Magnesium binding by terrestrial humic acids

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Environmental context. The behaviour of magnesium, which is an essential element for all living organisms, in terrestrial environments is influenced by natural organic matter. This study shows that magnesium binding by terrestrial humic acids exhibits a pronounced ionic strength-dependence indicating a strong preference for electrostatic binding to humic acids. This interaction is expected to influence the mobility of humic substances and their associated trace elements.

Abstract. Magnesium binding by three terrestrial humic acids was investigated at pH 8 and 25 °C as a function of Mg2+ activity and ionic strength using NaCl as the background electrolyte. The Mg2+ activity in solution was directly measured with an Mg2+-selective electrode in the titration experiments. In addition, coagulation experiments using Ca2+ and Mg2+ as the coagulants were carried out at pH 8. For the titration data, the NICA–Donnan model was used to quantitatively describe Mg2+ binding to the humic acids considering electrostatic and specific Mg2+ binding. Mg2+ binding to humic acids was found to be strongly affected by ionic strength variations indicating that Mg2+ binding largely arose from electrostatic (nonspecific) interactions with negatively charged functional groups of the humic acids. Data modelling suggested that the relative contribution of specific binding increased with decreasing Mg2+ activity and was related to functional groups with low proton affinities. For all three humic acids studied, the fitted Mg2+ affinity constants for specific binding were lower than the respective Ca2+ affinities. Corresponding to the observed differences in cation binding and the known differences in ion hydration, Ca2+ was observed to be the stronger coagulant as compared with Mg2+. The results suggest that Mg2+ may influence the mobility of trace elements that are strongly bound to humic acids such as mercury, although Mg2+ is not expected to directly compete with strongly sorbing elements for specific binding.

Additional keywords: calcium, carboxylic groups, coagulation, electrostatic binding, ionic strength, NICA–Donnan model, NOM, specific binding.

Received 18 April 2018, accepted 2 June 2018, published online 24 August 2018

Introduction

Magnesium (Mg) is an essential element for all living organisms. For instance, it is of vital importance for the functioning of ATP in living cells and plants that require magnesium for the synthesis of chlorophyll, in which a magnesium cation is coordinated in the centre of the porphyrin ring (Hawkesford et al. 2012). The biosphere’s high demand for magnesium is met by its high abundance in the Earth’s crust. Magnesium is contained in a large variety of minerals present in terrestrial environments (Stahr 2016), for example, in sedimentary phases such as carbonate minerals (e.g. magnesite and dolomite), in primary and secondary mineral phases, such as silicates (e.g. olivines, pyroxenes, amphiboles, serpentines, clays, and mica such as biotite), in oxides and hydroxides (e.g. periclase and brucite), as well as in chlorides and sulphate salts. When released from minerals owing to weathering, magnesium is supplied as Mg2+ to the biosphere. The cation Mg2+ is characterised by a small ionic radius (57 pm at coordination number CN = 4, 72 pm at CN = 6, and 89 pm at CN = 8) (Haynes et al. 2014). In aqueous medium, Mg2+ exhibits a strong tendency to form a well-ordered octahedral inner hydration shell (Mg(H2O)62+) as indicated by its large negative values for hydration enthalpy (ΔHm = −1922 kJ mol−1) and entropy (ΔSm = −183 J mol−1 K−1). These values are significantly larger than for Ca2+, which preferably forms a square antiprismatic inner hydration shell (Ca(H2O)82+) (Essington 2004; Persson 2010). For given numbers of coordinated water molecules, the binding energies of water have been reported to be higher for Mg2+ than for Ca2+ (Peschke et al. 1998) and the radius of the hydrated ion has been reported to be larger (rH (Mg2+) = 428 pm and rH (Ca2+) = 418 pm at CN = 6) (Nightingale 1959). Similar to Ca2+, Mg2+ is classified as a hard Lewis acid and prefers oxygen-donor ligands (Pearson 1963; Pearson 1968). Reported complex formation constants for aqueous 1:1 cation-ligand complexes of common natural oxygen-donor ligands, such as phosphate, carbonate and acetate, are fairly similar for Ca2+ and Mg2+ with values differing by less than 1 order of magnitude for both cations (Martell et al. 2004).

In soil solution, Mg2+ typically ranks as the second most abundant cation after Ca2+ and total dissolved magnesium has been reported to amount up to 30 mg L−1 in forested soils and 60 mg L−1 in arable soil (Kretzschmar 2016). The supply of dissolved Mg2+ arises from the high solubility of Mg-containing solid phases, especially Mg-containing silicates, which are ubiquitous in terrestrial environments. In calcareous soils, dolomite (CaMg(CO3)2) is expected to limit the amount of dissolved Mg2+, whereas in soils with pH below 7, the amount
of dissolved Mg$^{2+}$ is mostly limited owing to binding to cation exchange sites (Lindsay 1979). Natural organic matter (NOM) represents an important soil constituent contributing to cation exchange properties of a soil, particularly in top soil horizons enriched in NOM (Kögel-Knabner 2016; Tipping 2002). Despite the significance of Mg$^{2+}$ for vital processes in terrestrial systems, its binding to NOM has still been poorly studied. Whereas many datasets on Ca$^{2+}$ binding by fulvic and humic acids have been published, available data on Mg$^{2+}$ binding are lacking. In fact, the two publications on Mg$^{2+}$ binding by Van Dijk (1971) and Schnitzer and Skinner (1967) form the main basis for the Mg$^{2+}$–NOM affinity parameters of Model VII and the NICA–Donnan model (Milne et al. 2003; Tipping et al. 2011), which are widely used in geochemical modelling, e.g. by Marang et al. (2006), who used the Mg$^{2+}$ NICA–Donnan model parameters published by Milne et al. (2003) to model cobalt binding by Gorleben humic acid in a magnesium nitrate background electrolyte. As for the above mentioned Mg datasets, Van Dijk (1971) investigated the binding of various cations including Mg$^{2+}$ by different humic acids at pH 5 by indirectly measuring the drop in pH upon cation addition. Schnitzer and Skinner (1967) applied an ion-exchange method, known as Schubert’s method, to study the binding of divalent cations (Pb$^{2+}$, Ni$^{2+}$, Mn$^{2+}$, Co$^{2+}$, Ca$^{2+}$ and Mg$^{2+}$) by a fulvic acid at pH 3.5 and 5.0. Later, Pandey et al. (2000) confirmed that at pH 3.5, Ca$^{2+}$ was bound more strongly than Mg$^{2+}$ by humic acid. In a publication by Marang et al. (2006), Mg$^{2+}$ binding by humic and fulvic acid samples was investigated according to the procedure presented in the experimental section, but the authors only provided the data on Ca$^{2+}$ and stated that Mg$^{2+}$ behaved similarly. In a more recent publication by Yan et al. (2015), Mg$^{2+}$ binding by Suwannee River humic acid (SRHA) and fulvic acid (SRFA) was investigated at pH 5–10 and ionic strengths of 0.001–0.3 M using differential absorbanse spectroscopy. Their results revealed a strong influence of pH and ionic strength on the recorded UV-vis spectra. Despite the comprehensiveness of the presented dataset, a full quantification, as based on the experimental data, has not been provided (Yan et al. 2015).

Given the shortage of quantitative data on Mg$^{2+}$ binding to NOM and the significance of magnesium in terrestrial systems, the objective of this study was to provide quantitative data on Mg$^{2+}$ binding for terrestrial humic acids and to derive experimentally based Mg$^{2+}$ binding affinities. For this purpose, magnesium titration experiments were carried out using three well characterised humic acid samples. An Mg$^{2+}$ ion-selective electrode (ISE) was used to quantify Mg$^{2+}$ binding at pH 8 in 0.01 M, 0.03 M and 0.1 M NaCl. Mg$^{2+}$ affinities were derived from the experimental data for the three humic acid samples using the NICA–Donnan model. The results on Mg$^{2+}$ binding were compared with those of Ca$^{2+}$ binding to highlight the differences between these two major cations, including their impact on trace element mobility.

Experimental

Humic acids

Three terrestrial humic acids were used in this study: (i) Elliot soil humic acid (ESH, International Humic Substances Society (IHSS) Sample No. 1S102H) extracted from a Prairie soil near Joliet, Illinois, USA (ii) Pahokee peat humic acid (PPHA, IHSS Sample No. 1S103H) extracted from an agricultural peat soil in Florida, USA, and (iii) a purified humic acid extracted from the humified organic horizon of a forested Humic Gleysol at Unterrickenzopfen near Langenthal, Switzerland (PUHA). Details on the extraction, purification and characterisation of PUHA have been reported elsewhere (Christl et al. 2000). Information on the extraction and characterisation of ESHA and PPHA has been provided by the IHSS. According to published $^{13}$C-NMR spectroscopy data, the three humic acid samples are similar with respect to carboxyl carbon contents, but ESHA and PPHA contain about twice as much aromatic carbon as PUHA, which contains more alkyl and O-alkyl carbon (Christl et al. 2000; Thorn et al. 1989).

Mg$^{2+}$ activity measurements

Mg$^{2+}$ activities in solution were measured with an Mg$^{2+}$ ion-selective electrode (ISE, DX224-Mg magnesium half-cell, Mettler Toledo, Greifensee, Switzerland) and an Ag/AgCl reference electrode (6.0733.100, Metrohm). The reference electrode was placed into a salt bridge containing 0.1 M NaCl, which was continuously renewed by a peristaltic pump to avoid drifts in the reference electrode potential. For calibration, 40 mL of a 1 mM MgCl$_2$ solution was titrated with 10 mM Na$_2$-EDTA and 50 mM CO$_2$-free NaOH. The ionic strength was adjusted to 0.01 M, 0.03 M and 0.1 M, using NaCl as the background electrolyte for the preparation of solutions. The calibrations were performed in a thermostated room at 25 ± 1°C using an automated titration setup (Kinniburgh et al. 1995). The headspace of the titration vessel was continuously purged with water-saturated CO$_2$-free nitrogen gas. Additionally, separate solutions containing 3 mM and 10 mM MgCl$_2$ were prepared to cover Mg$^{2+}$ activities greater than $10^{-5}$ in the calibrations. Concurrent with the Mg$^{2+}$ activities, the proton activities in solution were measured with a pH electrode (6.0123.100, Metrohm). The readings of the Mg$^{2+}$ ISE were correlated with the activities of free Mg$^{2+}$, as calculated by the solution speciation program ECOSAT (Keizer and van Riemsdijk 2006), using the measured pH value and the known total amounts of all components as the input data. The stability constants for aqueous complexes were taken from the NIST Standard Reference Database 46 (Martell et al. 2004). In the resulting calibrations, the response of the Mg$^{2+}$ ISE correlated linearly to the calculated Mg$^{2+}$ activities in the range of $10^{-2.3}$ to $10^{-6.5}$, as shown in the example depicted in Fig. 1.

Magnesium binding by humic acids

Magnesium binding by PUHA, ESHA and PPHA was studied at pH 8 as a function of ionic strength at 25 ± 1°C in a CO$_2$-free nitrogen gas atmosphere by performing titration experiments. Four burettes containing 0.05 M NaOH, 0.05 M HCl, 1 mM MgCl$_2$ in either 0.01 M, 0.03 M or 0.1 M NaCl, and 0.1 M MgCl$_2$ were connected to an automated titration setup (Kinniburgh et al. 1995) that controlled the dosage of titrant solutions into the reaction vessel. A pH electrode (6.0123.100, Metrohm), an Mg$^{2+}$ ISE (DX224-Mg, Mettler Toledo), and an Ag/AgCl reference electrode (6.0733.100, Metrohm) placed into a salt bridge containing 0.1 M NaCl, which was continuously renewed during the experiment, were used for pH and Mg$^{2+}$ activity measurements. At the beginning of each experiment, the ~1 g L$^{-1}$ humic acid solution was first titrated to pH 10 with NaOH. Then the pH was decreased to the target pH of 8.0 by HCl addition and kept constant for 10 h before starting magnesium additions. During the experiment, the humic acid solution was stirred for 2 min after each addition of titrant.
Magnesium binding by terrestrial humic acids

**Fig. 1.** Performance of the Mg$^{2+}$ ion selective electrode (ISE) in 0.03 M NaCl at 25 ± 1°C as an example for ISE calibration. The electromotive force (emf) as measured against an Ag/AgCl reference electrode is linearly related to the calculated logarithm of Mg$^{2+}$ activities in solution in the range of 10^{-2.3} to 10^{-0.3}.

The pH value was automatically readjusted to the target value after each magnesium addition. Once the target pH value was reached within a range of ± 0.01 units, it was kept constant for an additional 20 min. The electrode readings were recorded once the potential drifts of the Mg$^{2+}$ ISE and the pH electrode were below 0.1 mV min^{-1} and 0.05 mV min^{-1}, respectively. These criteria corresponded to an overall equilibration time of ~1 h for each magnesium addition.

Additionally, proton and calcium binding of PPHA was studied in the same way as was done previously for PUHA and ESHA (Christl 2012; Christl and Kretzschmar 2001) to obtain an experimentally consistent set of proton, calcium and magnesium data for all three humic acids investigated. Briefly, 0.05 M NaOH and 0.05 M HCl were used as the titrants in the acid–base titration described above were conducted at pH 5.0 and 8.0 in a CO2-free nitrogen gas atmosphere at constant ionic strengths of 0.01–0.26 M, which was adjusted by NaCl. For calcium binding, calcium titration experiments similar to the magnesium titrations described above were conducted at pH 5.0 and 8.0 in 0.01 M and 0.1 M NaCl using CaCl$_2$ and a Ca$^{2+}$ ISE (Orion 9320, Thermo Scientific) instead of the MgCl$_2$ and a Mg$^{2+}$ ISE, respectively. Additional details on the acid–base titration and the calcium titrations, including Ca$^{2+}$ ISE calibration, can be found elsewhere (Christl 2012; Christl and Kretzschmar 2001).

**Data analysis and modelling of titration experiments**

From the magnesium titration data of PUHA, ESHA and PPHA, the amount of magnesium bound to humic acid was calculated for each data point as the difference between the total magnesium in solution and the sum of aqueous magnesium species calculated with the speciation program ECOSAT (Keizer and van Riemsdijk 2006) using the measured Mg$^{2+}$ activity, the pH, and the total concentrations of sodium and chloride as the input data. Similarly, calcium binding to PPHA was calculated. Molar H$^+$/Mg$^{2+}$ exchange ratios were derived for each data point from the amounts of acid and base required to readjust the pH after magnesium addition considering the entire solution speciation as well as the amount of magnesium bound to humic acid. From the acid–base titration recorded for PPHA, the number of protons bound by PPHA was calculated as the difference between total acid and base added and acid and base consumed by the bulk solution.

The experimental data on ionic strength-dependent magnesium binding to humic acids were fitted with the consistent NICA–Donnan model, which accounts for electrostatic (non-specific) interactions and specific cation binding of humic substances (Benedetti et al. 1995; Benedetti et al. 1996; Kinniburgh et al. 1996). Briefly, humic substances are regarded as a Donnan gel-like phase exhibiting a uniform potential. The negative charge of the gel phase, which results from deprotonation of functional groups, is balanced by the accumulation of cations. This results in increased cation concentrations in the Donnan gel, $c_{D,j}$, as compared with the respective cation concentration, $c_j$, in the bulk solution. Ionic strength effects on the volume of the Donnan gel, $V_D$, are considered using the semi-empirical equation $V_D = b \times \log I - b - 1$, where $b$ is the so-called Donnan parameter and $I$ is the ionic strength. The specific binding of cations by humic substances is described with a continuous affinity distribution considering two pools of binding sites, the $Q_1$ sites having lower affinities and the $Q_2$ sites having higher affinities. The widths of both affinity distributions are described by the model parameters $p$ ranging from zero to one and represent a measure for the heterogeneity of both pools of binding sites. The cation-specific non-ideality of binding is represented by the model parameters $n_j$, which were suggested to reflect lateral interactions and/or the stoichiometry of ion binding and were shown to correspond to the slope of the isotherms at low ion activity (Kinniburgh et al. 1999; Koopal et al. 1994). For further details on the model, the readers are encouraged to refer to Kinniburgh et al. (Kinniburgh et al. 1999). The optimisation of the NICA–Donnan model parameters was conducted with the computer program FIT (Kinniburgh and Tang 2004). The chemical speciation program ECOSAT (Keizer and van Riemsdijk 2006) was used to calculate the solution speciation from the experimental data to generate the required data input for parameter optimisation. For PUHA and ESHA, only two model parameters, the affinity constant $K_{1,Mg}$ and the non-ideality parameter $n_{1,Mg}$, were used as variable parameters, whereas previously published model parameters for both humic acids were fixed to published values (Christl 2012; Christl and Kretzschmar 2001; Christl et al. 2001; Maurer et al. 2012). This way, the consistency with proton, calcium, cadmium, copper and lead binding of PUHA as well as proton and calcium binding of ESHA was assured. For PPHA, the ionic strength-dependent proton binding data derived from acid–base titrations, the calcium binding data, and the magnesium binding data reported in this study were fitted simultaneously to receive a consistent set of model parameters that best described the ion binding of PPHA. Herein, $n_{1,Mg}$ had to be constrained and was fixed to the value fitted for PUHA. Best fits to the experimental data based on the Aikake information criterion (AIC) (Burnham and Anderson 2004; Christl et al. 2016) and corresponding model parameters (Table 1) are reported here.

**Coagulation experiments**

Coagulation of 0.5 g L^{-1} PUHA, as induced by Mg$^{2+}$ or Ca$^{2+}$ addition, was investigated at pH 8.0 ± 0.1. Total magnesium and calcium concentrations were varied from 0 mM to 10 mM. NaCl was used as the background electrolyte to adjust the ionic strength to either 0.03 M or 0.1 M. The pH was adjusted by adding aliquots of NaOH (0.05 M), before and immediately after calcium and magnesium addition, and again 1 h, 24 h.
The significance of electrostatic binding for Mg$^{2+}$

The direct comparison of experimental data on Mg$^{2+}$ cation on Ca$^{2+}$ result that molar H/$H$ arose from electrostatic binding. This finding agreed with the cations, Mg$^{2+}$
tication of binding sites being effective in the same manner for both

slope of the binding isotherms decreased with increasing Mg$^{2+}$
humic acids were fairly similar at a given ionic strength and ion

tively charged, functional groups of the humic acids (Fig. 3).

increased with increasing Mg$^{2+}$
activity and appeared to plateau at activities of

The supernatants were passed through 0.45 $\mu$m nylon membrane

For PUHA, PPHA and ESHA, Mg$^{2+}$ binding suggested that the observed difference arose from a weaker specific binding of Mg$^{2+}$ as the electrostatic interactions

and 48 h after calcium and magnesium addition. Then, the samples were centrifuged for 15 min at 3600 $\times$ g (Mistral 6000). The supernatants were passed through 0.45 $\mu$m nylon membrane filters (BGB), diluted, and acidified using HCl before the analysis for total dissolved organic carbon, magnesium and calcium using a TOC analyzer (DIMATOC 2000, DIMATEC) and a ICP-OES (5100 ICP-OES, Agilent Technologies).

Results and discussion

Effect of ionic strength on magnesium binding

For PUHA, PPHA and ESHA, Mg$^{2+}$ binding at pH 8 steadily increased with increasing Mg$^{2+}$ activity (Fig. 2). Herein, the slope of the binding isotherms decreased with increasing Mg$^{2+}$ activity and appeared to plateau at activities of $\approx 10^{-3}$, which indicated the maximum binding capacities of the three investigated terrestrial humic acid samples. The increase in ionic strength from 0.01 M to 0.03 M and further to 0.1 M resulted in a pronounced decrease of Mg$^{2+}$ binding for all three humic acids (Fig. 2). The strong ionic strength effect observed in the titration experiments indicated that Mg$^{2+}$ binding to humic acid mainly arose from electrostatic binding. This finding agreed with the result that molar H$^{+}$/Mg$^{2+}$ exchange ratios, as derived from the titration experiments, were clearly below the value of 2 for a fully charge-balanced stoichiometry, which indicated a preference of Mg$^{2+}$ to mainly interact with deprotonated, i.e. negatively charged, functional groups of the humic acids (Fig. 3).

The significance of electrostatic binding for Mg$^{2+}$ was further corroborated by the comparison with Ca$^{2+}$ binding (Fig. 2). Whereas the amounts of Mg$^{2+}$ and Ca$^{2+}$ bound to the studied humic acids were fairly similar at a given ionic strength and ion activities were greater than $10^{-3}$, which arose from the limitation of binding sites being effective in the same manner for both cations. Mg$^{2+}$ binding was clearly lower than Ca$^{2+}$ binding when ion activities were lower than $10^{-5}$. In a previous publication on Ca$^{2+}$ binding by terrestrial humic acids, it was shown that Ca$^{2+}$ was predominately bound by electrostatic interactions at high ion activities. However, at low ion activities, specific binding contributed increasingly to Ca$^{2+}$ binding (Christl 2012).

The direct comparison of experimental data on Mg$^{2+}$ and Ca$^{2+}$ binding suggested that the observed difference arose from a weaker specific binding of Mg$^{2+}$ as the electrostatic interactions

were effective for both cations at any given level of cation activity in solution. The strong ionic strength effect on Mg$^{2+}$ binding shown here was in agreement with the results presented for SRFA and SRHA using differential absorbance spectroscopy (Yan et al. 2015) and provides direct experimental proof of the significance of electrostatic interaction in Mg$^{2+}$ binding to humic acids, as has been proposed in the literature (Milne et al. 2003; Yan et al. 2015). For Ca$^{2+}$, it was demonstrated that the relative contribution of electrostatic

### Table 1. NICA–Donnan model parameters for the description of the ionic strength-dependent binding of H$^{+}$, Ca$^{2+}$ and Mg$^{2+}$ by PUHA, ESHA and PPHA

<table>
<thead>
<tr>
<th></th>
<th>PUHA</th>
<th>ESHA</th>
<th>PPHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donnan parameter $b$</td>
<td>$-0.40_{a}$</td>
<td>$-0.12_{a}$</td>
<td>$-0.20_{a}$</td>
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<tr>
<td>Site density $Q_1$ (mol kg$^{-1}$)</td>
<td>$3.1^{A}$</td>
<td>$3.0^{B}$</td>
<td>$3.3^{A}$</td>
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<td>Heterogeneity parameter $p_1$</td>
<td>$0.61^{a}$</td>
<td>$0.73_{a}$</td>
<td>$0.68_{a}$</td>
</tr>
<tr>
<td>H$^{+}$ affinity constant log $K_{1,Mg}$</td>
<td>$2.6^{A}$</td>
<td>$2.2^{B}$</td>
<td>$2.5_{B}$</td>
</tr>
<tr>
<td>H$^{+}$ non-ideality parameter $n_{1,Mg}$</td>
<td>$0.70^{a}$</td>
<td>$0.81^{B}$</td>
<td>$0.93_{a}$</td>
</tr>
<tr>
<td>Ca$^{2+}$ affinity constant log $K_{1,Ca}$</td>
<td>$-1.8^{B}$</td>
<td>$-1.7^{A}$</td>
<td>$-1.6_{A}$</td>
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<tr>
<td>Ca$^{2+}$ non-ideality parameter $n_{1,Ca}$</td>
<td>$0.53^{B}$</td>
<td>$0.50^{A}$</td>
<td>$0.64_{B}$</td>
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<tr>
<td>Mg$^{2+}$ affinity constant log $K_{1,Mg}$</td>
<td>$-2.9$</td>
<td>$-4.0$</td>
<td>$-2.4$</td>
</tr>
<tr>
<td>Mg$^{2+}$ non-ideality parameter $n_{1,Mg}$</td>
<td>$0.72$</td>
<td>n.d.</td>
<td>$0.72$</td>
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<tr>
<td>Site density $Q_2$ (mol kg$^{-1}$)</td>
<td>$2.7^{A}$</td>
<td>$3.8^{B}$</td>
<td>$2.7_{A}$</td>
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<tr>
<td>Heterogeneity parameter $p_2$</td>
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<td>$0.23^{B}$</td>
<td>$0.39^{B}$</td>
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<tr>
<td>H$^{+}$ affinity constant log $K_{2,Mg}$</td>
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<td>$8.2^{B}$</td>
<td>$8.0_{B}$</td>
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<tr>
<td>H$^{+}$ non-ideality parameter $n_{2,Mg}$</td>
<td>$0.80^{B}$</td>
<td>$0.88^{A}$</td>
<td>$0.83_{B}$</td>
</tr>
</tbody>
</table>

$^{A}$From Christl et al. (2001).
$^{B}$From Christl (2012).

![Fig. 2.](image_url)

Magnesium binding by (a) PUHA, (b) ESHA and (c) PPHA at pH 8 in 0.01 M, 0.03 M, and 0.1 M NaCl as a function of Mg$^{2+}$ activity in solution. For comparison, calcium binding at pH 8 in 0.01 M and 0.1 M NaCl is shown. Symbols represent data calculated from magnesium and calcium titrations of solutions containing ~1 g L$^{-1}$ PUHA, ESHA and PPHA. Lines depict fits using the NICA–Donnan model (see Table 1 for model parameters). Calcium data and fits for PUHA and ESHA were taken from Christl (2012).
Magnesium binding by terrestrial humic acids

interactions to Ca\(^{2+}\) binding by ESHA and PUHA increased with decreasing pH (Christl 2012). Since at pH 8, Mg\(^{2+}\) is bound less by specific interaction than Ca\(^{2+}\), as indicated by the data shown here, it can be assumed that the predominance of electrostatic binding for Mg\(^{2+}\) will also be increased at pH values lower than 8. This assumption is also supported by the low molar H\(^+/\)Mg\(^{2+}\) exchange ratios (Fig. 3), which indicated a weak competitiveness of Mg\(^{2+}\) for binding compared with H\(^+\) and consequently, a preferred association of Mg\(^{2+}\) with negatively charged, i.e. deprotonated, functional groups of humic acids.

**Modelling magnesium binding**

The relevance of electrostatic interactions for Mg\(^{2+}\) binding by terrestrial humic acids was further investigated by modelling the experimental data with the NICA–Donnan model, which has widely been used to describe cation binding to various NOM fractions that are characterised by their inherent heterogeneity with respect to the contained functional groups (Tipping 2002). In this model, the total binding of a cation is attributed to electrostatic (nonspecific) binding, which arises from the electrostatic attraction of a cation by negatively charged functional groups of NOM, and to specific cation binding to proton-reactive sites. The latter is described by two pools of sites, \(Q_1\) having a low affinity for protons and \(Q_2\) having a high affinity for protons. These pools were proposed to mainly represent carboxylic and phenolic moieties, respectively, being the quantitatively most prominent binding sites for protons and cations in NOM (Milne et al. 2001; Perdue and Ritchie 2003; Stevenson 1994; Tipping 2002). However, in some cases, e.g. for mercury binding, less abundant functional groups, such as reduced sulphur groups, have been shown be key for trace element binding to NOM (Nagy et al. 2011; Skyllberg et al. 2006; Skyllberg et al. 2003). A previous study on Ca\(^{2+}\) binding by PUHA and ESHA demonstrated that experimental data were described best with the NICA–Donnan model when considering only electrostatic interactions and specific binding to \(Q_1\) sites (Christl 2012). Including binding to \(Q_2\) sites did not improve the fit to the data. As new data on H\(^+\), Ca\(^{2+}\) and Mg\(^{2+}\) binding to PPHA were recorded in this study (Figs S1, S2 in the Supplementary Material and Fig. 2c), the PPHA dataset was used to verify the previous finding obtained for PUHA and ESHA. The simultaneous fitting of the entire PPHA dataset presented here revealed that the best fit was achieved when electrostatic interactions and specific binding to \(Q_1\) sites only were considered for both Ca\(^{2+}\) and Mg\(^{2+}\). The Ca\(^{2+}\) model parameters optimised for PPHA (log \(K_{1,Ca} = -1.6\) and \(n_{1,Ca} = 0.64\)) were similar to the previously published constants for PUHA and ESHA (Table 1). This result indicated that the Ca\(^{2+}\) binding properties of all three terrestrial humic acids were comparable. Additionally, the fitting of Mg\(^{2+}\) data for PUHA and ESHA (Fig. 2a, b) confirmed that \(Q_2\) sites were not needed to describe the experimental observations for Mg\(^{2+}\). This way, the number of required fitting parameters could be reduced. For PUHA, the best fit was achieved when interactions with \(Q_1\) sites were included in addition to electrostatic interactions. In the case of ESHA, the data was best explained by electrostatic accumulation of Mg\(^{2+}\) in the Donnan phase exclusively, and the corresponding fit results indicated an upper limit of Mg\(^{2+}\) affinities for \(Q_1\) sites of ESHA at log \(K_{1,Mg} \leq -4.0\) (Table 1). The Mg\(^{2+}\) affinities optimised for PUHA (log \(K_{1,Mg} = -2.9\) and PPHA (log \(K_{1,Mg} = -2.4\)) were similar (with \(n_{1,Mg} = 0.72\) for PUHA and PPHA), but were clearly lower than the respective Ca\(^{2+}\) affinities (Table 1). Correspondingly, the calculated fractions of Ca\(^{2+}\) bound to \(Q_1\) sites closely exceeded the fractions of Mg\(^{2+}\) bound to \(Q_1\) sites at ionic strengths of 0.01 M and 0.1 M at ion activities ranging from 10\(^{-8}\) to 10\(^{-3}\) (Fig. 4), which reconfirmed the preference of Mg\(^{2+}\) for electrostatic binding in the model calculations. Overall, the modelling results demonstrated that specific cation binding by the three studied terrestrial humic acids was considerably weaker for Mg\(^{2+}\) than for Ca\(^{2+}\). This

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Fig. 3. Molar H\(^+\)/Mg\(^{2+}\) exchange ratios for Mg\(^{2+}\) binding by (a) PUHA, (b) ESHA and (c) PPHA at ionic strengths of 0.01 M, 0.03 M and 0.1 M as a function of pH and Mg\(^{2+}\) activity in solution. Symbols represent data calculated from magnesium titrations. Lines depict predictions based on the NICA–Donnan model (see Table 1 for model parameters).
important outcome is in clear disagreement with the generic NICA–Donnan model parameters published by Milne et al. (2003) who proposed estimates for Mg$$^{2+}$$ affinity constants for humic acids that exceeded Ca$$^{2+}$$ affinities (log $$K_{1, \text{Mg}} = -0.6$$, log $$K_{1, \text{Ca}} = -1.37$$, log $$K_{2, \text{Mg}} = +0.6$$, log $$K_{2, \text{Ca}} = -0.43$$). Furthermore, specific binding to $$Q_2$$ sites was suggested by Milne et al. (2003) for both Mg$$^{2+}$$ and Ca$$^{2+}$$. The analysis of data on PUHA, PP HA and ESHA, however, provided evidence that specific binding of Mg$$^{2+}$$ and Ca$$^{2+}$$ to $$Q_2$$ sites was quantitatively negligible. As a consequence, cation binding sites with a high affinity for protons, such as the $$Q_2$$ sites, may contribute to Mg$$^{2+}$$ and Ca$$^{2+}$$ by electrostatic binding if deprotonated, i.e. only in the alkaline pH range. Concerning the application of the NICA–Donnan model parameters in geochemical predictions, the use of the published generic Mg$$^{2+}$$ model parameters for humic acids may overestimate Mg$$^{2+}$$ binding by humic acids and is therefore not recommended based on the findings presented here.

The outcome of data modelling with respect to the significance of ligands with high versus low proton affinity for Mg$$^{2+}$$ binding is supported by the comparison of established stability constants for aqueous complex formation of known ligands. For phenols, complexation of Ca$$^{2+}$$ as well as Mg$$^{2+}$$ is reported to be very weak (Martell et al. 2004) and quantitatively negligible at pH below 8.5, which is the pH range that is relevant for most terrestrial environments. Since the $$Q_2$$ sites are considered to mainly represent phenolic moieties being abundant in humic substances, the outcome of the modelling on the negligible role of $$Q_2$$ sites in specific Mg$$^{2+}$$ binding agrees with the known knowledge on complex formation of small organic ligands. Compared with phenols, slightly higher complex formation constants were found for amines, which also exhibit a high affinity for protons similar to phenols. However, the content of amines in NOM samples, such as humic acids, is known to be very low as $$^{15}$$N NMR spectroscopy proved that amide N is the predominant form of nitrogen in humic substances (Knicker et al. 1993; Knicker and Kögel-Knabner 1998). Concerning functional groups with a lower affinity for protons, as represented in the pool of $$Q_1$$ sites in the NICA–Donnan model, moieties other than the highly abundant carboxylic groups may also have to be considered for specific Mg$$^{2+}$$ binding. A comparison with published complex formation constants may again serve as the rationale for this consideration. The stability constants for 1:1 Mg$$^{2+}$$-ligand complexes were reported to be very similar for common O-donor ligands carrying a single negative charge such as acetate (log $$\beta_{11} = 1.26$$; at 25°C and I = 1 M), bicarbonate (log $$\beta_{11} = 1.01$$; at 25°C and I = 0 M), dihydrogen citrate (log $$\beta_{11} = 1.0$$; at 25°C and I = 0 M) and dihydrogen phosphate (log $$\beta_{11} = 1.65$$; at 37°C and I = 0 M) (Martell et al. 2004). For anions that carry two negative charges, the reported stability constants for 1:1 Mg$$^{2+}$$ complexes are considerably higher, including carbonate (log $$\beta_{11} = 2.92$$; at 25°C and I = 0 M), hydrogen citrate (log $$\beta_{11} = 2.59$$; at 25°C and I = 0 M), hydrogen phosphate (log $$\beta_{11} = 2.80$$; at 25°C and I = 0 M), sulphate (log $$\beta_{11} = 2.26$$; at 25°C and I = 0 M), and phthalate (log $$\beta_{11} = 2.52$$; at 25°C and I = 0 M) (Martell et al. 2004). Concerning sulphur, humic acids typically contain low amounts of this element. For the studied samples, sulphur contents amounted to 0.44 wt% for ESHA, 0.71 wt% for PPHA, and 0.4 wt% for PUHA (Christl et al. 2000; International Humic Substances Society (IHSS) 2017). A spectroscopic analysis of PUHA using S XANES identified 62% of the total sulphur in oxidation states 4.9–6.0, which indicated that a high fraction of sulphur was present as sulphonate and sulphate ester (Maurer et al. 2012). However, sulphonates and sulphate ester moieties can carry only one negative charge at pH 8 in contrast to inorganic sulphate and may thus not be among the low proton affinity groups having an enhanced affinity for Mg$$^{2+}$$. Consequently, dianion units, such as e.g. phthalate-like moieties and other dicarboxylic acid units as well as orthophosphate esters, which represent the most abundant form of organic phosphorous in soils (Jarosch et al. 2015), and phosphonates may be relevant as ligands for Mg$$^{2+}$$. Further information on the relevance of the above mentioned possible ligand groups may be derived from the molar H$$^+/$$Mg$$^{2+}$$ exchange ratios determined from the titration experiments. The ratios decreased from values above 0.5 at Mg$$^{2+}$$ activities below 10$$^{-6}$$ to values below 0.2 at Mg$$^{2+}$$ activities above 10$$^{-4}$$ for all three humic acids investigated (Fig. 3). Concerning the above mentioned potential ligands, the pK$$a_2$$ values are known to be lower for dicarboxylic acids (e.g. malonic acid: 5.70, phthalic acid: 5.41) than for phosphate (7.20) and monophosphonic acids (e.g. hydroxymethylphosphonic acid: pK$$a_2$$ = 7.36 at 25°C and I = 0 M; ethylphosphonic acid: pK$$a_2$$ = 7.8 at 25°C and I = 0.1 M; phenylphosphonic acid: pK$$a_2$$ = 7.47, at 25°C and I = 0 M) (Martell et al. 2004). In agreement with these differences in protonation, Mg$$^{2+}$$ complexation by P-containing moieties in humic acids may result in a proton release at pH 8 similar to the experimental observations at low Mg$$^{2+}$$ activities, whereas dicarboxylic acid moieties are expected to be fully deprotonated at pH 8 and, consequently, molar H$$^+/$$Mg$$^{2+}$$ exchange ratios would be close to zero. The latter also holds true for tricarboxylic acid moieties structurally similar to e.g. citric acid (pK$$a_3$$ = 6.40 at 25°C and I = 0 M) (Martell et al. 2004). The reported P contents are 0.24 wt% for ESHA and 0.03 wt% for PPHA (International Humic Substances Society (IHSS) 2017). The analysis of the total P conducted here for PUHA resulted in a similarly low P content (0.08 wt%). Considering the experimental conditions of the titration experiments, these low quantities of P-containing moieties were quantitatively sufficient to account for Mg$$^{2+}$$ binding only at very low Mg$$^{2+}$$ loadings, where molar H$$^+/$$Mg$$^{2+}$$ exchange ratios
The amounts of coagulated PUHA were calculated as the difference between pH 8 and ionic strengths of 0.03 M and 0.1 M as adjusted by NaCl addition. The coagulated PUHA were determined at 0.03 M and 0.1 M NaCl by direct measurement. Table 3 shows the results. The coagulation at pH 8 were highest. At Mg$^{2+}$ activities above $10^{-4.5}$, corresponding to conditions which may be representative for most terrestrial environments, the amounts of P-containing moieties are insufficient to dominate the specific Mg$^{2+}$ binding of the studied humic acids. In accordance with the very low molar H$^+/Mg^{2+}$ exchange ratios, more abundant carboxylic moieties may control Mg$^{2+}$ binding of humic acids under natural conditions in most cases.

Environmental implication

From the results of this study, it can be inferred that in terrestrial environments, Mg$^{2+}$ is bound to NOM predominantly by electrostatic interactions as Mg$^{2+}$ activities typically range above $10^{-4.5}$ (notice the difference to 100% in the calculated speciation for PUHA in Fig. 4). This predominance is expected to have a pronounced impact on the biogeochemical behaviour of magnesium and very likely, also on the fate of trace elements with a tendency to be strongly associated with NOM. The weak, electrostatic binding of Mg$^{2+}$ to NOM, which represents a major sorbent of cations in many soils, keeps the essential nutrient element magnesium in an easily bioavailable form. This way, NOM is likely to represent an important source for magnesium supply to organisms. However, Mg$^{2+}$ may also have a pronounced influence on the behaviour of NOM, especially when compared with Ca$^{2+}$. The stronger preference of Mg$^{2+}$ for electrostatic binding to NOM in combination with the tendency to form a stronger and slightly larger hydration shell makes Mg$^{2+}$ a weaker coagulant for NOM than Ca$^{2+}$, as shown for PUHA in Fig. 5. Similar differences between Ca$^{2+}$ and Mg$^{2+}$ have also been reported, e.g. for the aggregation of humic acid–Fe colloids and citrate-coated gold nanoparticles (Liao et al. 2017; Liu et al. 2013). Considering the levels of total calcium and magnesium in soil solution amount up to ~5 mM and ~1 mM (Kretzschmar (2016), respectively, higher magnesium to calcium ratios are expected to result in an enhanced dispersion and thus in an increased mobility of NOM. Therefore, the levels of both of the most abundant cations, Ca$^{2+}$ and Mg$^{2+}$, need to be considered for assessing the mobility of trace elements which are strongly associated with NOM, such as e.g. mercury, although Ca$^{2+}$ and Mg$^{2+}$ bind to NOM weakly and cannot directly compete with strongly sorbing trace element cations for specific binding to NOM.

Supplementary material

Experimental data and modelling results on the pH- and ionic strength-dependent charging behaviour of PPHA (Fig. S1), on Ca$^{2+}$ binding by PPHA at pH 5 and 8 in 0.01 M and 0.1 M NaCl (Fig. S2) and corresponding H$^+/Ca^{2+}$ exchange ratios (Fig. S3), as well as the amounts of Mg$^{2+}$ and Ca$^{2+}$ associated with Q, sites of ESHA and PPHA as calculated with the NICA–Donnan model (Fig. S4), and the amounts of Mg$^{2+}$ and Ca$^{2+}$ associated with coagulated and precipitated PUHA as measured in the coagulation experiment (Fig. S5) are provided as supplementary material. All data on Ca$^{2+}$ and Mg$^{2+}$ binding by PUHA, ESHA and PPHA, as reported in this paper, are also available in electronic form as a supplementary data file.

Conflicts of interest

The author declares no conflicts of interest.

Acknowledgements

This research did not receive any specific funding.

References


elemental compositions and stable isotopic ratios of ihs samples [verified 17 April 2018]


Keizer MG, van Riemersdijk WH (2006). ‘ECOSAT.’ (Department of Soil Quality: Wageningen University, The Netherlands)


Handling Editor: Montserrat Filella