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Characterizing the fluorescent properties and copper complexation of dissolved organic matter in saline-alkali soils using fluorescence excitation-emission matrix and parallel factor analysis

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Abstract

Purpose The goals of this study are to identify the individual fluorophore component in dissolved organic matter (DOM) of saline-alkali soils, to determine the binding capacities and stability constants of Cu (II) with fluorescent DOM components, and to analyze the potential impact on the complexation between Cu (II) and DOM in different soil environment.

Materials and methods DOM extracted from five saline-alkali soil samples were studied by fluorescence quenching titrations combined with three-dimensional excitation-emission matrix (3D-EEM) spectra and parallel factor analysis (PARAFAC). Modified Stern–Volmer model was used to calculate the conditional stability constant (K) and the percent of fluorophores ($f\%$) participating in the complexation reaction.

Results and discussion Three main fluorescent components in the DOM, e.g., fulvic-like (component 1), humic-like (component 2), and protein-like (component 3) were identified and characterized by fluorescence EEM-PARAFAC. Both the components 1 and 2 showed fluorescence quenching by gradual addition of Cu (II), while component 3 had no marked change in fluorescence intensity. The higher log K of DOM-Cu (II) complexes suggests that the DOM has a high Cu binding affinity. Meanwhile, humic-like components showed higher in the proportion of organic ligands than those of fulvic-like components in the DOM of saline-alkali soils.

Conclusions The fluorescence quenching titration using EEM-PARAFAC analysis allows for the assessment of metal ion interactions with specific fluorophores, and is a good approach to study the geochemical behavior of metal ions in saline-alkali soils and to provide a support for the management of saline-alkali soil environment.

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1 Introduction

Even at the lowest levels in the percent composition of soils, soil organic matter is well known to be responsible for major soil properties such as fertility, permeability, and structural stability, particularly its most soluble fractions, fulvic and humic acids (Nichols and Wright 2006). Dissolved organic matter (DOM) is an important component of soil organic matter, which is defined as the water soluble organic material that can pass through a 0.45-μm membrane filter, and consists of a complex mixture of relatively low molecular weight compounds bearing both polar and non-polar functional groups (Zsolnay 1996). DOM has the ability to form stable complexes with heavy metals and can facilitate their transport

toward the groundwater (Knoth de Zarruk et al. 2007). Most agricultural soils have a history of pesticide treatment, notably with copper (Cu), which is consequently found in many arable soils (Vavoulidou et al. 2005). Moreover, DOM in soil or water consists of a complicated mixture of organic substances containing a variety of different organic functional groups such as carboxyls (COOH), phenols or alcohols (OH), thiols (SH), and amines (NH_2) (Smith et al. 2002). These reactive functional groups in the molecules present in DOM account for their important environmental roles (Mounier et al. 2011). Without doubt, all of these functional groups mentioned above are also responsible for complexation with Cu (Karlsson et al. 2006). Metals transported by DOM through the soil into the surface and/or groundwater will undergo further reaction depending on the chemistry of the receiving waters (Christopher et al. 2002).

Fluorescence spectroscopy has been widely used to evaluate the structural and compositional changes in the soil DOM (Kalbitz et al. 1999; Plaza et al. 2006). Fluorescence excitation-emission matrices (EEMs) can record a whole range of incrementing excitation wavelengths and their corresponding emission data. Multiple peaks and their specific locations can be attributed to various fluorophores in complex DOM mixtures. Finally, the combined techniques of EEMs with parallel factor (PARAFAC) analysis can divide EEMs into fluorescent components (Stedmon and Bro 2008). Therefore, this approach provides a considerable advantage over the traditional methods of interpreting the multidimensional nature of EEM data sets. More detailed information about the DOM character of soil samples can be obtained by using the PARAFAC analysis.

Various methods and techniques have been used in studying DOM binding to metal ions. Among them, fluorescence spectroscopy has proven to be one of the most promising and productive one (Knoth de Zarruk et al. 2007; Yamashita and Jaffé 2008; Hur and Lee 2011). The fluorescence quenching technique has been applied to define conditional stability constants and binding capacities, which can be calculated by a single-site fluorescence quenching model (Ryan and Weber 1982), modified Stern–Volmer equation (Esteves da Silva et al. 1998), and Langmuir-type model (Pagnanelli et al. 2003). In order to quantify the stability constant and complexing capacity, the binding sites causing the fluorescence quenching, defined as the fluorescence binding sites, are assumed to form 1:1 complexes between the DOM and Cu (II). Based on these assumptions, the modified Stern–Volmer equation (Esteves da Silva et al. 1998) was used in our study to estimate the complexing parameters. Consequently, the combination of EEM quenching and PARAFAC analysis has been introduced to characterize the binding properties of DOM and metal ions (Ohno et al. 2008; Wu et al. 2011).

The pH is one of the most important factors affecting the lability of heavy metals in soil. Hydrolysis of metal ions at higher pH tends to increase sorption, because hydrolyzed species have lower solvation energies for surface binding than do aquo metal ions (James and Healy 1972). Increases in pH decrease surface potential and proton competition, and thus favor metal binding (Yin et al. 2002). McBride (1989) suggested that higher pH promotes dissolution of soil organic matter and increases total dissolved Cu concentration because of Cu-organic complex formation. Due to a very high pH in saline-alkali soils, the solubility of DOM may cause its conformational changes that may expose more binding sites. There have been no studies using PARAFAC to explore metals interactions with DOM from saline-alkali soils. The characterization of DOM and Cu (II) interaction in saline-alkali soils using EEM and EEM-PARAFAC analysis will significantly contribute to a better understanding of the geochemical behavior of Cu. The goals of this study are as follows: (a) to identify the individual fluorophore components in the DOM of saline-alkali soils using the PARAFAC model, (b) to determine the binding capacities and stability constants of Cu (II) with fluorescent DOM components using EEM-PARAFAC analysis combined with the modified Stern–Volmer equation, and (c) to analyze the potential impact on the complexation between Cu (II) and DOM in different soil environments.

2 Materials and methods

2.1 Study area and sampling collection

These studies were conducted at Lake Wuliangsuhai, (108° 43′~108° 57′E, 40° 27′~40° 03′N) located in the eastern Hetao Irrigation District in the Inner Mongolian Autonomous Region of China. This lake is the biggest fresh water body along the Yellow River Basin, covering a total area of 293 km^2 with an average total water volume of 250 to 300 million m^3. There is a sound agricultural irrigation and drainage network in the eastern Hetao Irrigation District. Lake Wuliangsuhai is an important constituent part of agricultural irrigation and drainage network, which plays a key role in water purification, and is also the primary water source for agricultural irrigation. Serious soil erosion and salinization occurred in the region as reported by Yu et al. (2010).

Saline-alkali soil samples were collected from the following five locations in the surrounding areas of Lake Wuliangsuhai for our study purposes: (i) AAF, an abandoned farmland; (ii) ASC, agricultural soils from corn fields; (iii) ASW, agricultural soils from wheat fields; (iv) SSE, a soil with vegetation named *Salicornia europaea*; and (v) GKF, a grown *Kalidium foliatum* community. The soil samples were collected from three different depths including 0–20, 20–40, and 40–60 cm; and named after its depth as $M_{0–20}$, $M_{20–40}$, and $M_{40–60}$, while M was replaced by one of these locations AAF, ASC,

ASW, SSE, and GKF. The soil samples were taken randomly within an area of 2-m radius from the marked plots using a tube sampler. Each soil sample was thoroughly mixed and homogenized after carefully removing the organic materials and roots. Physicochemical characteristics of the selected soils have been listed in Table 1.

Each soil sample was air-dried and sieved (100 mesh sieves). The DOM samples of all the five soils were extracted after 40 g of the soils were mixed with 100 mL of Milli-Q water and continuously shaken for 24 h at 25 °C. Duplicate or triplicate extractions and measurements were made depending on the availability of samples. The supernatants were collected each time. The supernatants were centrifuged for 10 min at 7000 rpm at 4 °C. Immediately after collection, DOM samples were filtered on a pre-combusted (5 h at 450 °C) glass fiber filter (Whatman GF/F, nominal pore size 0.45 μm). A vacuum suction filter machine was used to extract the DOM samples. The pH values and concentrations of DOM samples were measured by a Sartorius PB-10 pH meter and a total organic carbon analyzer (multi N/C 2100, Analytikjena), respectively.

2.2 Spectral analysis of DOM samples

Each DOM sample was diluted to 10 mg/L of dissolved organic carbon (DOC) concentration to make their comparisons possible and to avoid the inner filter effect by adding Milli-Q water to DOM samples to provide an optical density of 0.02 at 300 nm. The adjustments in pH values were performed by adding small volumes of 0.1 M of NaOH or HNO_3 solutions to the desired level (pH=8±0.05, T=25±0.1 °C). The EEM fluorescence spectra were measured in 1 cm quartz fluorescence cells at a constant temperature (~25 °C) and pH (8.0± 0.05), using a fluorescence spectrofluorometer (F-7000,

Hitachi, Japan) equipped with a 150-W Xenon arc lamp as the light source. The slit width was set at 5 nm for both the excitation and emission monochromators, and the scan speed was set at 1200 nm min^{-1}. The EEM spectra were obtained by scanning the emission (Em) wavelengths from 280 to 550 nm in 5 nm increments, while the excitation (Ex) wavelength increased from 220 to 450 nm in 5 nm increments. The EEM contour maps were obtained in which each fluorophore was characterized by an Ex/Em wavelength pair. Samples having absorbance greater than 0.02 at 350 nm (A_{350}>0.02 for 1 cm path length) were diluted with particle-free Nanopure Milli-Q water (also used as the blank in fluorescence measurements) to account for the inner filter effects. Any inner filter effects at wavelengths less than 350 nm were assumed to be negligible (Moran et al. 2000). An EEM of the Milli-Q water solution was obtained and subtracted from the EEM of each soil sample spectra in order to remove most of the Raman scatter peaks (Chen et al. 2003). Then the EEMs were normalized to daily-determined water Raman integrated area maximum fluorescence intensity (exCitation wavelength of 350 nm) according to the literature (Lawaetz and Stedmon 2009). Fluorescence intensity was calibrated in quinine sulfate units (QSU), where 1 QSU is the maximum fluorescence intensity of 0.01 mg L^{-1} of quinine (qs) in 1 N of H_2SO_4 at the excitation wavelength (Ex; nm)/emission wavelength (Em; nm)=350/450. Rayleigh scatter effects were removed from the data set by excluding any emission measurements made at wavelengths≤Ex +5 nm, and at wavelengths≥Ex +300 nm. Zero was added to the 3D-EEMs in the two triangle regions (Em≤Ex +5 nm, and ≥Ex + 300 nm) of the missing data.

Table 1 Physicochemical characteristics of the selected soils. The pH, EC, anion, and cation values are averages of two or three replicates (coefficient of variation <3 %)

Sampling	pH	EC mS m^{-1}	Ca^{2+} mmol kg^{-1}	Mg^{2+}	K^+	Na^+	SO_4^{2-}	Cl^-	TOC g kg^{-1}
AAF_{0-20}	8.08	3.31	1149.83	256.08	322.60	1053.56	34.38	75.33	0.55
AAF_{20-40}	8.19	3.90	1364.30	311.33	266.47	1356.88	20.37	33.69	0.97
AAF_{40-60}	8.29	2.81	590.91	230.41	205.90	507.61	8.21	16.24	0.65
ASC_{0-20}	8.12	2.29	577.02	232.86	254.04	519.80	6.41	8.82	0.86
ASC_{20-40}	8.10	2.25	529.86	279.01	212.60	505.27	6.52	10.07	0.63
ASC_{40-60}	8.08	2.26	507.54	253.96	66.60	498.43	6.50	10.10	0.8
ASW_{0-20}	7.94	2.27	579.49	216.72	78.30	213.39	2.38	3.11	1.16
ASW_{20-40}	7.94	2.32	538.42	234.22	86.57	238.68	3.15	3.02	1.06
ASW_{40-60}	7.96	2.30	490.67	239.95	74.97	291.20	3.47	3.12	0.75
SSE_{0-20}	8.77	13.11	1611.12	319.63	241.83	1889.07	17.70	45.42	0.49
SSE_{20-40}	8.80	5.92	1415.48	214.41	197.02	1510.46	13.92	32.89	0.15
SSE_{40-60}	8.58	3.77	158.41	101.30	165.67	246.92	7.58	15.74	0.34
GKF_{0-20}	8.74	21.10	1739.85	343.28	317.56	2198.31	44.64	137.79	0.93
GKF_{20-40}	8.57	6.26	1325.80	272.19	292.08	1647.45	37.19	118.43	0.97
GKF_{40-60}	8.44	3.80	1263.68	176.72	211.51	1315.16	32.62	108.85	0.36

2.3 Fluorescence quenching experiment

Prior to the quenching experiments, Cu was not detected in all the above samples determined by atomic absorption spectrophotometry (AA-6300, Shimadzu, Detection limit: ppb) at the same DOM concentrations (10 mg/L). Complexation studies of DOM samples with Cu (II) were carried out by adding Cu (NO$_3$)$_2$ solutions into the DOM samples. In order to avoid the concentration change, less than 5 % of total volume of the solution was added. The final Cu (II) concentrations of the solutions prepared were 0, 1, 2, 10, 20, 40, 100, and 200 μM L^{-1} for the eight aliquots of each soil DOM sample. All the samples were continuously shaken for 24 h at 25 °C to ensure that the complexation equilibrium was reached.

In order to quantify the complexation of Cu (II) ions and DOM, the modified Stern–Volmer equation was used to calculate the conditional stability constants and binding capacities. The modified Stern–Volmer equation is given as follows (Esteves da Silva et al. 1998).

$$I_0 \Big/ (I_0 - I) = 1 \Big/ (fK[\mathrm{Cu(II)}]) + 1 \Big/ f \qquad (1)$$

where I and I_0 are the fluorescence intensities of the DOM sample with and without the addition of Cu (II) ions, respectively. K is the conditional stability constant. The proportion of organic ligands which can interact with the free metal ions in DOM is represented by f. If the plot of $I_0/(I_0-I)$ vs. 1/[Cu (II)] is linear, K and f values can be estimated from the slope (1/fK) and intercept (1/f) (Lu and Jaffé 2001).

2.4 Parallel factor analysis

PARAFAC analysis is a statistical tool used to decompose multiway data into different components. It uses an alternating least-squares algorithm to minimize the sum of squared residuals in a trilinear model.

$$x_{ijk} = \sum_{f=1}^{F} a_{if} b_{jf} c_{kf} + \varepsilon_{ijk} \quad i=1,\ldots,I; j=1,\ldots,J; k=1,\ldots,K$$
$$(2)$$

where, F defines the number of components in the model; X_{ijk} is the fluorescence intensity of the sample i at emission wavelength j and excitation wavelength k; a_{if} is directly proportional to the concentration of the fth fluorophore in the ith sample (defined as scores); b_{jf} and c_{kf} are estimates of the emission and excitation spectra, respectively, for the fth fluorophore (defined as loadings). Finally, ε_{ijk} is the residual element representing the variability not accounted for by the model.

The analysis was carried out by Matlab 7.0 using the DOM Fluor toolbox (Stedmon and Bro 2008), and the split-half analysis was used to validate the identified components (Stedmon et al. 2003). The Rayleigh and Raman scatters were removed by the protocol of Bahram et al. (2006). Subsequently, Milli-Q water blank EEMs were subtracted from the sample EEMs to eliminate the Raman peaks. The data of EEMs have been calculated by using FL Solutions 2.1 for F-7000. Only the mean value of the data was analyzed using the PARA FAC model. The model was constrained to non-negative values. The outlier test was used to run a series of models from two to five components. The results from these initial models can be evaluated by two types of plots (loadings and leverages). The loadings and leverages plots showed that no samples were clear outliers with measurement errors. The split-half analysis and analysis of residuals and loadings showed that three components were identified for the dataset with some variability remaining in the residuals. The PARAFAC model with three components accounts for 99.12 % of the variance. A four-component PARAFAC model was rejected, as it could not be validated using the split-half analysis. The maximum fluorescence intensity of each component was represented by $F_{\max}$ (R.U., i.e., Raman units) (Stedmon and Markager 2005).

2.5 Statistical analyses

Statistical analyses were performed with SPSS 16.0 software (Statistical Program for Social Sciences). Regression and correlation analyses were used to examine the relationships between variables using SPSS software. Significance levels are reported as non-significant (NS) ($p > 0.05$), significant (*, $0.05 > p > 0.01$), or highly significant (**, $p < 0.01$). The linear model was validated by analysis of variance (ANOVA).

3 Results and discussion

3.1 Fluorescent components of soil DOM

A total of 120 EEMs of DOM-Cu (II) samples from the Lake Wuliangsuhai region were used for PARAFAC analysis. Compared to the traditional EEMs (Fig. S1, Electronic Supplementary Material), PARAFAC model can provide insightful information regarding the fluorescent chemical components of DOM. Three separate fluorescent components were identified for the soil DOM by the PARAFAC model, and the excitation and emission loadings including contour plots of each component are shown in Fig. 1. Component 1 had a primary fluorescence peak at an excitation/emission wavelength pair of 260/410 nm and a secondary peak at 330/410 nm, which indicates the presence of fulvic-like compounds. A red shift in the fluorescence wavelength for component 2 compared to component 1 occurred at 260/460 nm and 360/460 nm for the primary and secondary peaks, respectively. This may be due to the presence of humic materials which
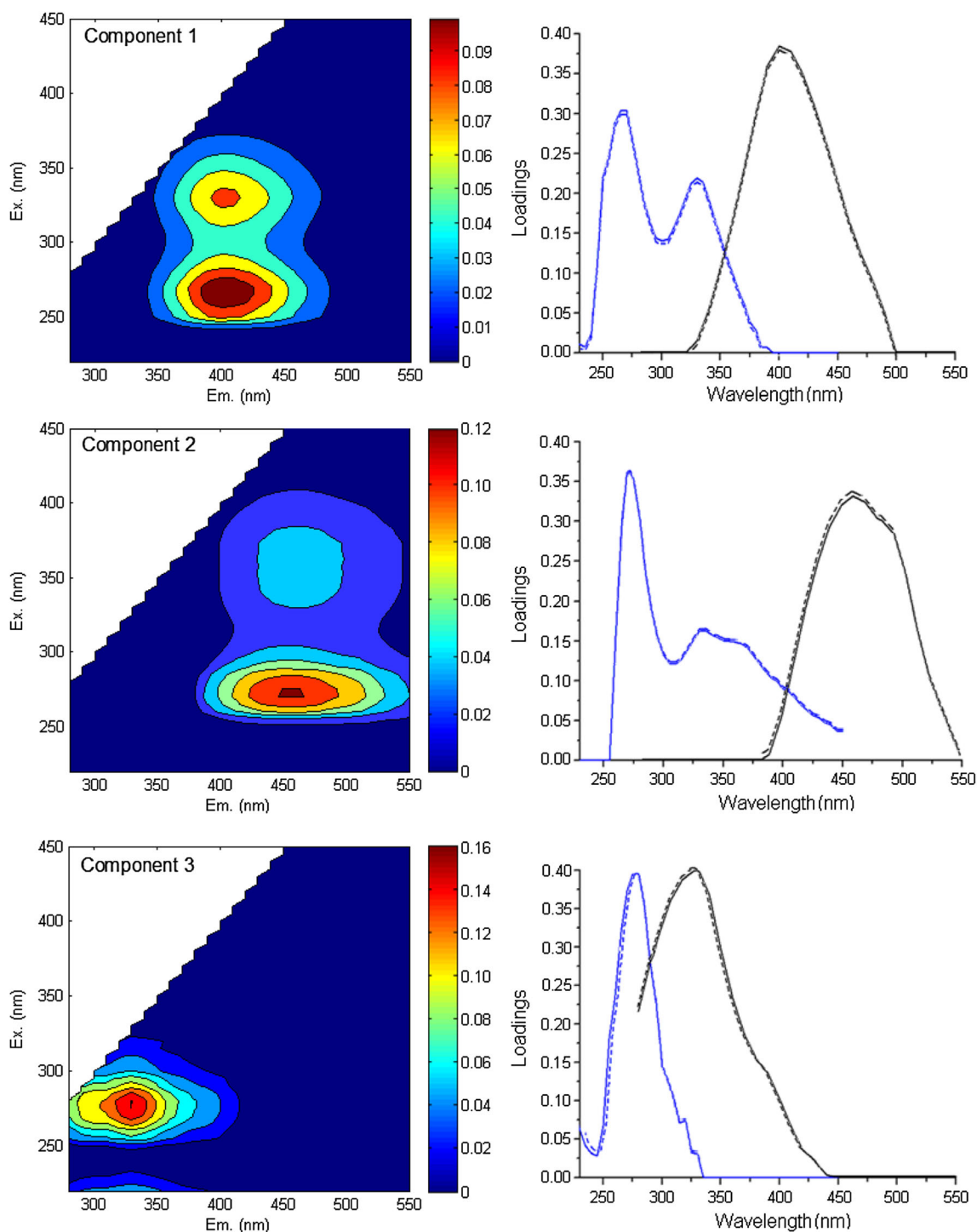
Fig. 1 Spectral representations of the three PARAFAC components (1–3). EEM representation (*left*) and excitation (*blue lines*) and emission (*black lines*) were estimated from two independent halves of the data set (*dotted lines*) and the complete data set (*solid lines*)

came from terrestrial sources and primarily consists of humic-like compounds (Yamashita et al. 2008). Senesi et al. (2003) suggested that the broader excitation and emission band and the longest emission wavelength of component 2 were associated with humic substances composed of high molecular weight aromatic organic compounds. Similarly, there was a significant positive correlation between components 1 and 2 ($r=0.9867$, $p<0.001$). Baghoth et al. (2011) indicated that component 1 correlated positively with component 2 ($r=0.97$,

$p < 0.001$). Components 1 and 2 were derived from terrestrial sources and represented humic-like substances with larger molecular weight aromatic humic compounds.

Component 3 presents a fluorescence peak at an excitation/emission wavelength pair of 275/340 nm and is often referred to as a tryptophan-like peak (Ohno and Bro 2006; Coble 2007). This type of fluorescence signal is often associated with microbial activities found in municipal solid waste and related to some biological products (Shao et al. 2009). However, the traditional EEMs did not detect the protein-like materials (e.g., peak B, peak D). The previous studies on synchronous fluorescence spectra indicated an obvious protein-like peak in the five soil DOM samples (Jiang et al. 2010; Guo et al. 2012). An index for assessing the relative contribution of microbially derived organic matter is the biological index or BIX (Huguet et al. 2009). The BIX is calculated by the ratio of emission intensities at λem 380 and 430 nm with excitation at λex 310 nm. BIX values of 1 or greater correspond to freshly produced DOM of biological or microbial origin, whereas values of 0.6 and lower contain little biological material. The BIX values ranged from 0.62 to 0.78 (Fig. S2, Electronic Supplementary Material), which could be viewed as freshly produced DOM of biological or microbial origin. In addition, the fluorescence index (FI) ranged from 1.55 to 1.78 (Fig. S2, Electronic Supplementary Material), indicated a microbially derived organic matter (McKnight et al. 2001).

All agricultural soil profiles in this study possessed high concentration of fulvic-like component 1 and relatively low concentration of protein-like component 3 (Fig. 2). The F_{max} values of components in agricultural soil profiles vary in the following order: component 1>component 2>component 3. In the three agricultural soils, the F_{max} values of components 1 and 2 at 20–40-cm profiles showed a clear increase compared to these at 0–20-cm profiles. The F_{max} values of components 1 and 2 increased by 153.78 and 197.52 % from the

AAF_{0-20} to the AAF_{20-40} soil, 29.92 and 27.66 % from the ASC_{0-20} to the ASC_{20-40} soil, 20.17 and 5.44 % from the ASW_{0-20} to the ASW_{20-40} soil. The most potential reason for this observation is that agricultural cultivation has resulted in mixing the surface soils with the next layer of underground soil. However, the protein-like component (component 3) showed a small variation. The highest F_{max} value of the short wavelength excited component 1 was found in SSE and GKF surface soils, respectively. Compared to component 1, a large decrease was observed for component 1 in SSE_{20-40} (e.g., 24.66 %) and SSE_{40-60} (e.g., 61.09 %), followed by component 2 (19.59 % for SSE_{20-40} and 52.79 % for SSE_{40-60}). With the increase in soil depth in the SSE soil, the F_{max} value of component 3 increased gradually. Furthermore, component 1 in GKF_{20-40} and GKF_{40-60} had a large decrease in the F_{max} value (37.35 and 74.70 %, respectively). The longer wavelength excited component 2 also showed a significant decrease in the F_{max} value (32.22 and 72.17 % for GKF_{20-40} and GKF_{40-60}, respectively). Component 3 in the GKF soil obviously had no variation in the F_{max} value. In general, humic-like substances (components 1 and 2) occupied a dominant F_{max} value indicating the presence of relatively high level of humic substances. The protein-like materials in autochthonous DOM were present in relatively low concentration, which was reported to have been originated from the microbial activities. Protein-like components represent either intrinsically fluorescent molecules which constitute bioavailable organic fractions of the DOM or fluorescent products of microbial activity, existing on the bioavailable, labile organic fractions, or perhaps a mixture of both (Hudson et al. 2007). Determann et al. (1998) have reported that protein-like fluorescence peaks were directly related to the microbial activity (biomass and metabolism) of marine algae and bacteria. Yu et al. (2010) demonstrated that soils had a high
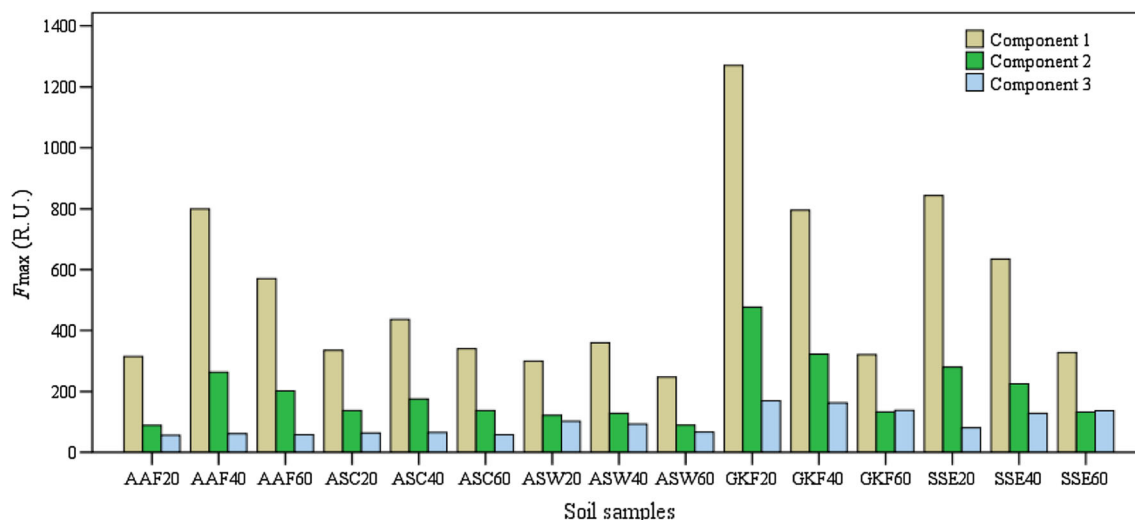

Fig. 2 The maximum fluorescence intensity of three components in 15 soil subsamples

exchangeable sodium percentage (ESP) with a low microbial activity and soil fertility. A high ESP content not only deteriorated the soil physicochemical properties but also repressed the microbial activity (enzyme activity, C mineralization, ammonification, nitrification, etc.) (Rietz and Haynes 2003; Yuan et al. 2007). García and Hernández (1997) reported that biological properties such as basal respiration and biomass C also had low values in derelict soils subject to erosion and also indicated a low biological activity. Meanwhile, high EC soil has a reduced biological activity resulting from their greater salt content (Sarig et al. 1993). Previous studies also specified that the soil in the Lake Wuliangsuhai region has undergone serious salinization and erosion in the past years (Yu et al. 2010). Humic-like substances were found to be the main components in soil DOM.

3.2 DOM binding capacities by Cu (II) ions

Figure 3 clearly shows that the F_{max} values of components 1 and 2 in AAF soil decreased gradually with increasing addition of Cu (II), indicating that Cu (II) ions interacted with the ligands of fluorescent humic-like and fulvic-like components.

However, the F_{max} value of component 3 showed no significant change when additional Cu (II) was added. An increasing F_{max} value of protein-like components can also be found in Fig. 3. Similar results also can be found in the other soil DOM samples. High levels of Cu (II) ions may cause the three-dimensional structural changes of protein molecules that resulted in the changes in quantum yields of protein fluorescence. Furthermore, the interactions between protein-like components and inorganic (metal ions) or other organic components may quench the fluorescence of protein-like components (Yamashita and Jaffé 2008). The increasing fluorescence intensity of protein-like component is likely to result from the addition of heavy metals in the DOM which stimulates some chromophores (Huang et al. 2009). Meanwhile, Wu et al. (2011) reported that fluorescent protein-like substances from municipal solid waste leachate DOM were quenched or enhanced by complexation of metal ions. The other four soils in this study also followed the same trend for the formation of DOM-Cu (II) complexes. To obtain a quantitative information on the complexation reaction, the modified Stern–Volmer model was used to calculate conditional stability constants, complexing capacity, and the percent of fluorophores (f %)
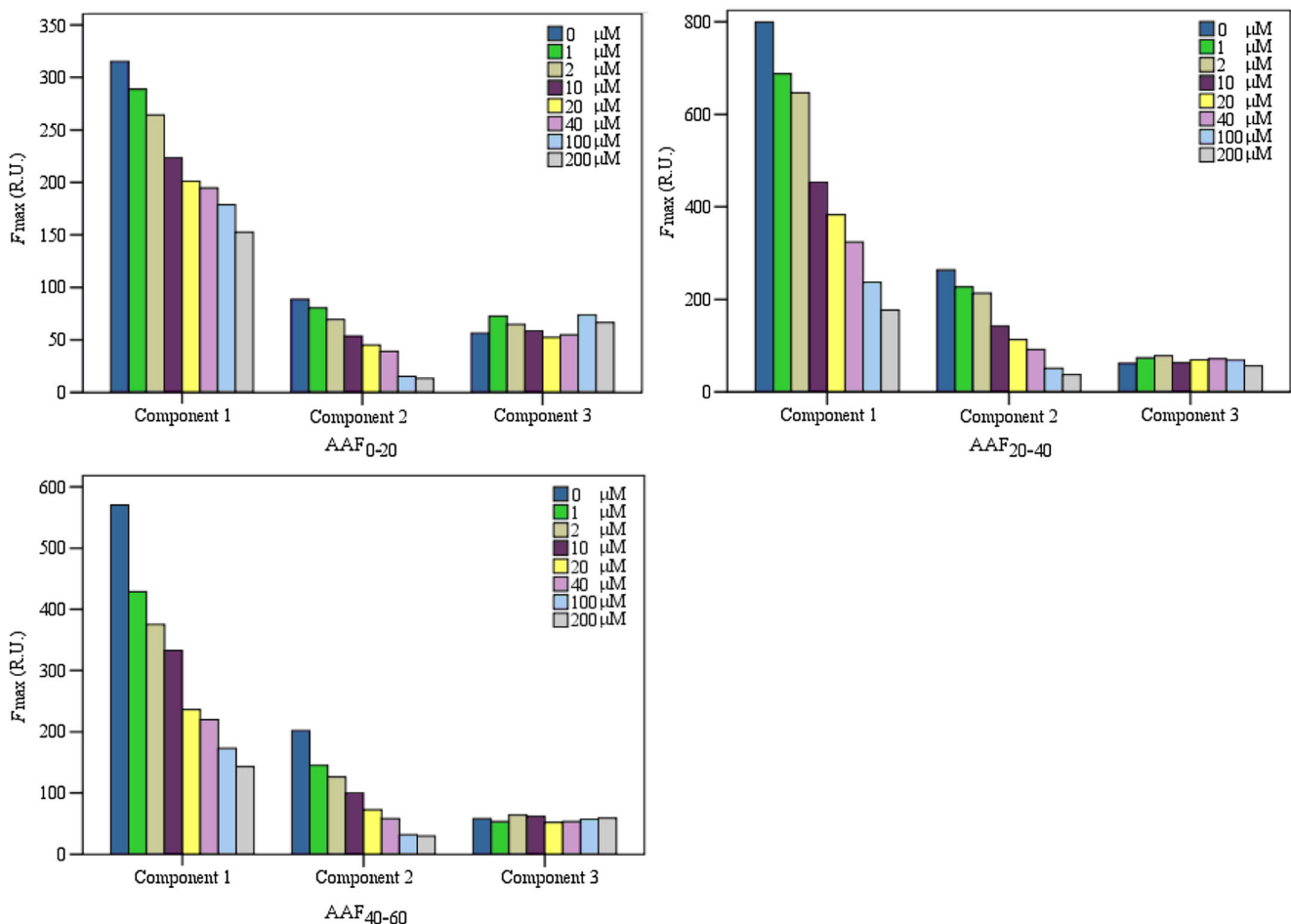

Fig. 3 The changes of mean F_{max} values for three fluorescent components by adding additional Cu (II) in AAF soil

which participate in the binding of Cu (II) ions. Table 2 lists the stability constants (log K) and the proportion of organic ligands (f %) for the formation of the Cu (II)-DOM complexes. The large values found for the correlation coefficients (R) indicate that the model fits very well to the experimental data sets for the DOM samples, as it was similar to previous works (Lu and Jaffé 2001; Plaza et al. 2006). However, due to the structural difference and possible reasons outlined above, the experimental data for component 3 were not modeled by the modified Stern–Volmer model.

The calculated log K values for humic-like substances in the five soil samples are higher than those found in the previous studies (Hernández et al. 2006; Knoth de Zarruk et al. 2007), suggesting that Cu (II) ions can be strongly bound to DOM through the formation of relatively stable inner-sphere coordination complexes (Senesi 1992). There is no systematic trend of log K values among the sampling sites. However, the log K values are evidently lower at 0–20 cm soil depth than those at 20–40 cm soil depth. Soil salinization and erosion, agricultural irrigation, and drainage led to the loss of large amounts of organic matters from the soils (Jiang et al. 2012; Yu et al. 2012), which greatly affects the binding affinity of the DOM by Cu (II) ions, and so the log K values of the top soils decreased (Guo et al. 2012). Most log K values of component 2 for Cu (II) binding are higher than that of component 1. As a result of different vegetation types, the structure and characteristic of DOM showed an obvious difference in all the five soils (Guo et al. 2012). Fluorescent DOM shows more complex structural components at longer

wavelengths than those at shorter wavelengths (Santos et al. 2010). These results show that there is a stronger Cu (II) binding affinity for the humic-like component than for the fulvic-like component in all the five soil samples in this study.

The binding affinity of the DOM by Cu (II) in saline-alkali soils was evidently higher than that in other soils (Hernández et al. 2006; Knoth de Zarruk et al. 2007). The high log K values for the soils may be due to the large presence of aromatic acidic functional groups (Hernández et al. 2006). An aromatic carboxyl group and adjacent phenolic OH group, or two adjacent aromatic carboxyl groups are known to form highly stable salicylate-like and phthalate-like ring structures with metal ions (Senesi 1992; Stevenson 1994). Lu and Allen (2002) found out that Cu (II) ions mainly combine with the phenolic OH groups of natural DOM. Hernández et al. (2006) reported that the Cu (II) binding capacities showed a significant positive correlation with COOH and phenolic OH group contents. The DOM samples from selected five surface soils had a broad band of stronger intensity centered between 1440 and 1400 cm^{-1} in FT-IR spectra (Guo et al. 2012), which was preferentially assigned to O—H deformation and C—O stretching of phenolic OH, C—H deformation of CH_2 and CH_3 groups and/or antisymmetric stretching of COO^- groups. However, the complexation of Cu (II) ions with phenolic OH and carboxyl groups for the selected DOM samples in saline-alkali soils needs further study.

The f % values of humic-like substances with Cu (II) ions ranged from 44.2 to 71.0 for fulvic-like component, and from 51.6 to 81.9 for humic-like component. Several interesting facts were noticed regarding the spatial and inter-components differences in the f % values. First, the f % values of humic-like component 2 were always higher than those of fulvic-like component 1, signifying that there was more organic ligands present in component 2 than in component 1. Senesi et al. (2003) demonstrated that the fluorescence signals of organic substances at short wavelengths are related to simple structural components with a low degree of aromatic polycondensation, whereas fluorescence signals at longer wavelengths are associated with complex structural components with a high degree of conjugation. Organic substances with a higher degree of aromatic polycondensation generally have higher chemical stability, thus increasing the residence time of organic matters in the soil environment (Santos et al. 2010). Therefore, humic-like component 2 may contain more binding sites and higher chemical stability than fulvic-like component 1. Second, the f % values of component 1 and component 2 have no systematic trend in the same soil types, respectively. Compared with component 1, the f % value of component 2 shows a large increase in the top soils of three agricultural soils. The f % value from component 1 to component 2 increased by 32.5, 14.7, and 9.9 for AAF$_{0-20}$, ASC$_{0-20}$, and ASW$_{0-20}$, respectively. However, an increase of the f % value from component 1 to component 2 is the smallest in SSE$_{0-20}$ and GKF$_{0-20}$ soils (8.9 and 6.2, respectively). Such differences suggest that the

Table 2 The log K and f % values of DOM-Cu (II) in five soils

Sampling	Component 1			Component 2		
	log K	f %	R	log K	f %	R
AAF$_{0-20}$	5.35	46.3	0.9941[**]	5.14	78.8	0.9859[**]
AAF$_{20-40}$	5.42	64.0	0.9871[**]	5.35	71.2	0.9879[**]
AAF$_{40-60}$	5.75	67.2	0.9941[**]	5.78	72.5	0.9710[**]
ASC$_{0-20}$	5.38	54.0	0.9892[**]	5.61	68.7	0.9859[**]
ASC$_{20-40}$	5.67	62.5	0.9635[**]	5.59	64.4	0.9733[**]
ASC$_{40-60}$	5.57	55.8	0.9811[**]	5.52	68.5	0.9837[**]
ASW$_{0-20}$	5.11	64.3	0.9973[**]	5.35	74.2	0.9949[**]
ASW$_{20-40}$	5.22	67.5	0.9924[**]	5.43	77.0	0.9915[**]
ASW$_{40-60}$	5.27	61.9	0.9957[**]	5.54	69.6	0.9859[**]
SSE$_{0-20}$	5.44	60.0	0.9947[**]	5.41	68.9	0.9943[**]
SSE$_{20-40}$	5.68	60.7	0.9739[**]	5.51	72.8	0.9953[**]
SSE$_{40-60}$	5.13	60.1	0.9966[**]	5.47	79.6	0.9814[**]
GKF$_{0-20}$	5.27	71.0	0.9931[**]	5.29	77.2	0.9967[**]
GKF$_{20-40}$	5.39	63.3	0.9951[**]	5.45	76.6	0.9928[**]
GKF$_{40-60}$	5.41	68.9	0.9909[**]	5.55	81.1	0.9869[**]

[**] Correlation is significant at the 0.01 level (2-tailed)

[*] Correlation is significant at the 0.05 level (2-tailed)

binding sites and the complexation processes are different between component 1 and component 2.

Many factors such as pH, Ca^{2+}, and Mg^{2+} ions may affect the complexation of DOM with metals. Hydrolysis is an important factor for hampering the complexation with metal ion by DOM (Lu and Jaffé 2001). The Ca^{2+} ion can significantly enhance the humic-like fluorescence, but the enhancement of fluorescence for protein-like substances is not obvious. Electron withdrawing groups such as halide ions easily causes fluorescence quenching to reduce the fluorescence quantum yields. The Lake Wuliangsuhai region has suffered serious soil erosion and salinization in the past years that resulted in higher concentrations of Ca^{2+} and Mg^{2+} ions in the soils (Yu et al. 2010; Guo et al. 2012). The competing effect of the cations, Ca^{2+} and Mg^{2+}, on the complexation of Cu (II) by fulvic and humic acids has been reported (Cabaniss 1992; Lamelas and Slaveykova 2007). The competition for Cu (II) ions reduces with increasing Ca^{2+} or Mg^{2+} concentrations (Lu and Jaffé 2001). Therefore, the presence of high concentration Ca^{2+} and Mg^{2+} ions in Wuliangsuhai soils may be a potential factor for the complexation of Cu (II) ions by fulvic-like and humic-like components. Moreover, Lu and Jaffé (2001) suggested that Ca^{2+} and Mg^{2+} ions were preferably bound by the carboxyl functional groups (COOH), especially at relatively high concentrations, resulting in a weakened apparent competition effect. All of these factors taken together can affect the geochemical behavior of Cu (II) ions in saline-alkali soils.

4 Conclusions

Fluorescence spectra can be used to explain the fluorescent components of soil DOM. Fulvic-like and humic-like components are the main fluorescent materials (organic compounds) in saline-alkali soils. The EEMs in conjunction with PARAFAC analysis have been used to trace the fluorescent components of soil DOM, which can estimate the relative concentrations of each fluorescent component in the DOM. Overall, three fluorescent components using the PARAFAC model were identified: one fulvic-like (component 1), one humic-like (component 2), and one protein-like (component 3). The dominance of fulvic-like and humic-like components has already been established. Fluorescence quenching titration with single excitation and emission wavelengths accounts for the complexation between DOM and Cu (II) ions. However, the fluorescence quenching titration using EEM-PARAFAC analysis allows for the assessment of metal interactions with specific fluorophores within the sample under study. The fulvic-like and humic-like components in soil DOM were quenched by increasing the Cu (II) concentrations during this study. The protein-like component (component 3) originated from autochthonous DOM, whose F_{max} values had no significant change by adding additional Cu (II) ions. This study indicates that fulvic-like and humic-like components have a high Cu (II) ion binding affinity. Humic-like components possess higher in the proportion of organic ligands than these of fulvic-like components in the DOM of saline-alkali soils. Meanwhile, different vegetation types can influence the structure and characteristic of DOM. Soil salinization and erosion, agricultural irrigation, and drainage greatly influenced the binding affinity of the DOM by Cu (II), and resulted in the log K values of the top soils decreased.

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