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Heterogeneity in metal binding by individual fluorescent components in a eutrophic algae-rich lake

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ABSTRACT

Dissolved organic matter (DOM) affects the toxicity, mobility and bioavailability of metals in aquatic environment. In this study, the interactions between two metals of environmental concern [Cu(II) and Fe (III)] with DOM in a euthrophic algae-rich lake (Lake Taihu, China), including dissolved natural organic matter (NOM) and algal extracellular polymeric substance (EPS), were studied using fluorescence excitation–emission matrix (EEM) quenching titration combined with parallel factor (PARAFAC) analysis. Obvious protein-like peaks were detected in algal EPS matrix, while both protein- and humic-like peaks can be found in NOM. PARAFAC analysis identified four fluorescent components, including one humic-, one tryptophan- and two tyrosine-like components, from 114 EEM samples. It was shown that fluorescent tyrosine- ($\log K_M > 5.21$) and humic-like substances ($\log K_M > 4.84$) in NOM fraction exhibited higher metal binding capacities than those in EPS matrix, while algal EPS was characterized with a high metal-tryptophan-like substances affinity ($\log K_M > 5.08$). Moreover, for the eutrophic algae-rich lakes, fluorescent tryptophan- and humic-like substances were responsible for Cu transportation, whereas the mobility of Fe would be related with the tyrosine-like substances. The results facilitate a further insight into the biogeochemical behaviors of metals in eutrophic algae-rich ecosystems as well as other related aquatic environments.

1. Introduction

Metal toxicity, mobility and bioavailability are controlled by their speciation (Yamashita and Jaffe, 2008; Baken et al., 2011). Dissolved organic matter (DOM) in aquatic environment can interact with metals and exhibit a substantial effect on metal speciation (Hur and Lee, 2011; Guo et al., 2012). Thus, insight into the interaction between metals and DOM is necessary to better understand the toxicity, mobility and bioavailability of metals.

Previous studies on the binding of metals to organic ligands in aquatic environment were mainly focused on the dissolved natural organic matters (NOM) from slough systems (Haitzer et al., 2002; Yamashita and Jaffe, 2008), lake waters (Xue and Sigg, 1999), ponds and reservoirs (Baken et al., 2011). However, in some aquatic ecosystems such as the eutrophic shallow lakes, algal blooms usually occur due to climatic changes and nutrient enrichment (Paerl et al., 2011). Extracellular polymeric substance (EPS), a high molecular weight biopolymer produced via excretion, secretion, sorption and cell lysis, etc., is the inevitable substance during algal growth (Klock et al., 2007), and may significantly contribute to the dissolved organic carbon pool in lakes (McIntyre and Gueguen, 2013). In addition, algal EPS was reported to be essential to protect algal cells from metal toxicity through binding process (Pereira et al., 2011). Therefore, investigations on metal–DOM interaction in these algae-rich waters should include the related information of not only NOM but also algal EPS for a more comprehensive understanding.

Numerous analytical techniques had been previously reported to probe the metal binding properties, which included ion selective electrode (ISE) (Saar and Weber, 1982), cathodic stripping voltammetry (CSV) (Yang and Vandenberg, 2009), one-step resin-exchange (Baken et al., 2011), and fluorescence quenching titration (McIntyre and Gueguen, 2013; Sheng et al., 2013). Among them, fluorescence excitation–emission matrix (EEM) quenching titration has been widely utilized due to its rapid, effective and sensitive nature. Through determining the different quenching

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degrees, one can obtain the metal binding information. However, the raw EEM spectra usually suffered many overlapped peaks, making the further analysis laborious and difficult based solely on the traditional visual inspection approach (Henderson et al., 2008; Guo et al., 2012). Recent studies showed that a multivariate analysis method named parallel factor (PARAFAC) analysis can be used to solve the peak overlapping problems by deconvoluting the complex EEMs into several independent components and can thus provide an enhanced understanding about metal–DOM interaction (Ohno et al., 2008; Yamashita and Jaffe, 2008; Xi et al., 2012; Ishii and Boyer, 2012). However, to our best knowledge, this technique has not been applied to investigate the metal binding difference between NOM and algal EPS in eutrophic algae-rich lakes.

Increasing attention is focused on China Lake Taihu (30°55′40″–31°32′58″N and 119°52′32″–120°36′10″E), a typical eutrophic shallow lake with an area of 2338 km^2 and a mean depth of 1.9 m, who is suffering serious eutrophication (Shen et al., 2007; Paerl et al., 2011). Large amounts of algal blooms occurred regularly each year from March to November throughout much of the lake (Li et al., 2011), and the algal species mainly consisted of cyanobacterial *Microcystis* spp. (Wang et al., 2012). In recent years, the inflow of increased untreated or partially treated wastewater from industry, agriculture and sewage resulted in the high Cu(II) and Fe(III) concentrations (Shen et al., 2007; Yu et al., 2012).

Therefore, the objectives of this study were (1) to investigate the composition difference between NOM and algal EPS, (2) to compare the spectral changes caused by metal addition between NOM and algal EPS, and (3) to obtain the metal binding heterogeneity of individual fluorescent components through using fluorescence quenching titration combined with PARAFAC analysis. For this purpose, the EPS matrix was specifically fractionated into loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) in terms of the binding strength with algal cells (Xu et al., 2013a, 2013b). Bloom samples were collected from lake Taihu, and two widely found metals [Cu(II) and Fe(III)] were used as fluorescent quenching agents for titration. The binding properties of the selected metals with fluorescent components in NOM, LB-EPS and TB-EPS fractions were respectively investigated. Results obtained here would provide a comprehensive view of the biogeochemical behaviors of metals in eutrophic shallow lakes as well as other related aquatic environments.

2. Materials and methods

2.1. Sample preparation

In this study, a total of 2.4 L of surface water samples (30 cm from the surface) containing 1.0 g L^{-1} algal biomass were collected for one time during the summer-fall period (16 August, 2012), since this period suffered the typical algal blooms (Qin et al., 2010). The concentrations of total suspended solids and volatile suspended solids were 1.15 and 1.02 g L^{-1}, respectively. The samples were collected by a plexiglass column (length 30 cm, inner diameter 10 cm) from Meiliang Bay, the north region of Lake Taihu (Fig. S1 in the Supporting information, SI), stored on ice and transported to laboratory within several hours. The samples were firstly filtered through the 0.45 μm polytetrafluoroethylene membranes (Xingya Purification Materials Co., Shanghai, China) for NOM measurement. Secondly, the residues were carefully scraped and dissolved with a 0.05% NaCl solution to the original volume and centrifuged at 5000*g* for 15 min, with the liquid collected carefully for measurement of LB-EPS. Thirdly, the harvested algae samples were re-suspended in NaCl solution, heated at 60 °C for 30 min and centrifuged at 15,000*g* for 20 min, with the liquid collected as TB-EPS fraction (Xu et al., 2013b).

2.2. Fluorescence titration

Titration experiments were carried out by adding 0.1 mol L^{-1} $Cu(NO_3)_2$ or $Fe(NO_3)_3$ to a series of brown sealed vials which contained 50 mL of diluted solution [dissolved organic carbon (DOC) < 10 mg L^{-1}] using an automatic syringe (Ohno et al., 2008; Yamashita and Jaffe, 2008). It was noted that no more than 50 μL of metal titrant was added, making the metal concentrations in the final solutions ranging from 0 to 100 μmol L^{-1}. The pH was maintained at 6.0 under which no precipitate was formed (Ohno et al., 2008). After metal addition, all solution samples were shaken for 24 h at room temperature to ensure complexation equilibrium (Yamashita and Jaffe, 2008). Each titration experiment was performed in duplicate.

2.3. Fluorescence EEM determination and PARAFAC analysis

Fluorescence EEMs were obtained using the Hitachi F-7000 fluorescence spectrometer (Hitachi High Technologies, Tokyo, Japan) in scan mode with a 700-voltage xenon lamp at room temperature. EEM spectra were gathered with scanning emission (Em) spectra from 250 to 550 nm at 2 nm increments by varying the excitation (Ex) wavelength from 200 to 450 nm at 10 nm increments. The spectra were recorded at a scan rate of 1200 nm min^{-1}, using Ex and Em slit bandwidths of 5 nm. Water Raman scatter peaks were eliminated by subtracting the EEM spectra of Milli-Q water blank.

The approach of PARAFAC modeling has been described in detail elsewhere (Ohno et al., 2008; Stedmon and Bro, 2008; Yamashita and Jaffe, 2008), and only a brief description will be given here. PARAFAC is a generalization of bilinear principal component analysis to higher order arrays, which decomposes *N*-way arrays into *N* loading matrices. Thus, if fluorescence EEMs are arranged in a three-way array *X* of dimensions $I \times J \times K$ (*I*: sample number; *J*: Em number; *K*: Ex number), PARAFAC can decompose them into three matrices *A* (the score matrix), *B* and *C* (loading matrices) with elements a_{if}, b_{jf}, and c_{kf}.

The PARAFAC analysis in this study was conducted in MATLAB 7.0 (Mathworks, Natick, MA) using the DOMFluor toolbox (http://www.models.life.ku.dk/). The non-negativity constraints were applied to allow only chemically relevant results. No outlier samples were found by leverage comparison, and a total of 114 fluorescence EEM data array (114 samples × 26 Ex × 151 Em) were obtained for PARAFAC analysis. The residual analysis, split half analysis, and visual inspection were applied to determine the correct numbers of components (Stedmon and Bro, 2008).

2.4. Complexation modeling

The Ryan–Weber and the modified Stern–Volmer model were two widely used methods to estimate the metal binding parameters. The Ryan–Weber model is dependent on the 1:1 metal/ligand complexes, but the linear relationship between metal-bound ligand concentration and the quenched DOM fluorescence intensity may not valid if stable non-fluorescent complexes were formed and/or more than two different binding sites existed (Hur and Lee, 2011). The modified Stern–Volmer equation can partially solve the problems associated with non-linear fluorescence response by estimating the contribution portion of unquenched fluorescence (Hays et al., 2004; Hur and Lee, 2011). For this reason, the modified Stern–Volmer equation is applied in this study, with the following forms:

$$\frac{F_o}{F_o - F} = \frac{1}{fK_M C_M} + \frac{1}{f} \quad (1)$$

here *F* and F_o are the measured fluorescence component scores (arbitrary units per unit DOC, A.U./mg/L) at the total metal concentration C_M (μmol/L) and the beginning of the titration (i.e., no metal addition), respectively. The parameter K_M was the conditional stability constant at the certain experimental condition, and *f* represents the fraction of the initial fluorescence, which is accessible to quencher. The parameters of *f* and K_M were solved by plotting $F_o/(F_o - F)$ against $1/C_M$.

2.5. Other physicochemical analysis

The algal biomass of sample was measured by drying samples at 60 °C for 2 days to obtain a constant weigh. The DOC content was measured by a TOC-VCPH analyzer (TOC-4000, Shimadzu, Japan), and the metal content was determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES, Prodigy, USA).

3. Results and discussion

3.1. Concentration comparison of organic matters and metals between NOM and algal EPS

Table 1 compares the concentrations of organic matters and metals between NOM and algal EPS. A high DOC concentration of 7.66 mg L^{-1} for NOM was found in this study, which was higher than those of Yao et al. (2011) who reported a DOC concentration ranging from 0.65 to 4.68 mg L^{-1} with a mean of 2.19 mg L^{-1} in lake Taihu. The contents of algae-borne organic matters were influenced by many factors including the algal biomass, algal species as well as growth stage (Henderson et al., 2008; Xu