Lability of copper bound to humic acid

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HIGHLIGHTS

- Isotopic dilution was used to assess the lability of Cu bound to humic acids.
- Time-dependent Cu fixation by humic acid was observed over 160 d.
- Around 40% of Cu bound to HA may be non-labile.
- Non-labile Cu in humic acids may affect predicted speciation by geochemical models.

ARTICLE INFO

ABSTRACT

Geochemical speciation models generally include the assumption that all metal bound to humic acid and fulvic acid (HA, FA) is labile. However, in the current study, we determined the presence of a soluble 'non-labile' Cu fraction bound to HA extracted from grassland and peat soils. This was quantified by determining isotopically-exchangeable Cu (E-value) and EDTA-extraction of HA-bound Cu, separated by size-exclusion chromatography (SEC) and assayed by coupled ICP-MS. Evidence of time-dependent Cu fixation by HA was found during the course of an incubation study (160 d); up to 50% of dissolved HA-bound Cu was not isotopically exchangeable. This result was supported by extraction with EDTA where approximately 40% of Cu remained bound to HA despite dissolution in 0.05 M Na_2-EDTA. The presence of a substantial non-labile metal fraction held by HA challenges the assumption of wholly reversible equilibrium which is central to current geochemical models of metal binding to humic substances.

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1. Introduction

Geochemical models, such as WHAM-VII (Tipping, 1998; Lofts and Tipping, 2011) and the NICA-Donnan Model (Kinniburgh et al., 1999), include the assumption that trace metals sorbed on humic substances are entirely labile – i.e. they participate in a reversible dynamic equilibrium with free metal ions. However, there is increasing evidence that a non-labile fraction may exist in sub-micron sized colloidal phases and bound to dissolved species. For example, in a study of 18 soils, Ma et al. (2006) found non-isotopically exchangeable Cu in the solution phase filtered through 0.2 μm cellulose acetate filters; they suggested the Cu was likely to be associated with dissolved organic matter. Similar suggestions have been made by the same research group (Lombi et al., 2003;

Nolan et al., 2009) for Ni, Cu and Zn. Jackson et al. (2005) in a study of dissolved organic matter (DOM) in river water, measured Ni and U distribution in a colloidal phase separated by size exclusion chromatography (SEC) or flow field-flow fractionation (FIFFF) and concluded that Ni was 'more labile' than U because Ni^{2+} appeared to interact with the SEC column gel phase and the FIFFF membrane to a greater degree. By contrast Laborda et al. (2011), used asymmetrical flow field flow fractionation (AsFIFFF) to separate dispersed colloidal phases of isotopically spiked compost material and, from measurements of the isotopic ratio within different size fractions including the free inorganic phase, they concluded that all Cu and Pb present was isotopically exchangeable.

It is accepted that metals can be held by very strong binding sites on humic substances through the formation of 'multidentate' complexes with carboxylic and phenolic groups or by specific binding with N and S groups which are known to preferentially bind metals such as Cr^{III}, Fe^{III}, Cu and Hg (Tipping, 2002). However, strong chemical bond formation does not necessarily result in non-labile complex formation and there have been very few

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studies that have attempted to determine the presence of such a metal fraction on dissolved humic substances. It is well established that isotopic dilution (ID) approaches and some chemical extractants (eg 0.05 M EDTA) can be used to estimate the labile fraction of trace metals in soils (Gabler et al., 2007; Degryse et al., 2009). However, to measure metal lability on a dissolved system containing a range of metal complex species requires a fractionation technique to separate high MW DOM-bound metal from low MW organic and inorganic complexes. This approach then also allows fractionation of spike isotopes introduced to follow the kinetics of metal assimilation throughout the various species and quantification of the isotopically exchangeable forms present. Size exclusion chromatography (SEC) coupled with inductively coupled plasma mass spectrometry (ICP-MS) has been applied to the study of trace element distributions within humic acids (Schmitt et al., 2001; Jackson et al., 2005; Liu and Cai, 2010; Liu et al., 2011; Kozai et al., 2013).

The aim of this investigation was to determine the lability of Cu bound to dissolved humic acid (HA) to test the hypothesis that Cu bound to dissolved HA is labile. To achieve this, two popular techniques for measuring labile metals in soils were modified and applied to two types of HAs fractionated using SEC-ICP-MS: extraction with EDTA and determination of isotopic exchangeability. Dissolved HA was incubated with an enriched stable Cu isotope and its subsequent extractability with EDTA determined from differences in isotopic abundance between forms bound to HAs and to low MW complexes with EDTA. Humic acid solutions were incubated with Cu (normal isotopic mix) for a period of time before they were spiked with an enriched stable Cu isotope. The proportion of dissolved HA-bound metal that was isotopically exchangeable (% E-values; Smolders et al., 1999; Young et al., 2000) was then determined from changes in isotope distribution between Cu complexes with HAs and the Tris buffer (Tris (hydroxymethyl) aminomethane) used as the chromatography eluent.

2. Materials and methods

2.1. Humic acid preparation and characterisation

Two humic acids were previously extracted and purified by Marshall et al. (1995) from a permanent grassland soil (G-HA; Nottinghamshire, UK) and Sphagnum moss peat (P-HA; North Yorkshire, UK). The ash content was measured by dry combustion at 500 °C for 8 h as 23.5 g kg^{-1} and 3.9 g kg^{-1} for G-HA and P-HA respectively. Total acid group concentrations determined by Marshall et al. (1995) were 5.50 mol$_c$ kg^{-1} for G-HA and 6.94 mol$_c$ kg^{-1} for P-HA using a modified Ba(OH)$_2$ method (Schnitzer and Khan, 1972) including a pre-dissolution step in 0.1 M NaOH to ensure complete hydration of the HA. In this study, the freeze dried HAs were dissolved in 0.5 M NaOH and pH adjusted to 7.0 with dilute nitric acid to provide working HA stock solutions of 16.0 g L^{-1}.

2.2. Size-exclusion chromatography (SEC-ICP-MS)

Fractionation of HA based on molecular size was undertaken using size-exclusion chromatography (SEC) with an ICS-3000 chromatography system (Thermo-Fisher Scientific) and a Superose 12 10/300 GL column (GE Healthcare) with a separation range from 1000 to 300 000 Da. A guard column (Hamilton, Peek PRP-X100) was installed prior to the SEC column. Humic acid samples of 100 μL were injected into an eluent comprising 0.1 M Tris buffer at pH 8.2 adjusted with nitric acid at a flow rate of 1 mL min^{-1}. The pH of the eluent was chosen to coincide broadly with the maximum buffer power of the Tris (pKa = 8.1) and the

equivalence-point of carboxyl groups on the HA. The column outflow was connected directly to the nebuliser of an ICP-MS (XSeries^{II}; Thermo-Fisher Scientific) operating in 'collision cell with kinetic energy discrimination' mode (7% hydrogen in helium) to reduce polyatomic interferences (e.g. ^{35}Cl-^{35}Cl interference with ^{70}Zn). The minimum and maximum quadrupole settle times were adjusted to 1000 μs, to minimize the effects of plasma flicker, and Cu isotopes (^{63}Cu & ^{65}Cu) were measured with a quadrupole dwell time of 40 ms. The instrument 'dead time correction factor' was set to 37.2 ns after establishing the value which gave a zero slope in a plot of isotope ratio against concentration of Pb in solutions of the Pb isotope standard NIST-981. The ICP-MS was directly calibrated with both Cu isotopes, precluding the need for internal or external mass discrimination. For pragmatic reasons (nebuliser capacity) we did not include an internal standard line downstream of the SEC column. For discrete sample analysis which may vary in matrix composition this is vital but considering (i) the use of standard additions for calibration (Section 2.5), (ii) prior knowledge of the total Cu concentration in each sample and (iii) the constancy of the eluent matrix, we did not consider that addition of an internal standard would improve reliability of data. In practice, we found very high reproducibility of chromatograms without an internal standard. A sample loop flushing step between each sample was standard on the SEC chromatography program to avoid carryover and cross-contamination. Chromatographic data were collected for 34 min but typically the data of interest were integrated between 5 and 25 min.

2.3. Determination of Cu extractable by EDTA from humic acid

A stable isotope of Cu with enriched isotopic abundance (IA) was purchased from ISOFLEX USA as a metal foil and dissolved in concentrated HNO$_3$. Dissolved humic acid, at concentrations of 4.0 g L^{-1} (G-HA) or 4.4 g L^{-1} (P-HA), were incubated with the diluted stable isotope solution: 168 μg L^{-1} ^{65}Cu (IA $\geqslant$ 99.0%). The Cu concentration was chosen to be high enough to give a measurable ICP-MS signal after complex-formation. The concentration of functional groups (total acidity) in the dissolved HA solutions (mol$_c$ L^{-1}) was calculated from the values provided by Marshall et al. (1995), as 0.022 mol$_c$ L^{-1} for G-HA and 0.031 mol$_c$ L^{-1} for P-HA, while the spiked isotope solution contained 2.6×10^{-6} mol$_c$ L^{-1} of Cu. After equilibration for 40 or 160 d at 18 °C the spiked solutions were diluted with 0.1 M Tris buffer at pH 8.2 (to give final concentrations of 1.0 g L^{-1} for G-HA and 1.1 g L^{-1} for P-HA) and equilibrated for 2 h with a range of Na$_2$-EDTA concentrations (0, 0.0005, 0.0025, 0.01, 0.025 and 0.05 M). Samples were analysed by SEC-ICP-MS to determine the concentrations of Cu complexes formed with EDTA, Tris and HA; all samples had Na$_2$-EDTA added exactly 2 h before the start of their chromatographic runs. The proportion of EDTA-extractable Cu was calculated as the amount of ^{65}Cu removed from the HA complexes by EDTA divided by the total ^{65}Cu added to the system. Estimation of Cu recovery from the chromatographic process is discussed in Section 2.5 and the procedure for chromatograph integration is discussed in Section 3.2.

2.4. Isotopically exchangeable Cu

Isotopic exchange kinetics and E-values for Cu (M_{E-HA}) were determined for G-HA (4.0 g L^{-1}) and P-HA (4.4 g L^{-1}) solutions previously incubated for 40 d with a spike prepared from a Cu ICP-MS standard solution of 240 μg L^{-1} (natural isotopic abundance). Immediately following further dilution with Tris buffer, the samples were spiked with sufficient enriched ^{65}Cu solution to give measureable increases in ICP-MS signal. The final concentration of added ^{65}Cu was 118.0 μg L^{-1}. This may seem quite a high spike