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Isolation and purification treatments change the metal-binding properties of humic acids: effect of HF/HCl treatment

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Environmental context. Studying the mechanism of binding between metals and natural organic matter is fundamental to understanding the transport and availability of these contaminants in the environment. The influence of sample treatment on the purification of organic matter showed significant differences in the interaction with metals. The results will contribute to improved modelling of metal binding to organic matter in soils, thereby providing a basis for a more realistic risk assessment.

ABSTRACT. We studied the changes in metal binding characteristics of extracted humic acids induced by HF/HCl treatment followed by dialysis, i.e. the last step of the International Humic Substances Society (IHSS) extraction protocol. We performed metal binding experiments with both the alkaline-extracted material (AE) and the fully purified (FP) humic acid using the electrochemical stripping technique (AGNES) and modelled the results using the NICA-Donnan model. The results showed an increase of free Zn, Cd and Pb concentrations of ,1 order of magnitude for the AE compared with the FP. These differences may be mostly explained by the different carbon content (51.3 % FP and 36.5 % AE) associated with an AE/FP carboxyl ratio of 0.5. Simulations using the NICA-Donnan model showed that halving the amount of carboxylic groups ($Q_{max,1}$) for the FP reduced this difference to 0.25 log units for Cd and Zn and to 0.15 log unit for Pb. There is a clear need for further research on the differences between purified v. less-disturbed natural organic material, which will contribute to improved modelling of metal binding to organic matter in soils, hence providing a basis for a more realistic risk assessment.

KEYWORDS Alkaline-extracted material, humic acid

1. Introduction

The solid solution partitioning and speciation of trace metals in terrestrial and aquatic environments is key to understanding their mobility, fate and bioavailability. During the last decades, great progress has been made in the development of advanced speciation models for the prediction of metal binding to the most important adsorbing constituents in natural systems, such as organic matter, Al, Fe and Mn-(hydr)oxides, clays and their assemblages^[1]. Areas of applications of these models include: (i) environmental fate^[2,3] (ii) ecotoxicity^[4] and metal deficiency in micronutrients^[5] and (iii) environmental risk assessment and regulation^[6–8].

Experimental and modelling studies indicate a prominent role of organic matter (OM) in metal binding in soils and surface waters^[1,9,10]. The most advanced models for metal binding to organic matter, especially the alkaline extract or ‘humic fraction’ thereof, are the Humic Ion Binding Models VII^[11], the Stockholm Humic Model (SHM)^[12] and the Non-Ideal Competitive Adsorption–Donnan (NICA–Donnan) model^[13]. Model parameters are derived from proton and metal binding experiments with purified humic and fulvic acids isolated from soils and surface waters in general using the extraction procedures suggested by the International Humic Substances Society (IHSS). However, besides the humic substances, other ligands of a more hydrophilic nature (including biological exudates and low-molecular organic acids) may have a role in the speciation of metals in terrestrial and aquatic systems^[14–16].

Thus, there are questions regarding the representativeness of purified humic substances for OM in natural systems, especially in soils where the extraction procedure includes an HF/HCl attack to destroy the more resistant aluminosilicate minerals. It has been suggested that the harsh chemical conditions of the soil humic substances isolation and purification may cause artefacts that influence their binding properties^[17], and that configurational aspects and interactions with inorganic colloidal materials leading to the formation of heteroaggregates may affect the binding properties of the humic materials^[18]. The purification of aquatic humic matter is considerably softer from the

chemical point of view and thus less criticised. Amongst other work, Ahmed et al.^[19] recently compared metal binding to aquatic humic acid (HA) and fulvic acid (FA) isolated according to the IHSS protocol with dissolved OM obtained by a milder procedure and found similar behaviour.

Geochemical modelling studies have shown that only a fraction of soil OM can be interpreted in concentrations of ‘reactive’ OM with equivalent binding properties to isolated and purified FA and HA. Several studies have related this fraction of reactive OM in soil with the extractable humic substances. The results vary depending on the types of soils; for example, Dijkstra et al.^[20] reported a fraction of extracted humic substances from eight sandy soils between 25 and 67 % of the total soil OM, whereas Lumsdon^[21] obtained values between 14 and 87 % for seven different soils, being the lowest extraction in the O-horizon of forest soil. Weng et al.^[22] used cation exchange capacity measurements to estimate that the average binding site density of a sandy soil is 36 % of that corresponding to the generic HA. Lopez et al.^[23] measured the acid–base properties of a peat soil and its humin, HA and FA fractions and concluded that the proton-binding properties of the peat soil are analogous to those of the humin, whereas the soil showed only 41 % of carboxylic groups but an identical amount of phenolic groups when compared with the dissolved HA.

Another interesting trend is the growing interest of agro-industry in selling alkaline soil OM extracts, especially those obtained from peat soils, as natural fertilisers to replace commercially used inorganic fertilisers. The commercial version of the ‘natural fertilisers’ is usually available in the form of ‘humic substances’ or ‘humic acid’, their purification being similar to the IHSS procedure^[24] but without HCl/HF treatment and dialysis. The environmental impact of these fertilisers is thus difficult to model because the extraction and purification procedures are different from the ones used in the scientific studies that have as their objective obtaining model parameters^[25].

The hypothesis of the present work is that the HF/HCl treatment followed by dialysis in the last step of the IHSS extraction protocol effectively changes the metal-binding characteristics of the

extracted organic matter, and that this may have a significant impact on predicted metal speciation in risk assessment studies.

To investigate this hypothesis, we measured the free zinc, lead and cadmium concentrations in the presence of humic matter, taking advantage of the nanomolar detection limit of the electrochemical stripping technique (absence of gradients and Nernstian equilibrium stripping, AGNES). The results are interpreted and discussed within the framework of the NICA-Donnan model. It is the first time that zinc-binding properties with humic matter at such low metal concentrations are presented, while lead and cadmium measurements are useful for comparison with previously published parameters.

2. Materials and methods

2.1. Chemicals and reagents

The chemicals used were of analytical reagent grade and were used as received. All solutions were prepared with ultrapure water (18.3 MO cm, Milli-Q systems, Millipore–Waters, USA). Nitric acid (65 %, Suprapur) and standard stock solutions of mercury nitrate ($1001 \pm 2 \text{ mg L}^{-1}$), cadmium nitrate ($999 \pm 2 \text{ mg L}^{-1}$), lead nitrate ($999 \pm 2 \text{ mg L}^{-1}$) and zinc nitrate ($999 \pm 2 \text{ mg L}^{-1}$) were purchased from Merck. Sodium nitrate electrolyte solution (0.01 M), MES (2-(N-morpholino)ethanesulfonic acid) and MOPS (3-(N-morpholino)propanesulfonic acid) buffer solutions were prepared from the solids (Merck Suprapur and Merck >99 % respectively). Solutions for volumetric titrations were bought from Fluka (0.10 M NaOH and 0.10 M HNO₃) and Roth (0.010 M NaOH) and used directly in the potentiometric titrations.

Solutions prepared from nitric acid (Merck Suprapur) and sodium hydroxide (0.1 M standard, Merck) were used to adjust the pH when necessary. Potassium thiocyanide, hydrochloric acid, potassium chloride and ammonium acetate were all from Merck.

2.2. Extraction and purification of HA sample

Peat samples were collected in the Mogi River region of Ribeirão Preto, São Paulo State, Brazil. The humic substances were extracted following

the IHSS procedure for soil organic matter^[25]. The alkaline extracted (AE) soil HA was taken before the HCl/HF treatment whereas the fully purified (FP) HA underwent the full purification procedure. In brief, the humic matter purification procedure for soils consists in separating the insoluble humin from the soluble humic and fulvic acids using a 1/10 mass ratio of 0.1 M NaOH for 4 h under an inert atmosphere. This step is followed by acidification to pH 1 using 1.0 M HCl to separate the soluble FA from the acid-insoluble HA. The HA is resuspended in 0.1 M NaOH and precipitated in 0.1 M HCl/0.3 M HF to destroy the remaining mineral phase. Then, the solid is dispersed in water to form a slurry, and transferred to a Visking dialysis tube where it is subsequently dialysed against distilled water.

2.3. Characterization of HA samples

Both HAs (AE and FP) were characterised by UV-vis spectroscopy in the visible region. The absorption in 465nm and 665nm (E_4/E_6 ratio) was determined by dissolving 2.0 mg of peat HA in 10 mL of 0.05 M NaHCO₃, followed by measurement of the absorbance at 465 and 665 nm using a Hitachi U2000 spectrometer. The elemental composition (C, H and N) was determined using a Thermo Finnigan Flash EA 1112 elemental analyser. ¹³C NMR analysis with crossed polarisation (CP) and magic angle spinning (MAS) was performed using a Bruker Avance III 400-MHz spectrometer, with a rotation of 5 kHz, contact time of 2 ms, relaxation waiting time of 5 s and 11 000 scans. Triplicate ash content determinations were performed by gravimetry, calcinating 1.0 g of sample at 650 °C for 4 h using an oven from Magnus Fornos Lda (Brazil).

Total iron and aluminium contents after HNO₃/HF/HCl digestion were determined by inductively coupled plasma atomic absorption spectroscopy (ICP-AES) (Thermo Fischer iCAP 6200 Duo). All analytical procedures were performed in triplicate for each sample. The standard reference material EnviroMAT SS-2 was acid-digested, then analysed using the same procedure as for the samples to check total metal content measurement accuracies, and the recoveries were within 93–105 % of expected values. Geochemically reactive

metal present in the AE sample was extracted using a 0.43 M HNO₃ extraction^[26] and concentrations were measured by ICP-AES and ICP-mass spectrometry (MS).

2.4. Potentiometric titrations of HA samples

All titrations were performed using a computer-controlled system under an argon atmosphere at 25 °C. The organic matter concentration was 0.25 g L⁻¹ while the ionic strength ($I = 0.01, 0.03, \text{ and } 0.1 \text{ M}$) was fixed using NaNO₃. The quoted I are the initial values before the addition of any titrant. In data analyses, actual I values were calculated for every data point, accounting for both background electrolyte ions and free H⁺ and OH⁻. The pH of the solutions was controlled during titrations by addition of 0.1 M HNO₃ and 0.1 and 0.01 M NaOH solutions. The pH was recorded with a pH Hamilton Polilyte laboratory combined glass electrode (part no. 238403/02), calibrated by performing a blank titration of the background electrolyte before sample titration. The suspension was then titrated by adding small volumes of titrant, and the pH was recorded as a function of the titrant volume added to the suspension. After each addition, a drift criterion for pH was used ($\Delta mV \text{ min}^{-1} < 0.1$) and a maximum time of 10 min was set for acquiring each data point. A similar procedure was followed for the blank solution titration. Three replicates were performed with fresh suspensions, in which each replicate was constituted by a forward and backward titration.

The same set-up was used to perform one-way titrations to estimate the functional group content of both AE and FP samples: samples were suspended in freshly prepared 0.1 M NaNO₃ and titrated under an inert atmosphere between pH 3.5 and 10 using 0.1 M NaOH. Determination of carboxylic and phenolic content was done assuming that all carboxylic functional groups are neutralised at pH 8, and that half of the phenolic groups are titrated between pH 8 and 10^[27].

2.5. Free metal determination in the presence of humic matter: electrochemical experiments

The free metal ion concentration in the presence of the HAs was quantified using the AGNES electroanalytical technique. This technique enables

direct determination of the free metal without the need for physical separation, while not being hampered by adsorption of OM at the electrode surface, giving a detection limit on the nanomolar range^[28,29].

An Ecochemie μ Autolab III and a PGStat 12 were used in conjunction with a Metrohm 663 VA stand (Metrohm, Switzerland). The set-up was controlled by GPES 4.9 software from EcoChemie (the Utrecht, Netherlands). A three-electrode configuration was used, comprising an Hg thin film plated onto a rotating glassy carbon disk (GC) (2-mm diameter, Metrohm) as a working electrode^[30], and a DriRef-5 Ag/AgCl reference electrode from World Precision Instruments (electrolyte leakage $< 8 \cdot 10^{-4} \text{ mL h}^{-1}$). A Denver Instrument (Model 15) and a Radiometer analytical combination pH electrode, calibrated with Titrisol buffers (Merck) were used to measure sample pH.

Metal titrations of 5 to 100 mg L⁻¹ HA were performed at $I = 0.01 \text{ M}$ and total metal concentrations in the interval from 10^{-7} to $5 \cdot 10^{-6} \text{ M}$. Cd^{II} and Zn^{II} titrations were carried out at pH 6.0, 7.0 and 8.0, whereas Pb^{II} titrations were carried out at pH 5.0, 6.0 and 6.5. Prior to each metal titration, a free metal calibration was performed. The limits of detection (LOD) calculated from the standard deviation of residuals ($3s_b/m$; s_b meaning standard deviation, m meaning mean) for 12 different calibrations were in the interval 0.4 to $3.3 \cdot 10^{-9} \text{ M}$ for Cd²⁺, 1.4 to $5.5 \cdot 10^{-9} \text{ M}$ for Pb²⁺, and 1.4 to $6.0 \cdot 10^{-9} \text{ M}$ for Zn²⁺.

The measurements were performed applying the following sets of deposition potential E_1 and deposition time t_1 : Cd: -0.68 V for 540 s; Pb: -0.48 V for 540 s; Zn: -1.1 V for 540 s. The stripping step was performed with stripping chronopotentiometry using an oxidising current I_s of $2 \times 10^{-6} \text{ A}$, applied until the potential reached -0.30 V. All solutions were purged for 15 min at the beginning of every experiment and for 20 s (assisted by mechanical stirring of the rotating electrode, 1000 rpm) after each measurement. All measurements were carried out at room temperature (23 °C). All measurements were performed in triplicate and no systematic variation was observed, indicating that chemical equilibrium was achieved in solution.

2.6. Determining NICA-Donnan proton- and metal-binding parameters of FP sample

Material specific NICA-Donnan parameters for proton and metal binding for FP HA were determined using the *PEST-ORCHESTRA* tool^[31]. The NICA-Donnan model distinguishes two different groups of binding sites, generally associated with carboxylic (Type 1 sites) and phenolic (Type 2 sites) functional groups. Non-specific electrostatic effects are taken into account with a Donnan model considering the humic particle as a permeable gel phase with a Donnan volume V_D related to I ^[31]. Here, we performed the optimisation using the relationship linking V_D and I through two parameters, a and b ^[32], which according to Companys et al.^[33] in their comparison of the Donnan and non-linear Poisson–Boltzmann approaches, gives a good approximation of the electrostatic interaction of HA.

First, unconstrained parameterisation was performed on the potentiometric titrations dataset, and eight parameters were optimised: a , b , $Q_{\max,1}$, $Q_{\max,2}$, $\log K_{H,1}$, $\log K_{H,2}$, m_1 and m_2 ^[31]. $Q_{\max,1}$ and $Q_{\max,2}$ are the proton site densities for the two considered groups of binding sites; m_1 and m_2 are the $n_{H,1}p_1$ and $n_{H,2}p_2$. In a second step, all parameters, except m_1 and m_2 , were fixed, and proton-binding data were used together with Cd- and Zn-binding data to optimise together p_1 , p_2 (p_1 and p_2 are a measure of the widths of the affinity distributions,

which describes the intrinsic heterogeneity of the humic material), $n_{H,1}$, $n_{H,2}$ and the metal-binding parameters, $\log K_{Cd,1}$, $\log K_{Cd,2}$, $n_{Cd,1}$, $n_{Cd,2}$, $\log K_{Zn,1}$, $\log K_{Zn,2}$, $n_{Zn,1}$, and $n_{Zn,2}$. Last, the Pb-binding dataset was used to optimise the four Pb-binding specific parameters ($\log K_{Pb,1}$, $\log K_{Pb,2}$, $n_{Pb,1}$ and $n_{Pb,2}$).

3. Results and Discussion

3.1. Characteristics of FP and AE humic acids

The comparison of the characteristics of both samples (Table S1, available as Supplementary material to this paper) provides information on the degree of humification and aromaticity of the material, which seems to be directly related to the metal binding ability of these substances^[34]. Elemental analysis of soil purified HA gives values of ,50–60 % C, 3–6 % H, 0.5–4 % N and 30–40 %

O. Although the FP is well within these ranges, it is clear that the carbon content of the AE (36.5 % total or 39.6 % ash-free) is well below the usual 50 % C. The ¹³C NMR results (Table S2) indicate that HF/HCl attack followed by dialysis yielded a decrease of O-alkyl aromatic content and low C contents in the FP sample when compared with the AE. The results corroborate the variation in aromaticity shown by the spectroscopic E_4/E_6 ratio (Table S1) where ratios lower than 4 are indicative of a greater presence of condensed aromatic structures, whereas values greater than 4 indicate a lack of such structures^[35]. More importantly, the carboxyl content of the FP is almost twice that of the AE, which should affect the metal-binding ability of these compounds. These results suggest that the purification procedure alters the structural characteristics of the extracted humic materials.^[36]

The ash content difference between the two HAs (cf. Table S1: AE = 7.9 % v. FP = 0.6 %) led us to perform an ICP-MS direct quantification and a 0.43 M HNO₃ extraction (at 2, 4 and 48 h) to evaluate the amount of Al and Fe present and extractable in the AE sample (Table 1). The results show that there is a considerable amount of Al and Fe in the AE sample, with 78 and 58 % Al and Fe recovery after a 48-h extraction. Other metals are present in negligible amounts, except for the Na remaining from the NaOH addition during the purification because the AE sample was not dialysed (Table S3).

Table 1. Total and extractable (0.43 M HNO₃) Al and Fe concentrations in the alkaline extracted humic acid (AE HA) sample

Fractions	Al (g kg ⁻¹)	Fe (g kg ⁻¹)
Total	24.9	2.5
Extractable 2h	16.7	1.37
Extractable 4h	17.4	1.32
Extractable 48h	19.4	1.44

The X-ray powder diffraction (XRD) analysis (Fig. S1) showed the presence of a considerable amount of aluminosilicates (predominantly hal-

loysite) but not iron minerals, indicating that the latter is probably amorphous iron or ionic iron strongly bound to the OM.

3.2. Proton-binding characteristics of FP and AE humic acids

Acid-base titrations at 0.01, 0.03 and 0.10 M I were performed for both FP (Fig. 1 and Table 2) and AE HAs. Both materials, particularly AE, showed poor titration repeatability (see Fig. S2) and a tendency to aggregate. Before performing a titration, the samples were dispersed at pH 10 overnight under a nitrogen atmosphere. It is possible that some unstable and poorly crystallised oxides may dissolve and precipitate during titration in acidic and basic media respectively. This poor repeatability prevented us from obtaining sufficiently reliable protonation data for the AE sample.

For the FP, we obtained good NICA-Donnan model fits and Fig. 1 shows that when the charge of the FP is related to the pH in the Donnan gel instead of to the pH in solution, the curves for the different ionic strengths merge into a single master curve, indicating that the electrostatics are well accounted for using the Donnan volume equation with a and b parameters.^[32] The NICA parameters obtained are within the range of those reported previously by Milne et al.^[36] except for $\log K_{H,1}$ and m_1 , which are slightly higher, probably owing to

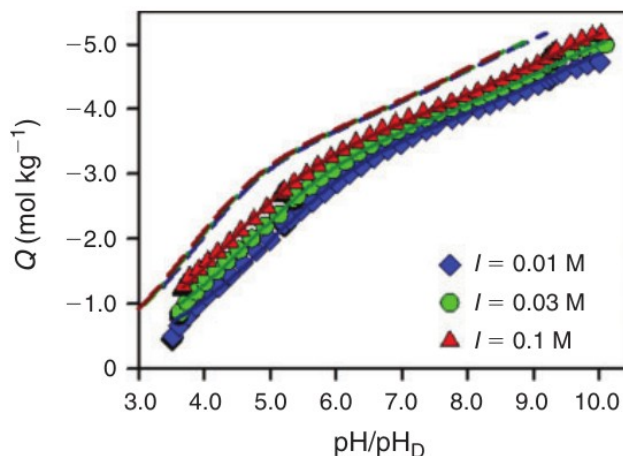


Fig. 1. Potentiometric titrations of fully purified humic acid (FP HA), at different I values: 0.01 M; 0.03 M; and 0.1 M (adjusted with NaNO_3). Solid lines are fit results, whereas dashed lines are the charge when plotted against pH in the Donnan gel (pH_D) (master curve) (lines are coloured dark blue, green and red accordingly with different coloured symbols for each I value).

the differences in the electrostatic fitting using a relationship between the Donnan volume and ionic strength with two parameters as compared with the one-parameter equation used by Milne.

To have a comparison between the two materials, we performed an end-point titration of both AE and FP, which yielded values of 2.2 and 4.0 mol kg^{-1} of carboxylic groups (titrated between pH

Table 2. Fitted set of NICA-Donnan parameters for proton and metal binding to fully purified humic acid (FP HA). Values in italics are from Milne et al.^[37] a and b , fitted set of NICA-Donnan parameters; $Q_{\text{max},1}$ and $Q_{\text{max},2}$ the proton site densities for the two considered groups of binding sites; p_1 and p_2 , a measure of the widths of the affinity distributions, which describes the intrinsic heterogeneity of the humic material

FP HA-specific parameters						
a	b	$Q_{\text{max},1}$ (mol kg^{-1})	p_1	$Q_{\text{max},2}$ (mol kg^{-1})	p_2	
0.60 ± 0.32	-0.27 ± 0.05	3.18 ± 0.92	0.79 ± 0.03	3.02 ± 2.40	0.44 ± 0.26	
Ion-specific parameters						
Ion i	$\log \tilde{K}_{i,1}$	$n_{i,1}$	$\log \tilde{K}_{i,2}$	$n_{i,2}$	RMSE	r^2
H	3.65 ± 0.23	0.84 ± 0.04	8.07 ± 0.77	0.65 ± 0.37	0.05	0.99
Cd	1.79 ± 0.10	0.56 ± 0.02	2.49 ± 1.30	0.71 ± 0.64	0.07	0.98
Cd ^[37]	-0.2	0.73	2.37	0.54	0.14	0.97
Pb	3.44 ± 0.11	0.58 ± 0.03	4.14 ± 1.78	0.69 ^A	0.10	0.96
Pb ^[37]	1.25	0.60	4.84	0.69	0.24	0.94
Zn	2.17 ± 0.11	0.69 ± 0.05	3.19 ± 2.78	0.27 ± 0.14	0.10	0.97
Zn ^[37]	0.11	0.67	2.39	0.27	0.17	0.92

^AGeneric value from Milne et al.^[38], value fixed in ORCHESTRA.

3.7 and 8, and corresponding approximately to the $Q_{\max,1}$) and values of 4.0 and 4.5 mol kg⁻¹ of phenolic groups respectively (twice the amount titrated between pH 8 and 10, corresponding approximately to the $Q_{\max,2}$). The FP values agree well with $Q_{\max,1}$ and $Q_{\max,2}$ obtained from the full titrations (Table 2). The AE shows approximately the same amount of phenolic groups as the FP but only half the amount of carboxylic groups, which agrees very well with the results obtained by NMR. Lopez et al.^[23] observed similar differences between the number of carboxylic sites of soil and purified HA fractions, which suggests that HF/HCl treatment followed by the 8-kD dialysis may be responsible for this difference.

3.3. Metal-binding parameters of FP HA

The results of Zn binding to FP obtained in the present work are the first presented for very low concentrations of Zn, with p[Zn] 9 to 7. These concentrations are much lower than those in the two studies used to obtain the generic NICA-Donnan parameters, which are in the range P[Zn] 4 to 6.^[37,38] Moreover, these two datasets, one obtained by resin exchange experiments^[39] and one obtained using the Donnan Membrane Technique (DMT) but in the presence of significant Ca²⁺ competition^[40], are small, having in total only 35 data points. The addition of the data points at low p[Zn] will therefore aid in obtaining more reliable model parameters.

Fig. 2 depicts the experimental data and modelling results for the Zn, Cd and Pb experiments at different pH values while Table 2 shows the optimised NICA-Donnan parameters. The $\log K_{H,1}$ is larger than the generic value (3.65 v. 2.93); hence, it is more appropriate to compare the difference between logarithm of bound metal ($\log K_{Me,1}$) and the proton ($\log K_{H,1}$) $\log K_{Me,1} - \log K_{H,1}$ rather than $\log K_{Me,1}$, which for Zn yields a binding constant for FP HA 20 times larger (1.3 log units) than the generic value. Similar differences are observed for the $\log K_{Me,1}$ of Cd and Pb, with differences compared with the generic values of 1.3 and 1.5 log units respectively. These differences are substantially larger than the variation in binding constants found so far.^[41] Electrostatic fitting using the *a* and *b* parameters instead of only one parameter yields a less acidic protonation constant, and concomitantly fewer metal ions bound in the Donnan phase in the acidic pH range (pH < 6). Therefore, the metal stability constants need to be larger in order to fit the experimental data, as observed in Table 2.

The *n* values for Zn are equal to the generic ones while the present *p*₁ value is significantly larger than the generic one (0.79 v. 0.62) and *p*₂ is similar (0.44 v. 0.41).

The values obtained for Type 2 parameters have likely systematically larger errors than those for Type 1 parameters. This because with a $\log K_{H,2}$ of 8.1, Type 2 sites should be largely protonated at

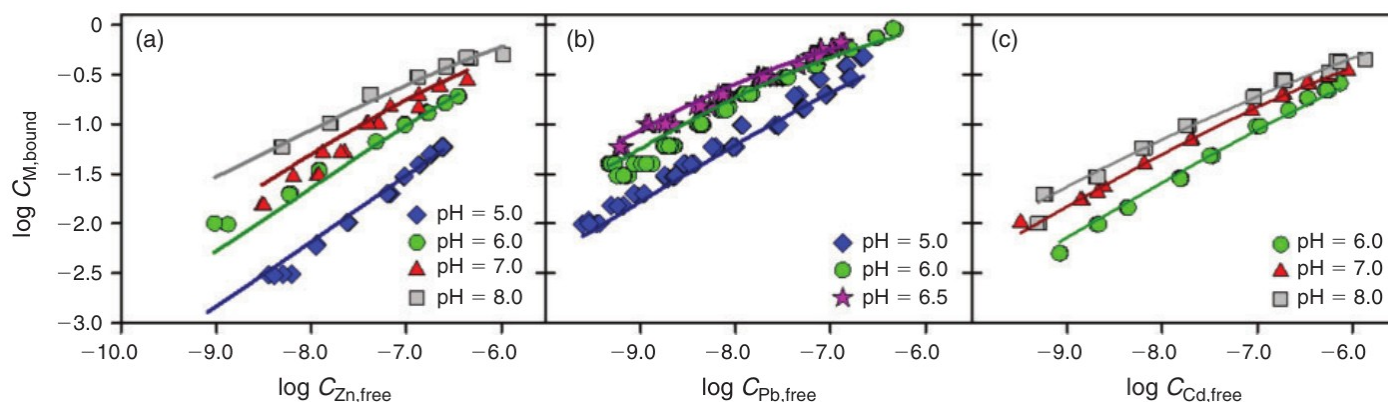


Fig. 2. Logarithm of bound metal concentration ((a) Zn; (b) Pb; (c) Cd) as a function of the logarithm of free metal concentration in the presence of fully purified humic acid (FP HA) determined by AGNES at ionic strength $I = 0.01$ M (adjusted with NaNO₃) at different pH values: 5.0; 6.0; 6.5; 7.0; and 8.0.

Solid lines are the optimisation results obtained by using the NICA-Donnan model (lines are coloured dark blue, green, pink, red and grey accordingly with different coloured symbols for each pH value).

the pH values studied, yielding larger uncertainties in the optimised Type 2 parameters. This is probably the case for most published datasets.

3.4. Comparison of metal-binding by FP and AE humic acids

In this section, we compare the free metal concentrations measured for AE v. FP under the same experimental conditions. Fig. 3 for Zn and Figs S3 and S4 for Cd and Pb respectively show measured free metal concentrations in the presence of AE as a function of those measured in the presence of FP (blue symbols). The 1:1 line represents the same amount of free metal; thus, points above the line have more free metal (less complexation).

The binding experiments show on average, 1 order of magnitude higher free metal concentration for AE as compared with FP for Zn and Cd and ~0.9 log units higher for Pb. This difference is more pronounced at lower metal-to-ligand (M/L) ratios and is smaller at higher M/L. This difference is similar for the three metals studied and independent of the pH within experimental error.

Part of these differences may be explained by the different carbon content of both materials (51.3 % for FP and 36.5 % for AE; Table S1) associated with a different chemical composition. This is even more evident from the NMR results (Table S2) and endpoint titration data showing that the carboxyl content of the FP is approximately twice that of the AE. Metal binding by the AE sample may also be suppressed by interaction

of AE with Al and Fe still present in the sample either in a mineral phase and as metal ions bound to the HA.

To investigate these hypotheses and their relative importance, we modelled the free metal concentrations in presence of FP for three scenarios using ORCHESTRA: (1) using the FP parameters as such (parameters from Table 2); (2) a scenario in which we reduced the value of $Q_{\max,1}$ by 50 % (to 1.59 mol kg⁻¹) to evaluate the effect on metal binding of a lower amount of carboxylic groups as determined in the AE sample; and (3) a scenario in which we additionally considered the competition of Al and Fe as present in the AE sample as measured in the 0.43 M HNO₃ extraction (Table 1, 48 h). Because no specific Al and Fe parameters are available for FP, we used their generic values in scenario 3 but scaled to the FP-specific proton parameters, thereby keeping the same relative strength of Al and Fe to proton binding. These metals were allowed to precipitate as ferrihydrite (logK = 3.19) or gibbsite (logK_{so} = 8.29).

Simulated free metal concentrations for scenario 1 (Fig. 3 for Zn and Figs S3 and S4 for Pb and Cd respectively) are close to the 1:1 line. The small differences are due to both uncertainties in measurements and modelling. The reduction of the amount of carboxylic groups in scenario 2 ($Q_{\max,1}$) has a strong effect on the complexation. The simulations show a marked decrease in the difference between the measured free metal in the presence of AE and the modelled free metal in this scen-

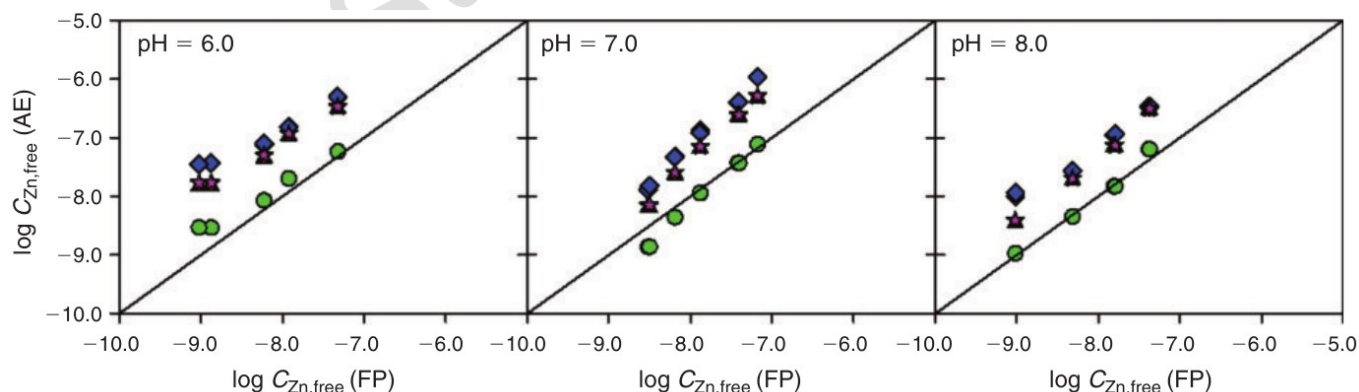


Fig. 3. Logarithm of free Zn²⁺ concentration in presence of alkaline extracted humic acid (AE HA) v. the logarithm of free Zn²⁺ concentration in the presence of fully purified (FP) HA for the same amount of organic material and at ionic strength $I = 0.01$ M for pH 6.0, 7.0, and 8.0.

Symbols represent: \diamond determined by AGNES; \circ fit results; \triangle adjusted Q_1 ; \star adjusted Q_1 with Al added.

ario, which for Zn and Cd decreases on average from ~1 to ~0.25–0.30, and for Pb from 0.9 to ~0.15. For Pb, the average reduction in the difference is somewhat misleading because for low M/L, the difference is still significant but at high M/L ratios, the difference disappears within experimental error. However, considering the competition of Al and Fe (scenario 3) has a negligible effect for Cd, and very minor effects for both Zn and Pb of ~0.02 and 0.05 log units respectively. Most of the difference in metal binding by FP can be explained by the larger number of carboxylic groups in the FP material.

3.5. Implications for environmental impact assessments

The results of this study indicate that the properties in terms of functional groups and aromaticity of humic materials present in OM are modified by the extraction and purification process and lead to increased metal binding by the fully purified material compared with alkaline extracted material. This implies that when applying ion-binding models for metal binding to OM that are parameterised based on purified HAs, metal binding is likely to be overestimated, and hence, bioavailability and mobility of metals may be underestimated. It is, however, common practice with the use of these models to consider only part of the OM being reactive in terms of a generic HA. This reactive fraction of soil OM is usually set at a fixed percentage between 30 and 100 by rule of thumb.^[1] The results of the present work show that when the number of carboxylic groups is reduced by 50 % to account for the difference between purified (FP) and the alkaline extracted material (AE) the predictions of metal binding to the more soil-like AE improves considerably. Regarding risk assessment studies, we demonstrate that it is indeed necessary to correct the $Q_{\max,1}$ provided by the general parameters using a measured value or a factor of at least 50 %. Failing to do so will lead to underestimation of free metal concentration values for Zn, Cd and Pb of one order of magnitude.

Our failure to obtain reproducible titrations of the AE material indicates that these fractions are not as stable as the FP material, which is already not particularly reproducible. The study of altern-

ative extraction techniques, like physical extractions instead of chemical extractions and the standardisation of softer chemical extractions may help us to understand the changes that OM undergoes from the soil matrix to the solution. One of these alternative methods is the replacement of the HF/HCl treatment by a filtration step for the removal of mineral fractions recently proposed by the IHSS^[25], which we are planning to study in the near future.

This problem is interesting in itself and more so since Lehman and Kleber^[42] questioned the reality of what is called humics in soils and suggested the adoption of a soil continuum model, stating that alkaline extraction is not representative for soil OM. There is a clear need for further research on the nature of differences in the reactivity between purified v. less-disturbed natural OM, which will contribute to improved modelling of metal binding to OM in soils and other media, hence providing a basis for a more realistic risk assessment.

Conflicts of interest The authors declare that they have no conflicts of interest.

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Supporting Information Available.

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