Sorption of cadmium(II) and copper(II) by soil humic acids: temperature effects and sorption heterogeneity

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Sorption of cadmium(II) and copper(II) by soil humic acids: temperature effects and sorption heterogeneity

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Sorption by humic acids is known to modify the bioavailability and toxicity of metals in soils and aquatic systems. The sorption of cadmium(II) and copper(II) to two soil humic acids was measured at pH 6.0 using ion-selective electrode potentiometric titration at different temperatures. Sorption reactions were studied with all components in aqueous solution, or with the humates in suspension. Adsorption reactions were described using a multiple site-binding model, and a model assuming a continuous log-normal distribution of adsorption constants. Adsorption of Cu^{2+} was more favourable than adsorption of Cd^{2+}. The log-normal distribution model provided the closest fit to observations and allowed parameterisation of adsorption data using a mean adsorption constant (log K_μ). Sorption of Cd^{2+} to dissolved humic acids increased slightly in extent and sorption affinity with increasing temperature, but the effect was small (log K_μ 2.96–3.15). A slightly greater temperature effect occurred for sorption of Cd^{2+} to solid-phase humic acids (log K_μ 1.30–2.08). Sorption of copper(II) to both aqueous- and colloidal-phase humates showed more pronounced temperature dependence, with extent of sorption, and sorption affinity, increasing with increasing temperature (log K_μ 3.4–4.9 in solution and 1.4–4.5 in suspension). The weaker adsorption of Cd^{2+} than Cu^{2+}, and smaller temperature effects for dissolved humates than suspended humates, suggested that the observed temperature effects had a kinetic, rather than thermodynamic, origin. For any metal-to-ligand ratio, free metal ion concentration, and by inference metal bioavailability, decreased with increasing temperature. The consistency of the data with kinetic rather than thermodynamic control of metal bioavailability suggests that equilibrium modelling approaches to estimating bioavailability may be insufficient.

Keywords: metal sorption; bioavailability; kinetics; adsorption models

1. Introduction

The complexation reactions between humic substances and polyvalent metal ions are important determinants of metal speciation in soils and aquatic ecosystems [1,2]. An understanding of these reactions is important in many contexts, for example: trace element geochemistry and mobility [3], or micronutrient nutrition of, or toxicity to, plants and aquatic fauna. Complexation with humic acid has been shown to decrease the uptake of cadmium by terrestrial plants in solution culture [4]. In soils, Cd uptake was shown to be reduced by binding of Cd to organic matter [5], and Cd uptake was correlated with fulvic-bound Cd but not humic-bound Cd in soil [6]. An analogous

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effect to that in soils was found in an aquatic system when humic acid, but not fulvic acid, reduced Cd and Zn toxicity to a freshwater unicellular alga [7]. In other aquatic and marine ecosystems, complexation by humic substances can reduce the toxicity of Cd [8] or Cu [9,10] to invertebrate fauna, although complexes of Cd with humic acid may be bioavailable to some (mollusc) species [9,11]. In addition, the response of humic substance–metal ion complexation reactions to changes in temperature is potentially important with respect to seasonal temperature changes in soils and aquatic systems, complexation of metal ions in hydrothermal systems and in the context of global climate change.

The trend in studies of the interactions between humic substance and metal ions has been to provide an increasingly quantitative thermodynamic description of these reactions. Early studies [12] used a range of analytical methods to verify the existence of soil organic matter–metal ion complexes. Later work [13–15] applied chemical thermodynamics, with varying degrees of rigour, to define stability constants for metal ion–humic substance complexation reactions. Currently, a wide range of models to describe these reactions exists, both semi-mechanistic [16,17] and stochastic [18,19].

Research which has examined the effects of temperature on sorption of metal ions to soils or soil components in any detail has generally focused on either whole soils [20] or inorganic soil components [21,22], although some studies present data on the effect of temperature on sorption of metal ions to soil organic matter. For example, one study observed an increase in the apparent sorption of Cd^{2+} by solid-phase humic acid as temperature increased, with reaction times up to 14 days [23]. By contrast, decreasing complexation of Ni^{2+} by dissolved humic acid as temperature increased has been observed (reaction time unspecified) [24]. In a more recent study [25], similar behaviour was observed for Ni^{2+}; with increasing temperature, Ni^{2+} complexation by dissolved humic acid decreased at pH 4, but complexation of Cu^{2+} increased. Finally, adsorption of Pb^{2+} by solid-phase humic acid was found to increase as temperature increased [26]. In some cases, the effects of temperature were interpreted in terms of reaction enthalpies, assuming that the sorption reactions had reached equilibrium [25,26]. Clearly, calculation of reaction enthalpies depends on the assumption of equilibrium, and also on the model (and therefore the equilibrium constant) chosen to represent metal–humic complexation reactions.

Chemisorption reactions involving naturally occurring solid phases can be very slow, with a proportion of the reaction continuing for periods longer than commonly allowed in studies that measure 'equilibrium' adsorption. Slow continuing sorption is more pronounced for strongly adsorbed ions than for more weakly adsorbed ions [27]. Many such studies, therefore, have not separated the effects of thermodynamics and kinetics on complexation reactions, and the causes of the observed temperature effects are not known unambiguously. Apparent increases in adsorption/complexation as temperature increases can be explained by higher temperature increasing reaction rates, even if equilibrium is not attained. Temperature effects may also reflect changes in aqueous speciation for the adsorbing ion(s) and changes in surface potential or surface activity [28].

Any observed temperature effects were expected to reflect differences in both sorption kinetics, and true thermodynamic differences. Cadmium ions adsorb relatively weakly to humic substances [29], possibly by outer-sphere mechanisms, and kinetic effects were therefore expected to be minimal. By contrast, copper ions form very strong complexes with humic substances [14], allowing the possibility of large kinetic effects [27]. Similarly, dissociation of aqueous phase copper–humate complexes has been shown to be retarded by conditions favouring a more condensed conformation of dissolved humic acids [30]. Any kinetic effects were thus expected to be more pronounced for metal ion sorption on solid-phase humic substances. We are then left with two questions: first, what is the effect of temperature on sorption of Cd^{2+} and Cu^{2+} by humic substances, especially since sorption affects the bioavailability of both elements? Second, what causes the temperature effects observed, since these may reflect either thermodynamic or kinetic effects,

Table 1. Analytical data for summit soil humic acid and Waimari peat humic acid [30].

Humic acid	Carbon (%)	Hydrogen (%)	Nitrogen (%)	Ash (%)
Summit	48.9	5.0	4.6	1.16
Waimari	54.2	3.6	3.1	1.30

or some combination of both? The aim of the study described here was therefore to examine the effect of temperature on cadmium (II) and copper (II) ion sorption to soil humic acids, both with all components in aqueous solution, or with suspended colloidal-phase humic acids.

2. Materials and methods

Humic acids were isolated from Summit Soil, Banks Peninsula, New Zealand and Waimari Peat, Canterbury, New Zealand using the methods adopted by the International Humic Substance Society (IHSS) for soil humic acids [31]. Summit Soil humic acid (SHA) is an IHSS reference humic substance. C, H, N and ash contents for these materials have been published previously (Table 1) [30]. Analytical grade reagents were used throughout; all water used was purified to a resistivity of 18.3 MΩ cm. All glassware and plastic ware was acid-washed (10% HNO_3) and rinsed thoroughly with purified water before use.

Isolated humic acids were converted to sodium humates by suspending humic acids in a minimum volume of water and adding NaOH solution until a steady pH of 7.0 was obtained. This solution was centrifuged to remove residual solids, and the sodium humate was obtained by freeze-drying. Calcium humates were prepared by adding $CaCl_2$ to a similarly prepared sodium humate solution at pH 7.0, so that the final concentration of Ca^{2+} was ~1 mol · L^{-1}. The pH of the resulting suspension was re-adjusted to 7.0 with NaOH; the solid calcium humate obtained was isolated by centrifugation. Soluble material was minimised in this calcium humate by alternately re-suspending in $CaCl_2$ solution and centrifuging (~20 cycles); excess salts were removed by suspension in deionised water and discarding the supernatant after centrifugation. The purified calcium humate was obtained in solid form by freeze drying the final suspension.

Sorption of Cd^{2+} or Cu^{2+} to sodium humates at constant temperature (20 or 40 °C), pH 6.00 and ionic strength (0.1 mol · L^{-1} $NaNO_3$) was measured by titrating solutions of sodium humate with metal ion, and determining free metal ion in solution using an ion-selective electrode (ISE) (Cd^{2+} – ISE, Orion 9448; Cu^{2+} – ISE, Orion 9429; double-junction reference, Orion 9002) and digital potentiometer (Orion 701A). Temperature was maintained at the desired value using a thermostatically controlled water bath which fed a jacketed titration vessel. Oxygen-free N_2 was purged through the continuously stirred solution at all times during titrations. Solution pH was maintained by additions of standardised NaOH solution following additions of metal ion solution, using an Orion 91-02 combination glass–Ag/AgCl reference electrode and Radiometer PHM 84 meter. Hydrogen ion release in complexation reactions was determined by measuring the volume of NaOH solution added in the course of a titration. The initial concentration of humic acid was ~0.1 g · L^{-1}; approximately 20 additions of metal ion were made in the ranges 0–0.45 mmol Cd · g $(HA)^{-1}$ or 0–0.65 mmol Cu · g $(HA)^{-1}$. Duplicate titrations were performed at each temperature.

Sorption of Cd^{2+} or Cu^{2+} to calcium humates at constant temperature (5, 20 or 40 °C), pH 6.0 and ionic strength [0.05 mol · L^{-1} $Ca(NO_3)_2$] was measured by batch titration. Portions of dry calcium humate (0.050 g) were weighed into 50 mL centrifuge tubes; to these were added 20 mL $Ca(NO_3)_2$ solution (0.05 mol · L^{-1}) containing metal ion in the ranges 0–0.06 mmol Cd · g

$(HA)^{-1}$ or 0–0.10 mmol $Cu \cdot g\,(HA)^{-1}$, in duplicate. The pH in each tube was adjusted to pH 6.0 by adding small aliquots of saturated $Ca(OH)_2$ solution. The tubes were shaken in an incubator at the desired temperature for 48 h; free metal ion concentrations were determined in the supernatant by ISE potentiometry following phase separation by centrifugation and filtration through a 0.45 μm membrane.

2.1. *Numerical methods*

The amount of metal ion bound to humic acids was determined from the difference between the known total amount of metal ion added to the system and the unbound metal ion concentration measured by ISE potentiometry. Formation constants for complexes of Cu^{2+} and Cd^{2+} with NO_3^- and OH^- [32], and single-ion activity coefficients [33] were used to correct calibration concentrations of unbound Cu^{2+} and Cd^{2+} to uncomplexed ion concentrations.

The complexation reaction between metal ions and heterogeneous substrates such as humic acids can be expressed by:

$$\sum_{i=1}^{n} HA_i + nM \rightleftharpoons \sum_{i=1}^{n} MA_i + nH, \tag{1}$$
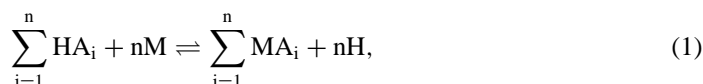
where n is the number of sorption sites; HA_i represents the ith sorption site; M represents the metal ion; and MA_i represents the ith metal–humate complex (charges omitted for clarity).

At constant pH, the equilibrium expressed in Equation (1) may be quantified by:

$$C_{MA} = C_L \sum_{i=1}^{n} \left(\frac{\frac{C_i}{C_L} K_i[M]}{1 + K_i[M]} \right), \tag{2}$$

where C_{MA} is the stoichiometric concentration of bound metal ion ($\equiv \sum_{i=1}^{n} [MA_i]$); C_L is the total ligand concentration ($\equiv \sum_{i=1}^{n} [MA_i] + \sum_{i=1}^{n} [HA_i] + \sum_{i=1}^{n} [A_i]$); C_i is the total concentration of the ith site (bound and unbound); K_i is a pH-dependent formation constant (implicitly including $[H^+]$) for the formation of complex MA_i; and $[M]$ is the concentration of unbound metal ion determined by ISE potentiometry.

Alternatively, a continuous log-normal distribution of humic acid sorption sites with respect to conditional metal–complex formation constant, K, can be assumed. The relevant equation for this assumption is [19]:

$$C_{MA} = C_L \frac{1}{\sigma \sqrt{2\pi}} \int_{-5\sigma}^{+5\sigma} \exp \frac{-1}{2} \left(\frac{\mu - \kappa}{\sigma} \right)^2 \frac{10^\kappa [M]}{1 + 10^\kappa [M]} d\kappa, \tag{3}$$

where μ is the mean of the distribution in $\log_{10} K$ with standard deviation σ; and κ ($\equiv \log_{10} K$) is the variable of integration.

Sorption data were parameterised according to Equation (2) (with $n = 1, 2$ or 3) and Equation (3) using a nonlinear optimisation program written in FORTRAN. This program used an adaptation of the multidimensional simplex method [34] to minimise an error sum-of-squares term with C_{MA} as the dependent variable. Iterative solution of the integral in Equation (3) was performed using the computationally efficient Romberg method [34].

3. Results and discussion

Isotherms for experiments at different temperatures are shown for sorption of Cd^{2+} by Summit sodium humate (Figure 1), Cd^{2+} by Summit calcium humate (Figure 2), Cu^{2+} by Summit sodium
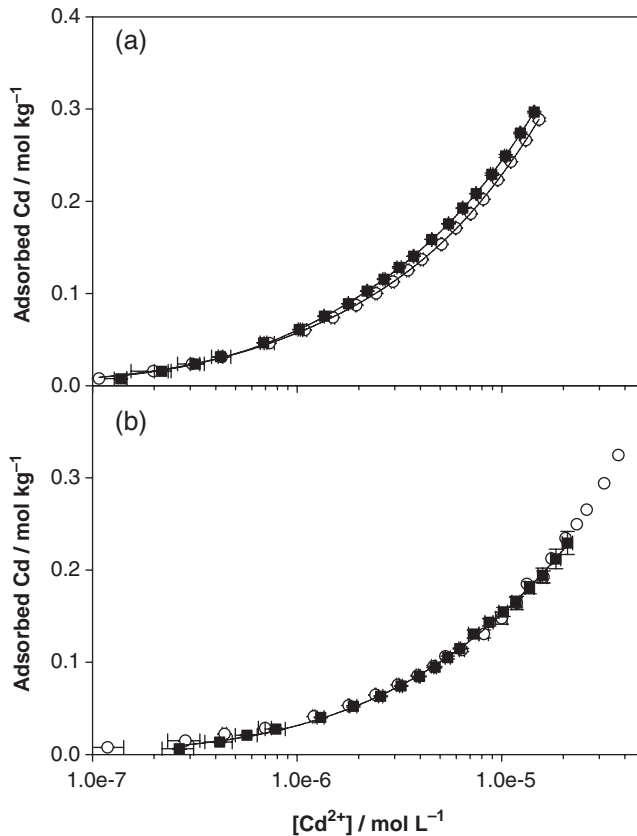
Figure 1. Isotherms for Cd^{2+} sorption by (a) Waimari peat and (b) Summit soil sodium humates at ($\bigcirc$) 20 °C and ($\blacksquare$) 40 °C. Error bars are standard deviations of duplicates.

humate (Figure 3) and Cu^{2+} by Summit calcium humate (Figure 4). A comparison of the data shows that Cu^{2+} adsorbs more strongly to dissolved or solid-phase humates than does Cd^{2+}, shown by substantially lower dissolved ion concentrations for equivalent amounts adsorbed under comparable reaction conditions. Stronger adsorption of Cu^{2+} than Cd^{2+} to humic substances has been observed frequently in previous research [29]. Bioavailability of Cu^{2+} and Cd^{2+} has been shown to be related to free metal ion concentration [5,35]. These results presented here therefore suggest that, in systems where humic substances control metal ion activity, bioavailability of Cu^{2+} and Cd^{2+} is dependent on temperature, with free metal ion concentrations decreasing as temperature increases.

The temperature dependence observed was more pronounced for sorption of Cu^{2+} than for Cd^{2+}, and there was a greater effect of temperature for sorption on solid-phase humate compared with dissolved humates. Important features of these data are the differences in agreement between and within replicate experiments, and the contrast observed in extent of temperature dependence, for the sorption reactions studied.

Experiments measuring cadmium sorption to both colloidal- and solid-phase humates (Figures 1 and 2) showed good agreement between replicates, and this is reflected in the closeness of values found for log K_μ and σ_K in the log-normal model (Table 2). The sorption isotherms for cadmium are also free of discontinuities, showing good internal consistency in data obtained from these titrations.
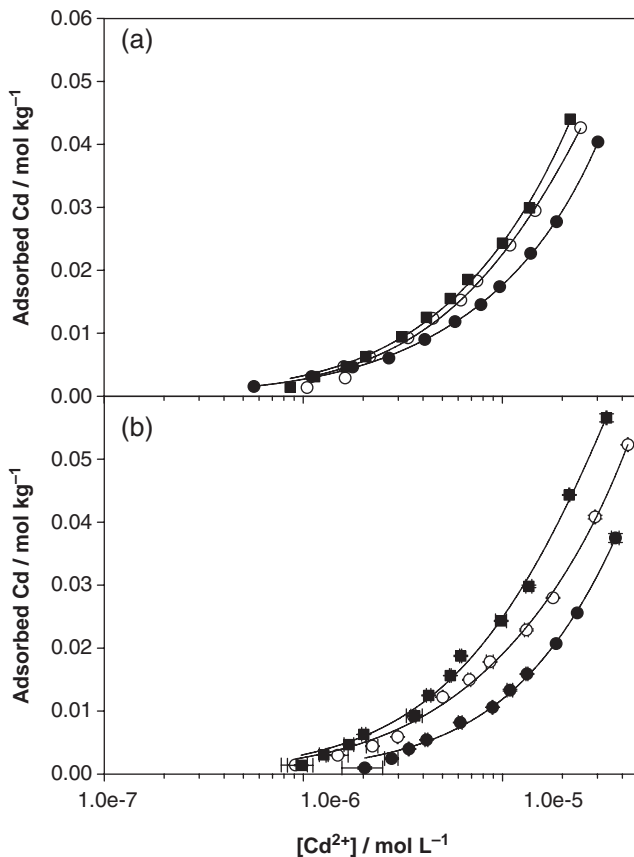
Figure 2.   Isotherms for Cd^{2+} sorption by (a) Waimari peat calcium humate and (b) Summit soil calcium humate at (●) 5 °C, (○) 20 °C and (■) 40 °C. Error bars are standard deviations of duplicates.

By contrast, the data obtained for copper sorption to colloidal- and solution-phase humates (Figures 3 and 4) exhibited poorer replication. Isotherms for sorption of Cu^{2+} by colloidal-phase calcium humates (Figure 4) also showed marked discontinuities within individual titrations.

Hydrogen ion release during titrations of aqueous-phase Na-humates was 0.6–0.8 mol H^+ per mol Cd^{2+} and 1.4 mol H^+ per mol Cu^{2+}. The incremental amount of H^+ released showed a slight decrease with increasing Cd^{2+} or Cu^{2+} addition over the course of titrations (data not shown). The amounts of hydrogen ion release suggest that these metal ions form either: (1) bidentate complexes either with already-deprotonated functional groups on humate ligands; or (2) a mixture of bidentate, monodentate and outer-sphere/electrostatic complexes. It is likely that the lower H^+ release during complexation of Cd^{2+} reflects scenario (2), and complexation of Cu^{2+} occurs by scenario (1).

A single-site adsorption model was not adequate to describe adsorption of Cd^{2+} or Cu^{2+} to humate ligands (Tables 3 and 4), although goodness-of-fit was better for sorption of Cd^{2+} than for Cu^{2+}. A two-site model was sufficient to describe adsorption of Cd^{2+}, with R^2 values ≥ 0.996 and residuals without significant autocorrelations (suggesting only random deviations of experimental data from fitted curves). A two-site model did not describe adsorption of Cu^{2+} as well as adsorption of Cd^{2+}; in some cases with very low R^2 values (Table 4), this was due to the high internal variability of the data (Figure 4). Where the data were of sufficient quality, a three-site model described adsorption of Cd^{2+} and Cu^{2+} well. In some sets of optimised parameters
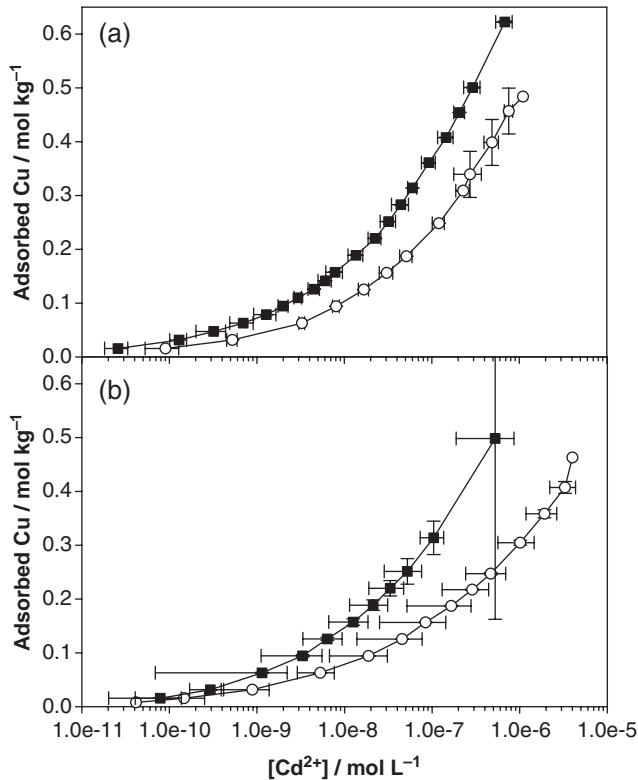
Figure 3. Isotherms for Cu^{2+} sorption by (a) Waimari peat and (b) Summit soil sodium humates at (○) 20 °C and (■) 40 °C. Error bars are standard deviations of duplicates; lines represent data fitted to Equation (2), $n = 3$.

for three-site models, however, there were either log K_3 values which were small enough that the site would not be involved in adsorption, or parameter values had high associated uncertainties, suggesting an over-parameterised model. Fit of adsorption data to the log-normal distribution model was very good, unless precluded by poor-quality data ($R^2 \geq 0.992$ for all Cd^{2+} adsorption and for adsorption of Cu^{2+} to Na-humates; Table 2).

It is evident from Figures 1 to 4 that the extent of temperature dependence of the metal–humate sorption reactions studied varies according to the sorption reaction. Two sensible comparisons of temperature dependence may be made: (1) between metal ion sorption to solution- or colloidal-phase humates; and (2) between sorption of Cd^{2+} and Cu^{2+} to humates.

Comparison of Figures 1 and 2 reveals that the effect of temperature was more marked for sorption of Cd^{2+} to colloidal-phase humates than for solution-phase; in solution, no significant difference was seen between sorption at 20 and 40 °C. A similar effect is apparent for sorption of Cu^{2+} (Figures 3 and 4). This effect is confirmed by the trends in mean formation constant (log K_μ) with temperature (Table 2). Dissociation of Cu^{2+} from complexes with dissolved humic acids has been shown to be slower if the pH and/or ionic strength of the solution had values which might be expected to result in a more condensed conformation for humic acid macromolecules [30]. A similar effect is likely to have occurred in these experiments. Diffusion of Cd^{2+} or Cu^{2+} towards sites on humic acids would be more restricted for solid-phase humic acids, with a more collapsed macromolecule, than for humic acids in solution. The need to overcome an activation energy for intraparticle diffusion conferred a relatively large temperature-dependence to adsorption of Cd^{2+} and Cu^{2+} on solid-phase humates.
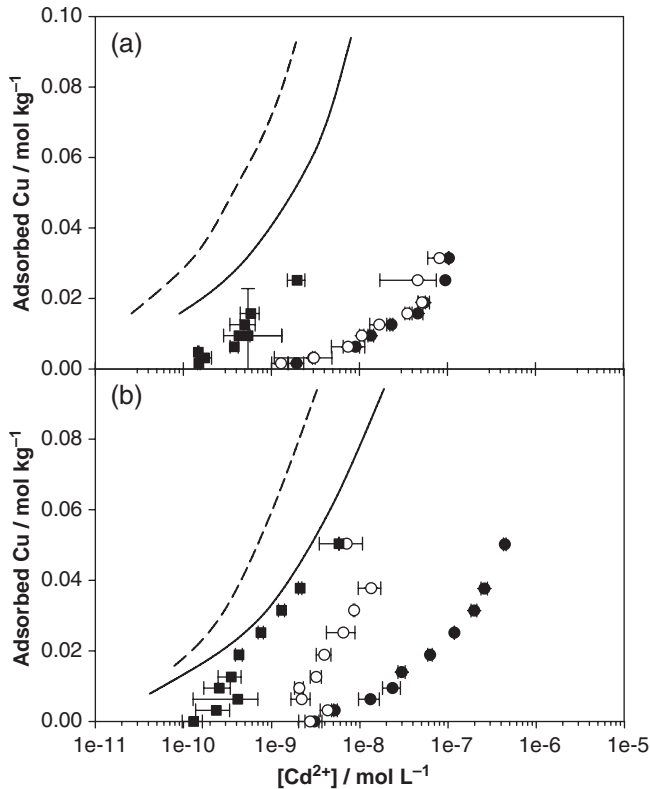
Figure 4. Isotherms for Cu^{2+} sorption by (a) Waimari peat and (b) Summit soil calcium humates at (●) 5 °C, (○) 20 °C and (■) 40 °C. Error bars are standard deviations of duplicates. Solid lines are smoothed data for sorption of Cu^{2+} by Na-humates, plotted on the same scale for comparison; ———, 20 °C; – – –, 40 °C.

Table 2. Refined parameter estimates following application of a continuous log-normal distribution model (Equation 3) to data for sorption of Cd^{2+} and Cu^{2+} to Na- and Ca-humates at pH 6.00 (values are mean ± standard deviation of duplicates).

System	Humate	Temperature (°C)	Fixed C_L (mmol·g^{-1})	log K_μ (± SD)	σ_K (± SD)	R^2
Cd^{2+}–Ca humate	Summit	5	1.95	2.08 (± 0.18)	0.83 (± 0.11)	0.995
Cd^{2+}–Ca humate	Summit	20	1.95	1.58 (± 0.03)	1.24 (± 0.02)	0.996
Cd^{2+}–Ca humate	Summit	40	1.95	1.88 (± 0.04)	1.16 (± 0.02)	0.993
Cd^{2+}–Na humate	Summit	20	1.95	2.96 (± 0.28)	1.23 (± 0.18)	0.999
Cd^{2+}–Na humate	Summit	40	1.95	3.09 (± 0.25)	1.13 (± 0.18)	0.999
Cd^{2+}–Ca humate	Waimari	5	2.60	1.30 (± 0.05)	1.28 (± 0.03)	0.999
Cd^{2+}–Ca humate	Waimari	20	2.60	1.57 (± 0.01)	1.21 (± 0.01)	0.994
Cd^{2+}–Ca humate	Waimari	40	2.60	1.73 (± 0.01)	1.15 (± 0.00)[a]	0.996
Cd^{2+}–Na humate	Waimari	20	2.60	3.09 (± 0.00)[a]	1.22 (± 0.01)	0.999
Cd^{2+}–Na humate	Waimari	40	2.60	3.15 (± 0.01)	1.21 (± 0.01)	0.999
Cu^{2+}–Ca humate	Summit	5	1.95	1.43 (± 1.30)	2.65 (± 1.03)	0.838
Cu^{2+}–Ca humate	Summit	20	1.95	3.34 (± 2.16)	1.97 (± 0.96)	0.291
Cu^{2+}–Ca humate	Summit	40	1.95	4.05[b]	2.07[b]	0.863
Cu^{2+}–Na humate	Summit	20	1.95	3.36 (± 0.11)	2.59 (± 0.13)	0.999
Cu^{2+}–Na humate	Summit	40	1.95	4.90 (± 0.33)	1.66 (± 0.65)	0.997
Cu^{2+}–Ca humate	Waimari	5	2.60	2.24 (± 0.42)	1.90 (± 0.14)	0.981
Cu^{2+}–Ca humate	Waimari	20	2.60	1.76 (± 0.72)	2.37 (± 0.55)	0.699
Cu^{2+}–Ca humate	Waimari	40	2.60	4.46[b]	1.66[b]	0.871
Cu^{2+}–Na humate	Waimari	20	2.60	4.10 (± 0.13)	2.10 (± 0.06)	0.999
Cu^{2+}–Na humate	Waimari	40	2.60	4.58 (± 0.10)	2.17 (± 0.01)	0.999

Note: [a]Model parameters from replicate experiments identical; [b]no replication.

Table 3. Refined parameter estimates (means $\pm$ SD of duplicates) from modelling sorption of Cd^{2+} to soil humates at pH 6.00 and 20 °C using discrete site-sorption models (Equation 2; $n = 1$–3).

Humate	Model (Equation no.)	Fixed C_L (mmol·g^{-1})	C_1/C_L (mmol·g^{-1})	$\log_{10} K_1$	C_2/C_L (mmol·g^{-1})	$\log_{10} K_2$	C_3/C_L (mmol·g^{-1})	$\log_{10} K_3$	R^2
					Adjustable parameters				
Na-Summit	2 ($n = 1$)	1.95	1[a]	3.89 ($\pm$ 0.06)	–	–	–	–	0.841
Na-Summit	2 ($n = 2$)	1.95	0.053 ($\pm$ 0.02)	5.7 ($\pm$ 0.3)	0.947[c]	3.61 ($\pm$ 0.06)	–	–	0.999
Na-Summit	2 ($n = 3$)	1.95	0.009 ($\pm$ 0.01)	6.7 ($\pm$ 0.6)	0.066 ($\pm$ 0.03)	5.3 ($\pm$ 0.4)	0.92[c]	3.55 ($\pm$ 0.09)	1.000
Na-Waimari	2 ($n = 1$)	2.60	1[a]	4.04 ($\pm$ 0.01)	–	–	–	–	0.861
Na-Waimari	2 ($n = 2$)	2.60	0.048 ($\pm$ 0.01)	5.78 ($\pm$ 0.13)	0.952[c]	3.75 ($\pm$ 0.05)	–	–	1.000
Na-Waimari	2 ($n = 3$)	2.60	0.033 ($\pm$ 0.01)	6.0 ($\pm$ 0.3)	0.30 ($\pm$ 0.05)	4.4 ($\pm$ 0.2)	0.67[c]	−48.1	1.000
Ca-Summit	2 ($n = 1$)	1.95	1[a]	2.90 ($\pm$ 0)[b]	–	–	–	–	0.932
Ca-Summit	2 ($n = 2$)	1.95	0.014 ($\pm$ 0.001)	4.90 ($\pm$ 0.02)	0.986[c]	2.63 ($\pm$ 0.02)	–	–	0.997
Ca-Summit	2 ($n = 3$)	1.95	0.014 ($\pm$ 0.001)	4.90 ($\pm$ 0.02)	0.49 ($\pm$ 0.4)	2.68 ($\pm$ 0.04)	0.495[c]	2.61 ($\pm$ 0.04)	0.997
Ca-Waimari	2 ($n = 1$)	2.60	1[a]	2.92 ($\pm$ 0.01)	–	–	–	–	0.944
Ca-Waimari	2 ($n = 2$)	2.60	0.027 ($\pm$ 0.0002)	4.61 ($\pm$ 0.01)	0.973[c]	2.22 ($\pm$ 0.01)	–	–	0.996
Ca-Waimari	2 ($n = 3$)	2.60	0.027 ($\pm$ 0.0004)	4.61 ($\pm$ 0.01)	0.244 ($\pm$ 0.06)	2.8 ($\pm$ 0.1)	0.729[c]	0.8 ($\pm$ 0.6)	0.996

Note: [a]For Equation (2), $n = 1$, with C_L fixed, $C_1 = C_L$ by definition; [b]model parameter values for duplicates were identical; [c]by subtraction.

Table 4. Refined parameter estimates (means of duplicates) from modelling sorption of Cu^{2+} to soil humates at pH 6.00 and 20 °C using discrete site-sorption models (Equation 2; $n = 1$–3).

Humate	Model (Equation no.)	Fixed C_L ($mmol \cdot g^{-1}$)	C_1/C_L ($mmol \cdot g^{-1}$)	$\log_{10} K_1$	C_2/C_L ($mmol \cdot g^{-1}$)	$\log_{10} K_2$	C_3/C_L ($mmol \cdot g^{-1}$)	$\log_{10} K_3$	R^2
					Adjustable parameters				
Na-Summit	2 ($n = 1$)	1.95	1[a]	5.07 ($\pm$ 0.06)	–	–	–	–	Undefined
Na-Summit	2 ($n = 2$)	1.95	0.12 ($\pm$ 0.01)	7.6 ($\pm$ 0.2)	0.880[b]	4.61 ($\pm$ 0.06)	–	–	0.983
Na-Summit	2 ($n = 3$)	1.95	0.054 ($\pm$ 0.02)	8.6 ($\pm$ 0.3)	0.110 ($\pm$ 0.001)	6.62 ($\pm$ 0.03)	0.836[b]	4.38 ($\pm$ 0.04)	0.998
Na-Waimari	2 ($n = 1$)	2.60	1[a]	5.59 ($\pm$ 0.07)	–	–	–	–	Undefined
Na-Waimari	2 ($n = 2$)	2.60	0.10 ($\pm$ 0.01)	7.8 ($\pm$ 0.1)	0.899[b]	5.2 ($\pm$ 0.1)	–	–	0.989
Na-Waimari	2 ($n = 3$)	2.60	0.03 ($\pm$ 0.01)	9.2 ($\pm$ 0.3)	0.11 ($\pm$0.01)	7.2 ($\pm$ 0.3)	0.867[b]	5.0 ($\pm$ 0.2)	0.999
Ca-Summit	2 ($n = 1$)	1.95	1[a]	6.1 ($\pm$ 0.4)	–	–	–	–	Undefined
Ca-Summit	2 ($n = 2$)	1.95	0.04 ($\pm$ 0.02)	8.0 ($\pm$ 0.2)	0.965[b]	−0.25 ($\pm$ 0.8)	–	–	0.342
Ca-Summit	2 ($n = 3$)	1.95	(regression did not converge)						
Ca-Waimari	2 ($n = 1$)	2.60	1[a]	5.5 ($\pm$ 0.3)	–	–	–	–	Undefined
Ca-Waimari	2 ($n = 2$)	2.60	0.017 ($\pm$ 0.002)	7.8 ($\pm$ 0.5)	0.983[b]	−142 ($\pm$ 201)	–	–	0.719
Ca-Waimari	2 ($n = 3$)	2.60	(regression did not converge)						

Note: [a]For Equation (2), $n = 1$, with C_L fixed, $C_1 = C_L$ by definition; [b]by subtraction.

If data for adsorption of Cd^{2+} on Na- or Ca-humates (Figures 1 and 2) are compared with data for adsorption of Cu^{2+}, it is apparent that reactions involving Cu^{2+} show greater temperature dependence, quantified by the values of mean adsorption constant (log K_μ) in Table 2. A possible explanation for this is that, because adsorption of Cu^{2+} is more thermodynamically favourable, humate macromolecules can change conformation to allow adsorption which would be unfavourable for a more weakly adsorbed ion such as Cd^{2+}. More pronounced temperature effects for ions which were strongly adsorbed to soil, relative to more weakly adsorbed ions, have been observed previously [27]. The observation that increased forward reaction time for complexation of Cu^{2+} by humates decreased the rate of subsequent dissociation has also been attributed to changes in humate macromolecule conformation, induced by Cu^{2+} complexation, which allow formation of more stable complexes [30]. Overall, the differences observed between sorption of Cu^{2+} and Cd^{2+}, and between aqueous-phase (Na) and colloidal-phase (Ca) humates suggest that the temperature dependence of these reactions reflects differences in reaction rates. It is possible that the observed temperature effects also have a thermodynamic origin, such as true differences in equilibrium constant, but the kinetic explanation is more consistent with the data.

The relative lack of agreement between and within replicate titrations for copper sorption experiments, when compared with cadmium sorption, may reflect, in part, shortcomings in the experimental techniques. The primary source of experimental error is likely to have been the ion-selective electrode determination of unbound Cu^{2+} concentrations. Although several authors have reported calibrations of Cu^{2+} ISEs below the Nernstian response range [36,37], calibration in the presence of humic substances is still subject to uncertainty. Small differences in the amount of Cu^{2+} bound to humic substances, when such a high proportion of total metal ion is bound, lead to proportionally larger differences in observed unbound metal concentrations. In addition, the discontinuities observed in batch titration of colloidal-phase calcium humates with Cu^{2+} probably reflect the titration procedure itself. In a continuous titration, any (bound metal, free metal) datum depends on the previous state of the system; such a dependence, however, does not exist in a batch titration experiment and as a result errors are more random. The magnitude of these errors was substantially smaller for titrations of humates with Cd^{2+}, due to the higher ion concentrations being determined, leading to the observed agreement within and between replicate experiments.

4. Conclusions

Sorption of Cd^{2+} and Cu^{2+} by humates in either the aqueous or colloidal phases shows varying degrees of temperature dependence. For any metal-to-ligand ratio, free metal ion concentration, and by inference metal bioavailability, decreased with increasing temperature, with this effect being greater for sorption of Cu^{2+} compared with Cd^{2+}, and also greater for sorption on colloidal-phase (Ca) humates than for aqueous-phase (Na) humates. These differences in temperature dependence for the systems studied are consistent with kinetic control of the position of apparent equilibrium for these reactions, and probably reflect rate-limiting diffusion or conformational change for humic macromolecules. The likelihood that kinetics, rather than equilibrium thermodynamics, control the temperature dependence of free metal ion concentrations in metal–humic systems, means that predicting bioavailability may be difficult. For example, geochemical solubility or surface complexation models, or approaches such as the biotic ligand model, implicitly assume thermodynamic equilibrium. More attention may need to be given to such modelling approaches if temperature effects on metal bioavailability reflect kinetic rather than thermodynamic control.

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