.0 meq/100 g, and water holding capacity (WHC) of 46.5 %.

Spiking and pH amendment of the soil

Test soils were prepared for the experiment as described in Ardestani and van Gestel (2013). In brief, soils were spiked with cadmium nitrate ($Cd(NO_3)_2 \times 4H_2O$, 99 % pure, Sigma-Aldrich GmbH) dissolved in deionized water. The selected nominal cadmium concentrations of 5 and 20 mg Cd kg^{-1} soil were below the reported EC_{10} and EC_{50} values of 6–19 and 40–90 mg kg^{-1} dry soil, respectively (Van Gestel and van Diepen 1997; Van Gestel and Hensbergen 1997; Van Gestel and Mol 2003) for the effect of cadmium on the reproduction of the springtail *F. candida* in different artificial and natural soils after 4–6 weeks of exposure. The reason that exposure concentrations were selected based on springtail tests is that these animals are among the most abundant soil organisms, and *F. candida* is widely used in soil toxicity tests. Nominal soil pH_{CaCl_2} values of 4.5, 5.5, and 6.5 were used in the present study (see Ardestani and van Gestel 2013 for more details on pH amendment).

Conditions for equilibrating the soils

All treated and control soils were equilibrated for 2 weeks at 20 °C (75 % relative humidity) in sealed plastic containers prior to the test. Twice a week, test soils were turned completely inside the containers to not allow anoxic conditions to develop at the bottom of the container. The containers were checked for aeration and moisture loss at the beginning and after the equilibration. For equilibrium, approximately 20 g of moist test soil samples were incubated in 100-mL glass test jars.

Test procedure

At day zero (2 weeks after spiking the soils), 3, 11, 21, 24, and 30 weeks after starting the experiment, test soils were sampled for extracting pore water, using two replicate test jars for each treatment and control. Total soil concentrations, water- and CaCl_2 -extractable cadmium concentrations, were only measured at the beginning of the experiment (day zero) and after 3 weeks. Additional glass jars were prepared for pH measurements.

Soil analysis

Soil properties

Soil properties were measured according to Ardestani and van Gestel (2013). Cation exchange capacity (CEC) of the test soils was determined using the silver-thiourea method (Dohrmann 2006). After mixing soil samples with silver nitrate and thiourea, shaking, and centrifugation processes, solutions were analyzed for residual silver by flame AAS (Perkin Elmer, AAnalyst 100).

For measuring dissolved organic carbon (DOC), a TOC Analyzer (HiPerTOC of Thermo, type 2008.122, equipped with ThEuS software package, version 1.4 (0022)) was used.

The pH values of the water ($\text{pH}_{\text{H}_2\text{O}}$) and CaCl_2 ($\text{pH}_{\text{CaCl}_2}$) extracts were measured using a Consort P907 pH meter. Soil pH, CEC, Ca, and DOC levels (the latter two in pore water samples) were measured at the beginning ($T = 0$) and 3 ($T = 3$) and 30 weeks ($T = 30$) after the start of the experiment.

Cadmium concentrations in the soil

Cadmium concentrations were measured following the method used in Ardestani and van Gestel (2013). Briefly, for the total concentration measurement, approximately 130 mg of dried soil was mixed with 2 mL of aqua regia (HNO_3/HCl , 4:1) and incubated for 7 h at 140 °C. After incubation, the samples were cooled to room temperature, and 8 mL of de-ionized water was added. The samples were kept at 4 °C until graphite furnace atomic absorption spectrophotometer (AAS) analysis (Perkin Elmer 5100 PC). Two replicate jars were sampled for each cadmium concentration and pH treatment.

Cadmium and calcium concentrations in soil extracts and soil pore water

For the CaCl_2 exchangeable concentrations, 5 g of dried soil was mixed with 25 mL of 0.01 M CaCl_2 and then shaken at 200 rpm for 2 h and left overnight. Thereafter, the samples were filtered through 0.45- μm pore membranes and kept at 4 °C before determination of cadmium concentrations by graphite furnace AAS.

For measuring the pore water concentrations, soil samples were saturated with water and equilibrated for 4 days. To collect pore water, approximately 20 g of water-saturated soil were placed in plastic containers with a 0.45- μm membrane filter and two paper filters (method described by Hobbelen et al. 2004). Thereafter, samples were centrifuged for 45 min at 2000 g, and 2-mL pore water was collected and analyzed for cadmium using graphite furnace AAS.

Calcium concentrations in pore water samples were also measured using flame AAS (Perkin Elmer, AAnalyst 100).

Data analysis

Using the measured soil pore water and total soil concentrations, sorption of cadmium to the test soils was described by a Freundlich isotherm:

$$C_s = K_f * C_w^n \quad (1)$$

where C_s is the measured cadmium concentration in soil (mg Cd kg^{-1} dry soil), K_f the Freundlich sorption constant (L kg^{-1}), C_w the measured cadmium concentration in the pore water (mg Cd L^{-1}), and n the shape parameter of the Freundlich isotherm. Estimates of K_f and n were obtained by linear regression in a double logarithmic plot of C_s versus C_w . A value of $n > 1$ is indicative of saturation effects in the solution, while when $n < 1$ binding sites in the soil may become saturated at high solution cadmium concentrations.

Cadmium speciation was calculated for all treatments with cadmium concentrations measured in the pore water and calculated pH_{pw} values (see below) using the Visual MINTEQ program (<http://www.lwr.kth.se/English/OurSoftware/vminteq>; Gustafsson 2007). Cadmium speciation for the pore water samples was calculated for the beginning of the test and 3 and 30 weeks after the start of the test. Since, for practical reasons, it was not possible to measure soil pH at all sampling times, no cadmium speciation calculations could be made for the other sampling times.

To calculate partition coefficients based on the soil ligand model (Rachou and Sauvé 2008), the method of Li et al. (2009) was applied. In brief, the model assumes competition of cadmium with other cations for binding to soil particles:



At equilibrium:

$$K_{\text{M-SL}} = [\text{M-SL}] / (\{\text{M}^{n+}\} \times [\text{SL}^-]) \quad (3)$$

where $\{\text{M}^{n+}\}$ is the activity of cadmium/cations in the solution (mol L^{-1}), $[\text{SL}^-]$ the concentration of binding sites on the soil surface (mol kg^{-1} dry soil), $[\text{M-SL}]$ the concentration of cadmium/cations on the soil surface (mol kg^{-1} dry soil). $K_{\text{M-SL}}$ is the binding constant, which is a measure of the affinity of

cadmium/cations to the binding sites on the soil ($L\ mol^{-1}$). In case of dissociation of cadmium/cations from the surface of soil particles to the pore water, the dissociation constant (K'_{M-SL} , $mol\ L^{-1}$) can be calculated as

$$K'_{M-SL} = (\{M^{n+}\} \times [SL^{-}]) / [M-SL] \tag{4}$$

The total concentration of binding sites on the soil surface is given by

$$S_{Tot} = [SL^{-}] + [Cd-SL] + [Ca-SL] + [H-SL] \tag{5}$$

where S_{Tot} is the total cation binding density (concentration) on the soil surface ($mol\ kg^{-1}$ dry soil) and $[Cd-SL]$, $[Ca-SL]$ and $[H-SL]$ the concentrations of cadmium, calcium, and protons bound to the active binding sites on the surface of soil particles ($mol\ kg^{-1}$ dry soil). In the present study, the effects of Ca^{2+} and protons, as two major cations affecting metal availability, were taken into account in all equations. Considering Eq. (4) and (5) and further rearrangements results in the following equation:

$$[Cd-SL] / \{Cd^{2+}\} = S_{Tot} / (K'_{Cd-SL} + \{Cd^{2+}\} + K_{Ca-SL} \times \{Ca^{2+}\} \times K'_{Cd-SL} + K_{H-SL} \times \{H^{+}\} \times K'_{Cd-SL}) \tag{6}$$

The ratio between the total cadmium concentration in soil ($mg\ kg^{-1}$) and cadmium activity in pore water ($mg\ L^{-1}$) is the soil–water partition coefficient (k_p , $L\ kg^{-1}$). The total cadmium in soil is assumed to be equal $[Cd-SL]$. Equation (6) can be rewritten as follows:

$$1 / k_p = \{Cd^{2+}\} / [Cd-SL] = (K'_{Cd-SL} / S_{Tot}) + ((K_{Ca-SL} \times K'_{Cd-SL} / S_{Tot}) \times \{Ca^{2+}\}) + ((K_{H-SL} \times K'_{Cd-SL} / S_{Tot}) \times \{H^{+}\}) \tag{7}$$

Using Eq. (7) and multiple linear regression, considering measured cadmium partitioning coefficients and soil and pore water characteristics, the binding constants indicating the affinity for binding of the major cations (Ca^{2+} and H^{+}) to the active sites on the soil surface could be obtained. Using this method in the present study, it was not possible to calculate conditional binding constant for cadmium. All analyses were run in SPSS package 21.0 for Windows (SPSS Inc., Chicago, Illinois, USA).

Results

Soil properties

Supporting Information, Tables S1 and S2 show the physical–chemical properties of the test soils measured at beginning = 0 and T = 3 weeks after the start of the experiment. Soil pH decreased with time, especially at the two lower pH levels. At the highest pH levels, however, changes were minor (Table S1). The pH_{H_2O} values ranged between 5.53 and 6.83 at test = 0 and between 5.05 and 6.60 at T = 3 weeks for the different cadmium/pH treatments. The pH_{CaCl_2} values were between 5.21 and 6.33 for T = 0 and between 4.51 and 6.41 three weeks after the start of the test (Table S1). At a certain time point, the measured pH_{CaCl_2} values (at pH 4.5 and 5.5) were quite similar to each other. pH_{H_2O} was always higher than pH_{CaCl_2} . In few cases, especially at pH 4.5, the pH values were slightly higher at higher compared to lower exposure concentrations ($p > 0.05$).

pH_{pw} values were calculated using a regression equation described by De Vries et al. (2005) ($pH_{pw} = 1.046\ pH_{H_2O} - 0.285$). The estimated pH_{pw} values ranged between 5.50 and 6.86 for T = 0, between 5.00 and 6.62 after 3 weeks, and between 4.81 and 6.50 at the end of the test (Table S1). The pH_{pw} decreased with time and the differences between pH levels were clearer at T = 0 compared to T = 3 weeks. The estimated pH_{pw} values were used in metal speciation calculations.

The CEC values ranged between 5.09 and 7.28 $cmol\ kg^{-1}$ for T = 0 and between 5.08 and 7.37 $cmol\ kg^{-1}$ three weeks after the start of the test (T = 3) and were highest at pH 6.5 (Table S2). Calcium concentrations in the pore water ranged between 281 and 483 $mg\ L^{-1}$ for T = 0 and between 277 and 478 $mg\ L^{-1}$ after three weeks and were lowest at pH 5.5 and highest at pH 4.5. DOC concentrations in the pore water ranged between 95.2 and 168 $mg\ L^{-1}$ at the beginning of the experiment and between 42.8 and 129 $mg\ L^{-1}$ 3 weeks after the start of the test (Table S2).

Total concentrations of cadmium in soil

Total cadmium concentrations measured in the soil-amended pH-adjusted soils at the start and after 3 weeks of incubation are presented in the Supporting Information, Table S3. Cadmium recovery for all test soils was >80 %. Measured soil concentrations ranged from 4.01 to 4.59 $mg\ Cd\ kg^{-1}$ dry soil for the lower cadmium treatment and from 16.1 to 18.7 $mg\ Cd\ kg^{-1}$ dry soil for the higher cadmium treatment, and they did not differ for the two sampling times. All results are expressed on the basis of measured cadmium concentrations.

Cadmium concentrations in soil extracts

Extractable cadmium concentrations increased with increasing soil concentrations and were always higher in 0.01 M CaCl₂ than in water extracts (Supporting Information, Table S4). Water extractable concentrations ranged between 2.07 and 20 µg Cd L⁻¹ for the lower and between 10.5 and 55.4 µg Cd L⁻¹ for the higher cadmium treatment. The CaCl₂ extractable cadmium concentrations varied between 10.8 and 179 µg Cd L⁻¹ for the lower and between 49.8 and 652 µg Cd L⁻¹ for the higher exposure level. The highest extractable cadmium concentrations were observed at pH 4.5, the lowest at pH 6.5. Except some cases, especially at pH 5.5, extractable cadmium concentrations decreased with time (Table S4).

Cadmium concentrations in soil pore water

In the pore water, cadmium concentrations increased with increasing exposure concentrations and also increased with decreasing pH at all sampling time points (Supporting Information, Table S5; Fig. 1). Pore water concentrations increased with time and ranged between 7.41 and 939 µg Cd L⁻¹ for the lower and between 43.7 and 4288 µg Cd L⁻¹ for the higher cadmium treatment (Table S5). The only exception was the lower cadmium concentration for T = 3 weeks at pH 6.5 compared to T = 0. The highest pore water concentrations were always observed at the end of the test (T = 30 weeks).

Cadmium sorption

For all pH levels, cadmium concentrations in the pore water increased with increasing total soil concentrations (Supporting Information, Figure S1). This is reflected by the Freundlich isotherms for which the R² values were above 0.97 for all pH levels and time points. It should be mentioned that these R² values should be used with caution because they are only based on three data points. Table 1 shows the Freundlich sorption constants k_f and the shape parameter n for all test soils. Except for pH 6.5, sorption constants increased with time, and they increased with increasing pH levels. The k_f values ranged between 4.26 and 934 L kg⁻¹ for different pH levels (with corresponding n values of 1.04 and 1.36). The n parameter was lower than 1 for time points up to T = 21 weeks, except for T = 3 weeks at pH 4.5, and were higher than 1 for T = 24 and T = 30 weeks.

Cadmium speciation and free Cd²⁺ ion concentrations

The free cadmium ion concentrations in the pore water calculated with Visual Minteq ranged between 0.004 and 1.12 µmol L⁻¹ at T = 0, between 0.008 and 2.07 µmol L⁻¹

at T = 3 weeks, and between 0.01 and 5.34 µmol L⁻¹ at T = 30 weeks for the lower cadmium treatments at different pH levels (Supporting Information, Table S6). For the higher cadmium treatments, the values ranged between 0.02 and 5.23 µmol L⁻¹ at T = 0, between 0.05 and 9.33 µmol L⁻¹ at T = 3 weeks, and between 0.06 and 26.5 µmol L⁻¹ at T = 30 weeks (Table S6). The Cd²⁺ ion concentrations increased with time for all cadmium treatments and decreased with increasing pH. The free cadmium ion concentrations varied between 1.12 and 26.5 µmol L⁻¹ at pH 4.5, between 0.04 and 4.66 µmol L⁻¹ at pH 5.5, and between 0.004 and 0.06 µmol L⁻¹ at pH 6.5 (Table S6).

Cadmium speciation in the pore water showed that the percentage of free cadmium ions decreased to 5–16 % with increasing pH. At the same time, the percentage of cadmium bound to DOC (usually fulvic acid) increased up to 94 %. Visual Minteq calculations also suggested that the concentration of free cadmium ions slightly increased with time from 50 % at T = 0 to 70 % at T = 30 weeks. In this case, the contribution of Cd-DOC complexation decreased to 20–30 %.

Modeling cadmium sorption by a semi-mechanistic approach

The soil–water partition coefficients (k_p, L kg⁻¹), calculated using Eq. (7), by dividing the total cadmium concentration in soil (mg kg⁻¹) with the cadmium concentration in pore water (mg L⁻¹), ranged between 4.21 and 612 L kg⁻¹ for all time points. The k_p values decreased with time and increased with increasing pH levels. Approx. 49 % of the variability in the experimental k_p data could be explained by Ca²⁺ and H⁺ that were included in Eq. (7):

$$1/k_p = 9 \times 10^{-5} + 0.037 \times \{Ca^{2+}\} + 10.036 \times \{H^+\} (R^2 = 0.49, F = 7.259, P = 0.006) \quad (8)$$

After removing two data points from the pH 4.5 soil at T = 30 weeks, approx. 67 % of the variability could be explained by including Ca²⁺ and H⁺ in Eq. (7).

Using Eq. (8), the k_p was calculated from the experimental results used for the model to test the model (auto-validation). Figure 2 shows the relationship between the predicted and measured k_p values. A relatively strong correlation was found between these two parameters with R² of 0.58 (P < 0.001). The affinity constants (log K values) for the binding of Ca²⁺ and protons (H⁺) to the binding sites on the soil surface were calculated from Eqs. (7) and (8) using all data (those that are supported with measured pH values). The resulting values were 2.61 L mol⁻¹ for log K_{Ca-SL} and 5.05 L mol⁻¹ for log K_{H-SL}.

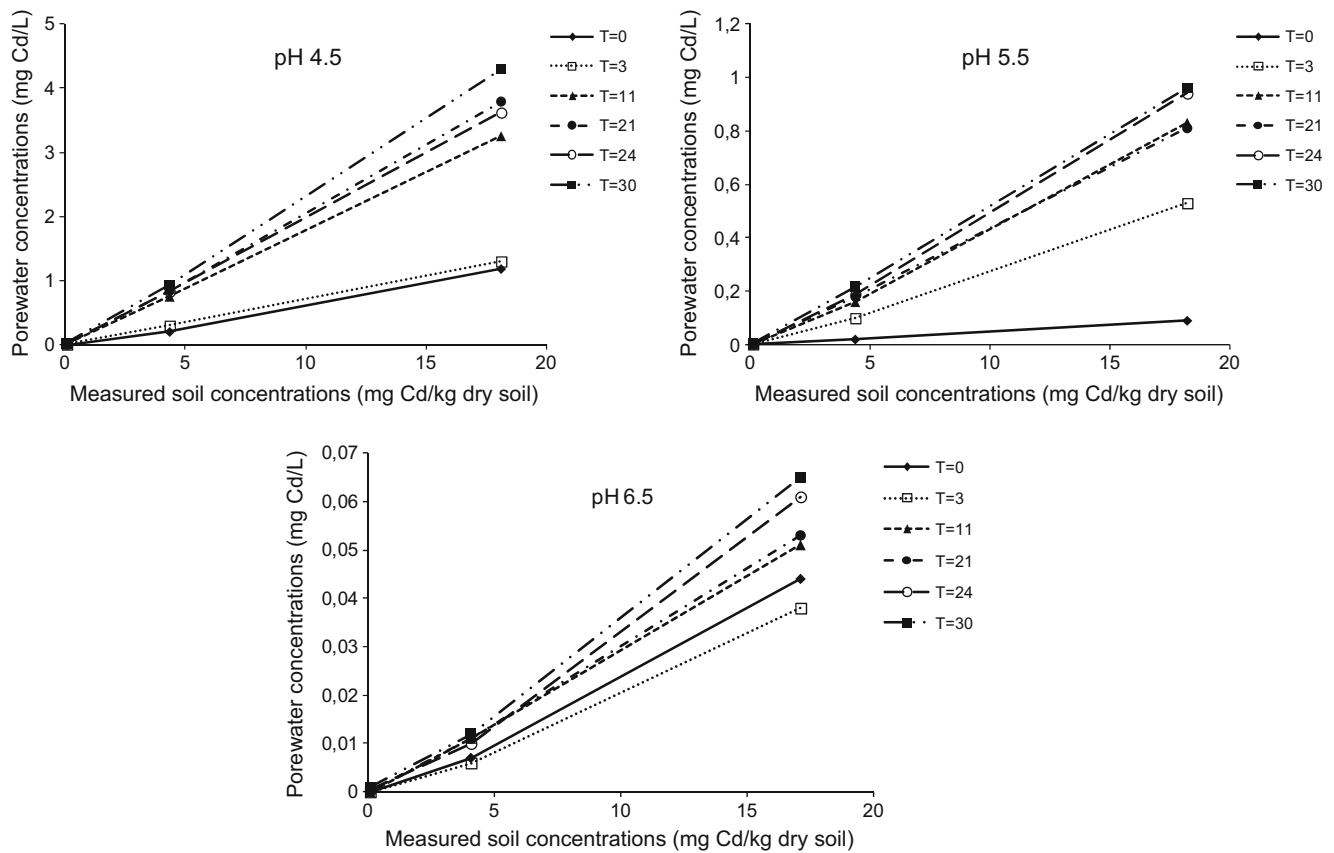


Fig. 1 The relationship between cadmium concentrations measured in the pore water (mg Cd L^{-1}) and total cadmium concentrations in LUFA 2.2 soil (mg Cd kg^{-1} dry soil) at the beginning ($T=0$) and 3 ($T=3$), 11 ($T=11$), 21 ($T=21$), 24 ($T=24$), and 30 ($T=30$) weeks after the start in

soil at three tested pH levels. Soils were spiked with cadmium nitrate at nominal concentrations of 5 and 20 mg Cd kg^{-1} dry soil and analysis was started after 2 weeks equilibration of the spiked soils

Table 1 Freundlich sorption constants ($k_f, \text{L kg}^{-1}$) for cadmium partitioning in LUFA 2.2 soil (Speyer, Germany) at the beginning ($T=0$) and 3 ($T=3$), 11 ($T=11$), 21 ($T=21$), 24 ($T=24$), and 30 ($T=30$) weeks after the start of the test. Soils were spiked with cadmium nitrate at nominal concentrations of 5 and 20 mg Cd kg^{-1} dry soil, and soil pH was adjusted to 4.5, 5.5, or 6.5 2 weeks before the test was started. Measured soil concentrations were used for $T=0$ and $T=3$ and average soil concentrations (mean of $T=0$ and $T=3$) were used for other sampling times

pH ^a	T=0	T=3	T=11	T=21	T=24	T=30
4.5	$k_f=15.9$ $n=0.93$	$k_f=13.1$ $n=1.04$	$k_f=5.73$ $n=0.98$	$k_f=5.05$ $n=0.96$	$k_f=5.03$ $n=1.02$	$k_f=4.26$ $n=1.04$
5.5	$k_f=176$ $n=0.94$	$k_f=30.2$ $n=0.86$	$k_f=21.2$ $n=0.86$	$k_f=21.8$ $n=0.92$	$k_f=19.8$ $n=0.94$	$k_f=19.5$ $n=1.02$
6.5	$k_f=246$ $n=0.83$	$k_f=215$ $n=0.79$	$k_f=290$ $n=0.95$	$k_f=222$ $n=0.89$	$k_f=417$ $n=1.08$	$k_f=934$ $n=1.36$

^a Nominal pH levels
 n shape parameter for cadmium sorption

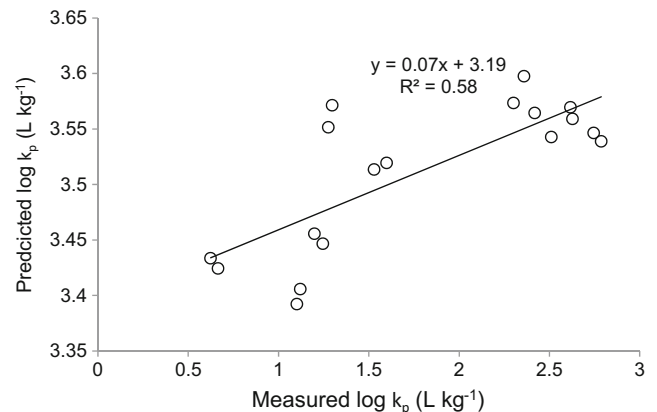


Fig. 2 The relationship between the predicted and measured $\log k_p$ (soil-water partition coefficient, L kg^{-1}) values for the sorption of cadmium to spiked LUFA 2.2 soil amended at three pH levels. The soil pH levels were adjusted to 4.5, 5.5, and 6.5 and test soils were equilibrated up to 30 weeks ($T=30$). The data used to draw this figure are from the beginning of the experiment ($T=0$), and from 3 ($T=3$) and 30 weeks ($T=30$) after the start of the test. Measurements started 2 weeks after spiking the soils with $\text{Cd}(\text{NO}_3)_2$ at two concentrations and adjusting soil pH

Discussion

Cadmium availability and soil properties

One of the important factors affecting cadmium partition is soil pH. The results of this study showed that measured pH values decreased with time for all cadmium treatments (Table S1). The reason for this decrease is, however, not completely known, but increase in the soil microbial activity might be one of the possible factors affecting pH levels. pH changes in soils are both biotic and abiotic processes in the soil, consuming or releasing H^+ through redox reactions and fermentation, and/or from rainfall that leaches bases in soil and thus lowering the pH (Sylvia et al. 2005). The majority of soil microbes thrive at neutral pH (6–7) due to the high availability of most nutrients in this pH range, but there are examples of microbes (especially fungi) that can tolerate much wider pH ranges from 1 to 13 (Sylvia et al. 2005; Rousk et al. 2009). Alterations in pH can render essential microbial enzymes inactive and/or denature proteins within the cells, limiting or preventing microbial activity (Sylvia et al. 2005). pH changes can also effect microbes in their access to metals and organics that react differently under varied pH regimes (Sylvia et al. 2005). Decreasing pH can also be related to the time-dependent interaction of cadmium with the soil, since cadmium concentrations in the extracts and pore water increased with time. The main reason for observing this time-coarse decrease in pH needs to be further investigated. The effect of soil properties must be studied to understand speciation, bioavailability, and toxicity of metals in soil. In the present study, the influence of soil pH on cadmium availability in LUFA 2.2 natural soil was investigated using an aging experiment. The results showed that soil pH affected cadmium availability with lower extractable concentrations measured at pH 6.5 (Table S4). Moreover, cadmium concentrations in the soil pore water decreased with increasing pH (Table S5). This reduction in cadmium availability is in line with other studies (Crommentuijn et al. 1997; Lock and Janssen 2001; Van Gestel and Mol 2003; Bur et al. 2010), indicating the important role of pH on the availability of metals.

In soils, the equilibrium processes of metals between pore water and solid phases are rather complex (Tipping et al. 2003). Although increasing soil pH generally leads to a decrease in metal solubility, it may increase dissolved metal concentrations due to the formation of metal complexes with DOC in the pore water. In the present study, a decreasing trend of DOC in the pore water with time was observed (Table S2). Also, Visual Minteq calculations showed that the concentration of complexes of cadmium with DOC (fulvic acids) in the pore water did not change with time at higher pH and the percentage of DOC-Cd complex formation also decreased at lower pH. Thus, the above assumption may not have been true

in this experiment, which may be attributed to the decrease in pH levels with time which also influences other parameters in soil. This needs to be further investigated in future experiments.

Cadmium sorption in natural LUFA 2.2 soils at different pH levels

The results of the present study showed that pH considerably influenced cadmium sorption. Measured cadmium concentrations in the pore water decreased with increasing pH (Table S5), indicating higher sorption of cadmium to the binding sites at the soil surface at pH 6.5. This resulted in higher k_f values at higher pH levels (Table 1). When considering soil organic matter as a major soil component controlling cadmium sorption, most cadmium may be bound to the adsorption sites on this soil fraction in the soil solid phase at higher pH. However, other binding sites of the soil, such as clay, oxides, hydroxides, and carbonates, to which cadmium may be bound, should also be considered. CEC is a measure of the amount of available sorption sites on soil clay, Al and Fe oxyhydroxides, and organic matter (Janssen et al. 1997). In the present study, CEC was higher at higher pH levels (Table S2). Among the pore water characteristics influencing metal sorption, complex-forming components are important factors of which DOC is known for its high degree of complexation with free metal ions, thus keeping them in the soil solution. The higher measured DOC level at higher soil pH (Table S2) may have caused higher complexation of cadmium with DOC, thereby reducing its (bio)availability at pH 6.5. On the other hand, higher Ca levels at lower pH (Table S2) may have resulted in higher competition of Ca^{2+} ions with Cd^{2+} ions for the sorption sites, leading to replacement of Cd^{2+} with Ca^{2+} at binding sites on the soil surface and, as a consequence, higher cadmium concentrations in the pore water at pH 4.5. At low pH, the excess H^+ ions forces Ca^{2+} into solution while at high pH, the excess Ca^{2+} is bound to the soil because of the availability of binding sites earlier occupied by the H^+ ions that are taken away by the CO_3^- used to adjust soil pH. The higher Cd^{2+} concentration in the pore water especially is the result of the higher H^+ concentration, which forces both Cd^{2+} and Ca^{2+} into the solution.

Measured cadmium concentrations in the soil pore water increased with time (up to approximately half a year). This was contrary to previous studies, where it was shown that for cationic metals, the concentration of added dissolved metals in the soil solution generally decreased with increasing contact time with the soil, due to stronger binding or fixation (e.g., Ma et al. 2006). This led to a reduced metal mobility and bioavailability in soil. Our results did not support these findings. Speciation calculations also showed that more Cd^{2+} ions

remained in the pore water which might indicate the loose binding of cadmium with DOC upon long-term equilibration. The few studies present in the literature on the role of aging on metal toxicity and at different test conditions show a higher toxicity of metals in laboratory-amended compared to field-contaminated soils (e.g., Smit and van Gestel 1996, 1998; Scott-Fordsmand et al. 2000; Smolders et al. 2009). Moreover, it may be concluded that toxicity decreases with time due to lowering metal availability (e.g., metal bound to the soil particles). This may confirm that total soil concentration does not explain toxicity. In this case, the effects of soil properties, of which pH is an important one, should be taken into account and their effects may change the results of the tests. Smit et al. (1997) and Smit and van Gestel (1998) compared zinc toxicity immediately after spiking and after aging for up to 26 months under field or laboratory conditions. Zinc toxicity decreased considerably after aging; however, as a parallel increase in pH of at least one unit was observed, it remained unclear what the relative contribution to the zinc toxicity was of this pH change and of aging. Therefore, generalizing the results of one study to a larger scale for a complete risk assessment program of a specific area should be considered with caution (Smolders et al. 2009). Smolders et al. (2009) concluded that the effects of leaching/aging and soil type should be taken into account when comparing toxicity tests using different experimental conditions. The latter authors applied a leaching/aging factor to correct the results and make them more comparable with standard tests. This also strengthens the argument that metal toxicity and availability assessed in soils spiked with soluble metal salts without a post-spiking leaching and aging treatment may be overestimated (Smolders et al. 2004). In most ecotoxicity tests, freshly metal-spiked soils are used. In this type of experiments, the soils have not been aged long enough to reach equilibrium. This makes it difficult to interpret the data within the framework of the risk assessment of contaminated soils. In addition, leaching is another important factor which should be taken into account in toxicity tests. Leaching will decrease the availability of metal ions not only by washing out and increasing the mobility of free metal ions from the soil but also by removing the counter ions (such as nitrate or chloride). It has been shown that the soil solutions of amended soils that have not been leached have elevated ionic strength and markedly decreased pH compared with field-contaminated soils (Smolders et al. 2009). This is due to the specific sorption of metal cations, thereby releasing protons and other cations, such as Al^{3+} or Ca^{2+} , to the soil solution.

Experimental aging of metal-salt-amended soils with free drainage (i.e., including natural leaching) decreased the concentrations of metals in soil solution up to one order of magnitude and generally decreased the fraction of added metal that was isotopically exchangeable. The processes behind this aging were likely to be related to fixation of metals in Fe or Mn

oxyhydroxides or the gradual formation of precipitates (Smolders et al. 2009). The results of the present study should be further elaborated with both chemical and biological measurements considering a combination of leaching–aging effect on cadmium sorption.

The soil ligand model approach for cadmium sorption

Our developed SLM model showed that the soil–water partition coefficient (k_p) values could successfully be predicted and showed relatively strong correlation with the measured values when considering the influence of major cations (Ca^{2+} and H^+) on cadmium sorption (Fig. 2). These results are in agreement with previous research on metal sorption and toxicity (Sauvé et al. 2000b; Impellitteri et al. 2003; Thakali et al. 2006; Li et al. 2008; Li et al. 2009). In these studies, a competing effect of major cations on the activity of the free metal ions for binding to the ligands present in the soil solution (soil organism/soil solid phase) has been reported. Stockdale et al. (2010) and Tipping and Lofts (2013) were able to model metal levels in field-collected aquatic invertebrates using speciation models. They studied the influence of major ions at biological uptake sites, considering organisms as humic acids to assess the relationships between metal exposure, chemical speciation, and internal metal concentrations. In addition, higher conditional binding constants were found for protons compared to other major cations (Ardestani et al. 2014), indicating their importance in competing with the free metal ions for binding to the surface of the soil solid phase.

The conditional binding constants ($\log K$ values) calculated in the present study for calcium ($\log K_{\text{Ca-SL}} = 2.61$) and protons ($\log K_{\text{H-SL}} = 5.05$) to bind to the binding sites at the soil surface are in agreement with the values reported in the literature. Li et al. (2009) estimated $\log K_{\text{Ca-SL}}$ and $\log K_{\text{H-SL}}$ of 1.47 and 5.59 L mol^{-1} , respectively, when investigating cadmium partitioning in nine test soils collected from different sites in China. Rachou and Sauvé (2008) reported $\log K_{\text{Ca-SL}}$ and $\log K_{\text{H-SL}}$ values of -0.84 and 4.1 L mol^{-1} , respectively. They could also estimate $\log K_{\text{Cd-SL}}$ of 4.4 L mol^{-1} for the binding of cadmium to the surface of the soil solid phase. The similarity of the conditional binding constants calculated based on our semi-mechanistic SLM compared to the few reported values for binding of major cations to the binding sites of soil shows that our SLM could successfully be applied to describe cadmium sorption in natural soils.

Conclusions

The effect of pH and time on cadmium availability in natural LUFA 2.2 soil was investigated in the present study by performing an aging test. The results showed that cadmium concentrations in the pore water increased with time for all soil

pH levels, while these concentrations decreased with pH. This was attributed to the strong effect of soil pH on pore water concentrations and the finding that pH varied over time. Moreover, the Freundlich k_f values were highest for pH 6.5. These findings indicate the importance of soil properties as well as time in modulating the sorption of added cadmium. pH and time showed to be two important factors influencing cadmium availability in long-term tests; however, the interaction of these factors determines cadmium sorption and the effect of each factor cannot be singled out. Calcium and protons showed to be two major cations affecting cadmium availability and should be taken into account when investigating cadmium sorption in long-term tests. The calculated affinity constants of Ca^{2+} and H^+ were similar to the previously reported values in the literature for their binding to the active sites on the surfaces of the soil solid phase based on the soil ligand modeling approach. This showed the applicability of our model to predict cadmium availability in natural soils, which was in line with the concept of the biotic ligand model. Further work is needed to assess the importance of long-term aging in terms of bioavailability and toxicity and identification of aging under experimental conditions which gives more reliable results for inclusion in ecological risk assessment programs.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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