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Fluorescence spectroscopic studies of natural organic matter fractions

Jie Chen [a], Eugene J. LeBoeuf [b], Sheng Dai [c], Baohua Gu [a,*]

[a] *Environmental Sciences Division, Oak Ridge National Laboratory (ORNL), P.O. Box 2008, Oak Ridge, TN 37831-6036, USA*
[b] *Department of Civil and Environmental Engineering, Vanderbilt University, Box 1831, Station B, Nashville, TN 37235, USA*
[c] *Chemical and Analytical Sciences Division, ORNL, P.O. Box 2008, Oak Ridge, TN 37831-6201, USA*

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Abstract

Because of the well-known molecular complexity and heterogeneity of natural organic matter (NOM), an aquatic bulk NOM was fractionated into well-defined polyphenolic-rich and carbohydrate-rich subfractions. These fractions were systematically characterized by fluorescence emission, three dimensional excitation-emission matrices, and synchronous-scan excitation spectroscopy in comparison with those of the reference International Humic Substances Society soil humic acid and Suwannee River fulvic acid. Results indicate that fluorescence spectroscopy can be useful to qualitatively differentiate not only NOM compounds from varying origins but also NOM subcomponents with varying compositions and functional properties. The polyphenolic-rich NOM-PP fraction exhibited a much more intense fluorescence and a red shift of peak position in comparison with the carbohydrate-rich NOM-CH fraction. Results also indicate that synchronous excitation spectra were able to provide improved peak resolution and structural signatures such as peak positioning, shift, and intensity among various NOM components as compared with those of the emission and excitation spectra. In particular, the synchronous spectral peak intensity and its red shift in the region of about 450–480 nm may be used to indicate the presence or absence of high molecular weight and polycondensed humic organic components, or the multicomponent nature of NOM or NOM subcomponents.
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1. Introduction

Humic substances or natural organic matter (NOM) are heterogeneous mixtures of a variety of organic compounds, consisting of aromatic, aliphatic, phenolic, and quinolic functional groups with varying molecular sizes and properties (Aiken et al., 1985; Suffet and MacCarthy, 1989). In addition, the size, chemical composition, structure, and functional groups of NOM may vary greatly, depending on the origin and age of the material (Aiken et al., 1985; Chin et al., 1994; Gu et al., 1995; Chin et al., 1998). Because NOM is known to play important roles in the interaction and transport of many toxic organic or inorganic chemicals and in nutrient cycling throughout the environment (Choppin, 1992; Chen et al., 1996; Chorover et al., 1999; Burgos et al., 2000; LeBoeuf and Weber, 2000a, b), detailed chemical and physical characterization of NOM is particularly useful for studying these reactions and processes. More specifically, a better understanding of the structural and functional properties of NOM may greatly improve our understanding of the underlying mechanisms responsible for the complexation, reduction, bioavailabiltiy, and

* Corresponding author. Tel.: +1-865-574-7286; fax: +1-865-576-8543.
E-mail address: b26@ornl.gov (B. Gu).

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mobilization or immobilization of metals and toxic organic chemicals with NOM. This, in turn, may improve our predictive capabilities of the behavior of NOM and environmental pollutants in natural ecosystems.

The sensitivity and nondestructive nature of fluorescence techniques are well suited for studies of the chemical and physical properties of NOM (Senesi et al., 1991; Pullin and Cabaniss, 1995; McKnight et al., 2001). In general, only a small amount of aqueous sample at a low concentration (typically <20 mg/l) is required for fluorescence analysis, as opposed to conventional preparative techniques that require large volumes of samples in order to concentrate and isolate sufficient quantities of NOM for chemical and structural characterization. More importantly, unlike UV/visible absorption, the intrinsic fluorescence of humic substances contains information relating to structure, functional groups, conformation, and heterogeneity, as well as dynamic properties related to their intramolecular and intermolecular interactions (Mobed et al., 1996). It has been shown that the fluorescence of dissolved organic matter accounts for a significant portion of the fluorescence in both natural fresh and oceanic water samples (Stewart and Wetzel, 1980; Lochmuller and Saavedra, 1986; Ewald and Belin, 1987; Mopper and Schultz, 1993; Coble, 1996). As early as 1971, Datta et al. (1971) applied the fluorescence technique in an attempt to obtain the structural information of humic materials. Their results indicated that humic substances, regardless of their origin, exhibited characteristic fluorescence spectra which were attributed to the presence of aromatic fluorophores with electron-donating functional groups. Ghosh and Schnitzer (1980) studied the effects of pH and ionic strength on the fluorescence excitation spectra of fulvic acid and humic acid. They noted that spectra of fulvic acids always exhibit greater fluorescence intensity than spectra of humic acids, and the intensity decreased with decreasing molecular weight and pH and increasing ionic strength. Similarly, in a study of a freshwater lake, Stewart and Wetzel (1980) showed that larger molecular weight aquatic humic fractions had a greater absorbance but lower fluorescence than smaller molecular weight fractions. Miano et al. (1988) systematically studied the fluorescence spectra of humic and fulvic acids of various origins and reported that the excitation spectra could be used to distinguish the humic acids from fulvic acids in an aqueous solution. The broad emission spectra showed maximum intensity wavelengths that were lower for humic than for fulvic acids, indicating a greater degree of condensed aromatic character in humic acids. Synchronous-scan excitation fluorescence spectroscopy has also been used for the study of humic substances and/or their interactions with metals and organic contaminants and often shows complex spectral line shapes that depend on the type of humic substances (Senesi, 1990; Senesi et al., 1991; Miano and Senesi, 1992; Pullin and Cabaniss, 1995). Although the exact nature of the fluorescing structural groups is not completely resolved, a classification of humic substances was proposed on the basis of their fluorescence behavior (Senesi et al., 1991). More recently, McKnight et al. (2001) proposed use of a fluorescence index to differentiate the microbially derived fulvic acids from those of the terrestrially derived fulvic acids.

Because of the heterogeneous nature of NOM and its complex chemical structure, however, significant spectral overlapping and peak shifting and broadening often occur, making the identification and interpretation of spectral signatures difficult. Although a fluorescence index is able to discern fulvic acids, it may not adequately differentiate the humic acid fraction because of its peak shift of both excitation and emission fluorescence. The fractionation of the bulk NOM into some well-defined subcomponents has been reported to offer advantages in characterizing NOM and providing improved understanding of the structural and functional properties of NOM (Gu et al., 1995; Leenheer et al., 1995a, b; Chen et al., 2002). By employing fractionation using cross-linked polyvinyl pyrrolidone (PVP) polymers, two subfractions of an aquatic NOM and a reference soil HA were recently studied in great detail for their structural characteristics by a range of wet-chemical and spectroscopic techniques (Chen et al., 2002). Their results indicated that the polyphenolic-rich (NOM-PP) fraction was characterized by its relatively high contents of phenolic and ketonic functional groups compared to the carbohydrate-rich (NOM-CH) fraction and the soil HA. However, the soil HA contained a much higher amount of polycondensed aromatic structures and showed higher electron paramagnetic spin counts than the NOM-PP and NOM-CH fractions. The present study was undertaken to apply the fluorescence technique to further characterize the structural and functional properties of the NOM-PP and NOM-CH fractions in comparison with two of the reference NOM materials obtained from the International Humic Substances Society (IHSS), as part of a larger study of the role of NOM structural properties on its interaction with contaminant metals (Gu et al., 2000). Three different fluorescence measurement techniques including emission, excitation-emission matrix (EEM), and synchronous-scan excitation techniques were employed and compared for their sensitivity and resolution to reveal structural signatures of NOM and its subcomponents.

2. Experimental

2.1. Materials and methods

The NOM samples used in this study include two subfractions (NOM-PP and NOM-CH) from a total

aquatic NOM (hereinafter referred to as GT-NOM) obtained from a wetland pond (Gu et al., 1994, 2002), a soil humic acid (Soil HA) and a Suwannee River fulvic acid (SRFA) obtained from IHSS. The reference SRFA was used to represent dissolved organic matter in groundwater and aquatic environments, whereas the soil HA was used to represent a high molecular weight NOM fraction that is rich in polycondensed aromatic moieties. Both of these reference humic substances have been studied extensively (Senesi et al., 1991; Pullin and Cabaniss, 1995; Chen et al., 2002). The method of fractionation and purification of NOM-PP and NOM-CH has been described in detail elsewhere (Lowe, 1975; Chen et al., 2002). Briefly, this method fractionates the sample on the basis of its adsorptive behavior on a cross-linked PVP polymer, where components rich in aromatic or phenolic C=C moieties preferentially adsorb on PVP under acidic conditions, while organic components not adsorbed by PVP are comprised primarily of low-molecular-weight carbohydrates, proteins, amino acids, and uronic acids. The fractionation of the bulk NOM thus offers advantages by selectively separating one group of organic compounds (or subcomponents) from the others on the basis of their physical and chemical properties. After fractionation, the NOM-PP and NOM-CH were found to consist of ~70% and ~20% of the bulk NOM, respectively.

2.2. Fluorescence spectral analysis

The following NOM samples were prepared for fluorescence measurement. Stock solutions of NOM-PP, NOM-CH, soil HA, and SRFA were prepared from freeze-dried solids without further purification. Each sample was first dissolved in purified water and filtered using a 0.45-μm Millipore-syringe filter to remove undissolved particulate materials. Solution pH was adjusted to 5, 7, and 10 using dilute NaOH or HCl in a 0.01 M KCl background. The final total organic carbon (TOC) concentration was made up to 20 mg C/l. Approximately 1.4 ml of the sample was placed in a Spectrosul Far UV quartz triangular fluorescence cell for the fluorescence analysis. The use of a triangular fluorescence cell minimized inner filtering and absorption effects, which could occur with the use of a rectangular cell so that the absorption correction must be performed in order for accurate representation and comparison of fluorescence spectra among NOM samples (Mobed et al., 1996).

All fluorescence spectra were recorded on a Fluorolog fluorescence spectrophotometer equipped with both excitation and emission monochromators (Johin-Yvon-SPEX instruments, New Jersey). A 450-W Xenon arc lamp was used as the excitation source. A series of emission spectra were collected over a range of excitation wavelengths to provide a complete representation of the fluorescence of a sample in the form of an EEM, in which fluorescence intensity was presented as a function of excitation wavelength on one axis and emission wavelength on the other. A wavelength step size of 10 nm was used for the collection of EEM spectra. The excitation wavelength range was from 290 to 480 nm, and the emission wavelength range was from 400 to 600 nm.

In addition, emission spectra of each NOM sample were collected from 400 to 600 nm at a fixed excitation wavelength of 340 nm, and synchronous-scan excitation spectra were obtained by measuring the fluorescence intensity while simultaneously scanning over both the excitation (λ_{ex}) and emission (λ_{em}) wavelengths while keeping a constant optimized wavelength difference $\Delta\lambda = \lambda_{em} - \lambda_{ex}$. The synchronous fluorescence spectra were used because of its advantages over conventional excitation or emission modes to provide additional information on structural signatures of NOM or NOM subfractions (Senesi, 1990; Pullin and Cabaniss, 1995). The excitation monochromator was scanned from 300 to 600 nm, and the optimized wavelength difference ($\Delta\lambda = 20$ nm) was kept constant as the emission spectrum was recorded. The emission and synchronous spectra were taken with a 5-nm slit width on both excitation and emission monochromators. The wavelength step size was 1 nm, and spectrum intensity was measured as the average of three signal samplings.

3. Results and discussion

3.1. Emission and excitation spectra

As commonly observed for humic and fulvic substances, fluorescence emission spectra of NOM and its subfractions appear to be broad and featureless (Fig. 1) (Miano et al., 1988; Senesi, 1990; Pullin and Cabaniss, 1995; McKnight et al., 2001). The spectra of the reference SRFA and GT-NOM (not shown) resembled each other, suggesting similar chemical characteristics between the SRFA and GT-NOM organic materials, as reported previously (Gu et al., 1994, 1995). With the exception of soil HA (Fig. 1d), each aquatic NOM or its subfractions showed an intense fluorescence as a result of its major fluorophore with its emission peak at ~440–450 nm. The soil HA exhibited about a twofold lower peak fluorescence intensity than those of the aquatic SRFA and NOM-PP (Table 1). More importantly, the emission maxima of the fluorophore of soil HA shifted to a longer wavelength at ~502 nm (or a red shift). On the other hand, the peak emission of the NOM-CH (rich in carbohydrate NOM components) occurred at a shorter wavelength, ~440 nm (Fig. 1b), and its intensity decreased substantially as compared with those of NOM-PP and SRFA (Table 1). The emission spectra of

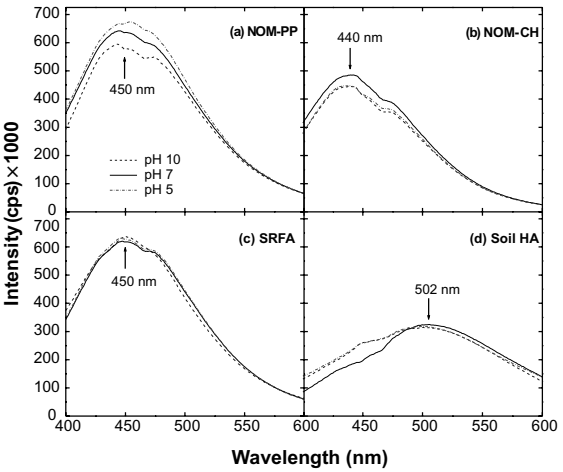

Fig. 1. Fluorescence emission spectra of two subfractions of an aquatic NOM (NOM-PP and NOM-CH) and two IHSS reference humics (SRFA and soil HA) at varying pH conditions. Samples were dissolved in 0.01 M KCl solution with a TOC concentration of 20 mg/l. Spectra of NOM-PP, NOM-CH, and Soil HA at pH 7 were reported in Chen et al. (2002) and used for comparison.

NOM-PP (enriched with polyphenolics) resembled SRFA and its parent material GT-NOM (not shown), likely because NOM-PP comprises ~70% of GT-NOM. Solution pH appeared to have little or no effect on emission spectral characteristics of NOM and its subcomponents.

Based on the wet-chemical and spectroscopic characterization of these NOM compounds (Table 2) and by comparing with the emission spectrum of soil HA, a general conclusion may be drawn to suggest that the peak emission wavelength of NOM shifted from shorter to longer wavelengths with increased molecular size and aromatic content (i.e., Soil HA > NOM-PP > NOM-CH). In addition, the fluorescence intensity of NOM (normalized to organic C content) appears to be influenced by the molecular weight and polycondensation of aromatic compounds within NOM macromolecules. Relatively low-molecular-weight polyphenolic compounds (NOM-PP) gave the most intense emission fluorescence as compared with those of soil HA and NOM-CH components. The NOM-CH gave a relatively low emission intensity, likely due to the depletion of its aromatic and/or polyphenolic contents, and also due to the enrichment of its carbohydrate materials which do not give fluorescence. Although the soil HA is known to be enriched with aromatic contents, its low fluorescence

Table 2
Relative order of molecular sizes and structural properties of NOM fractions[a]

Property	Relative order
Molecular weight	Soil HA ≫ NOM-PP ⩾ SRFA > NOM-CH
Aromaticity	Soil HA > NOM-PP ⩾ SRFA > NOM-CH
Phenolics	NOM-PP > Soil HA > NOM-CH[b]
UV/Vis absorptivity	Soil HA > NOM-PP > SRFA > NOM-CH
Carboxyls/hydroxyls	NOM-PP > NOM-CH > Soil HA[b]

[a] Generalized properties from Chen et al. (2002), Gu et al. (1994, 1995), Aiken et al. (1985), Mobed et al. (1996) and Zhou et al. (2001).
[b] Not available for SRFA.

Table 1
Peak position, intensity, and fluorescence index of two subfractions of an aquatic NOM (NOM-PP and NOM-CH) and two IHSS reference humics (SRFA and soil HA) at varying pH conditions

	pH	Emission		Synchronous-scan		Fluorescence index[a]
		Position, λ (nm)	Intensity (×1000 cps)	Position, λ (nm)	Intensity (×1000 cps)	
NOM-PP	5	450	670	400	230	1.43
	7	450	645	400	245	1.44
	10	450	590	400	305	1.40
NOM-CH	5	440	485	390	145	1.79
	7	440	450	390	95	1.74
	10	440	450	390	105	1.78
SRFA	5	450	630	400	200	1.41
	7	450	620	400	210	1.41
	10	450	635	400	280	1.52
Soil HA	5	502	320	470	255	0.81
	7	502	325	470	215	0.61
	10	502	315	470	260	0.83

[a] Fluorescence index is the ratio of emission intensity at 450 and 500 nm with an excitation wavelength at 340 nm.

intensity may be partially attributed to its highly substituted aromatic structural features, its inter- and/or intramolecular bonding, and self-quenching within humic macromolecules (Miano et al., 1988; Senesi, 1990). Senesi (1990) also attributed these effects to the greater proximity of aromatic chromophores and the consequent greater probability of deactivation of excited states by internal quenching in higher molecular weight organic molecules.

The maximum emission intensity of these organic materials, however, also depends on the excitation wavelength and varies among each individual NOM component or subfraction. As shown in Fig. 2, results of the three-dimensional EEM spectra indicate that the maximum emission intensity of the NOM-PP, SRFA, and NOM-CH (all aquatic NOM) occurred at an excitation wavelength of ~340 nm. This was the excitation wavelength used to collect and compare all emission spectra of NOM and its subfractions, as discussed above (Fig. 1). However, the EEM spectrum of the soil HA (Fig. 2d) showed two distinct emission maxima at excitation wavelengths of 415 and 465 nm, respectively, indicating the presence of two major fluorophores at longer excitation wavelengths than those of SRFA, NOM-PP, and NOM-CH fractions at ~340 nm (Fig. 2a–c). These observations offer additional explanations to the observed relatively low emission intensity of the soil HA (Fig. 1d). In other words, a much higher emission intensity of the soil HA would have been observed if a longer excitation wavelength (e.g., at 415 nm) were used for the measurement. These characteristics of NOM (peak positioning and intensity) may be potentially useful in discerning various NOM components or NOM from various origins (Mobed et al., 1996; McKnight et al., 2001).

McKnight et al. (2001) recently proposed to use a fluorescence index to distinguish the microbially derived fulvic acids from the terrestrially derived fulvic acids. The index is defined as the ratio of fluorescence emission intensity at wavelength 450 nm to that at 500 nm (or E_{450}/E_{500}). The microbially derived organic materials generally contain low fluorophores and a low aromaticity with a fluorescence index value of ~1.9. However, terrestrially derived fulvic acids commonly consist of a relatively high aromaticity and fluorophores, and show a fluorescence index value of ~1.4. Although the emission spectra of the present study were measured at an excitation wavelength of 340 nm (instead of 370 nm), the calculated fluorescence indices of GT-NOM, SRFA, and NOM-PP ranged from 1.4 to ~1.5 (Table 1, last column) and are therefore in good agreement with findings by these authors. In particular, the NOM-CH fraction showed a fluorescence index of ~1.8 (Table 1), which is consistent with those of microbially derived fulvic acids (~1.9) (McKnight et al., 2001). Although NOM-CH was derived from terrestrial GT-NOM, the fractionation technique employed enriched the NOM-CH fraction with carbohydrates (similar to microbially derived materials), but depleted it of polyphenolic NOM components. However, a large discrepancy occurred when calculating the fluorescence index of soil HA, which ranged from 0.6 to ~0.8 (Table 1). Apparently, the

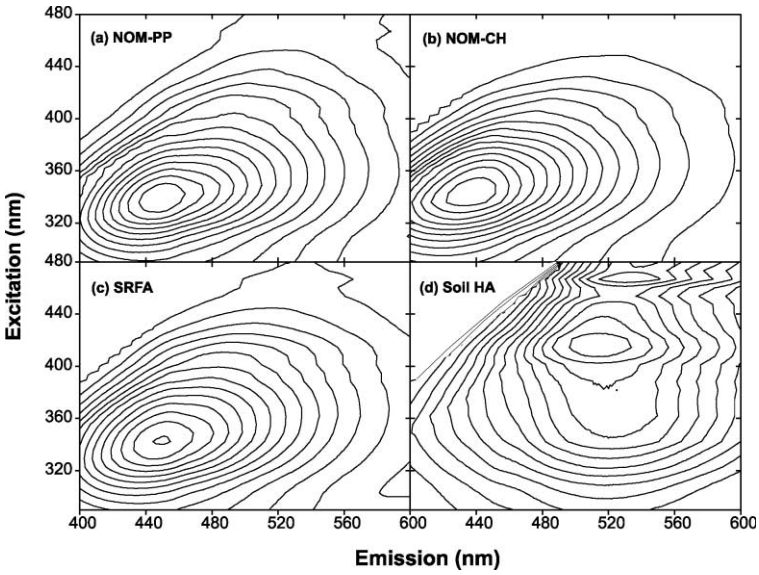

Fig. 2. Fluorescence excitation–emission matrix spectra of two subfractions of an aquatic NOM (NOM-PP and NOM-CH) and two IHSS reference humics (SRFA and soil HA). Samples were dissolved in 0.01 M KCl with a TOC concentration of 20 mg/l.

fluorescence index may be best applied to those of aquatic humic substances and fulvic acids. Such a discrepancy is primarily caused by the arbitrary definition of the fluorescence index, so that the ratio of E_{450}/E_{500} changes as the peak position of fluorescence emission shifts to a longer wavelength (502 nm, or a red shift) with the content of aromaticity and polycondensation of humic materials, as mentioned previously. Additionally, it has been reported that a distinct red shift accompanied by a broadening of the emission peak also occurs with an increase in molecular weight of humic substances (Senesi, 1990). It therefore appears that the emission peak position may be a better index to discern various sources of humic substances with varying fluorophores and molecular sizes. In the present study, we found that the peak emission positions of NOM-CH, NOM-PP, and Soil HA increased consistently from ~440, 450, to 502 nm, respectively.

3.2. Synchronous-scan excitation spectra

Synchronous-scan excitation spectra (Fig. 3) appeared to provide improved peak resolution and increased selectivity or structural signature among various NOM components compared with those of emission and excitation spectra of NOM (Fig. 1). The soil HA exhibited three major line shapes at about 466, 480, and 489 nm (Fig. 3d), which could be attributed to various electron-withdrawing substituents in the humic material (Miano et al., 1988; Senesi, 1990; Miano and Senesi, 1992). In addition to its red shift, the fluorescence peak of soil HA was much better defined (or more narrow) as opposed to those of broad and featureless emission spectra (Fig. 1). However, the carbohydrate-rich NOM-CH fraction still appeared to be broad and featureless (Fig. 3b), probably resulting from residual, low-molecular weight phenolic and carboxylic NOM components. As observed from its emission spectroscopy, the synchronous-scan spectral peak of NOM-CH occurred at a shorter wavelength (at ~390 nm), and its fluorescence intensity was much lower than those of soil HA, NOM-PP, and SRFA. Although the synchronous-scan spectra of NOM-PP and SRFA also appeared to be broad, they showed distinct signatures of electron-withdrawing substituents such as polyphenolic and aromatic NOM components (Fig. 3a and c), and the spectra were better resolved than those of the emission spectra (Fig. 1). The major fluorescence peak occurred at about 400 nm, which could be attributed to those of phenolic fluorophores in the polyphenolic-rich NOM-PP and SRFA. A series of shoulders appeared between 440 and 480 nm, however, which matched very well with those of soil HA (Fig. 3d). These observations indicate the polydispersity of NOM-PP and SRFA, in which both low- and high-molecular-weight organic substituents are present; the shoulders between 440 and 480 nm may be indicative of the presence of some humic-like organic compounds (with high molecular weight or polycondensation).

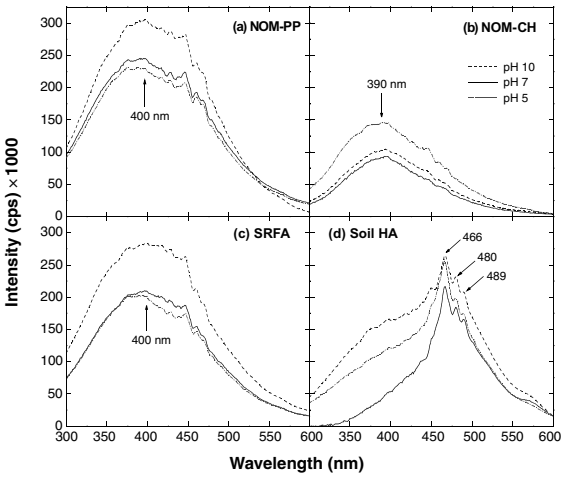

Fig. 3. Synchronous fluorescence spectra of two subfractions of an aquatic NOM (NOM-PP and NOM-CH) and two IHSS reference humics (SRFA and soil HA) recorded at $\Delta\lambda = 20$ nm with the excitation monochromator scanned from 300 to 600 nm. Spectra of NOM-PP, NOM-CH, and Soil HA at pH 7 were reported in Chen et al. (2002) and used for comparison.

Although pH did not appear to significantly affect fluorescence emission spectra (Fig. 1), a pronounced effect of pH was observed in the synchronous-scan spectra of these NOM components (Fig. 3). With the exception of the NOM-CH fraction, the synchronous spectral intensity of SRFA, NOM-PP, and soil HA generally increased with an increase of solution pH, particularly in the region of about 350–450 nm (Fig. 3a, c and d). The fluorescence intensity increased considerably at pH 10, but an opposite trend was observed for the NOM-CH fraction (Fig. 3b). These observations are not surprising, considering that the phenolic organic content of these NOM components were in the order of NOM-PP $\geqslant$ SRFA $>$ soil HA $>$ NOM-CH (Table 2) and are therefore consistent with this observation (Fig. 3). It is known that the fluorescence spectra of most aromatic compounds that contain acidic or basic functional groups are sensitive to pH; some functional groups (e.g., phenols) become stronger acids on excitation, whereas others become more basic (e.g., carboxylic acids) (Senesi, 1990). Therefore, the increase in excitation intensity with pH may be related to the increased ionization of phenolic hydroxyls causing the decreased particle association and decoiling of macromolecular structures, (e.g., disruption of inter- and intramolecular hydrogen bonds) (Laane, 1982; Senesi, 1990). The electron donating groups such as hydroxyl and methoxyl

groups have also been reported to enhance fluorescence by increasing the transition probability between the singlet and ground state (Senesi, 1990). The effect of solution pH could thus be associated in particular with the ionization of phenolic hydroxyl groups in NOM-PP and SRFA, resulting in a greatly increased fluorescence intensity of the main excitation peak at ~400 nm at pH 10. On the other hand, the NOM-CH contained a much smaller amount of phenolic functional groups and exhibited the lowest free radical content, but a relatively high amount of carbohydrate and electron-withdrawing carboxylic groups (Chen et al., 2002). These structural properties of NOM-CH could have contributed to an observed low fluorescence intensity of the NOM-CH at pH 10. Previous studies also indicated that the electron-withdrawing carboxyl functional groups could weaken the fluorescence intensity, particularly when these carboxylic groups are para-oriented to the hydroxylic OH groups (Senesi, 1990).

Similar observations have been reported previously by Miano et al. (1988) and Pullin and Cabaniss (1995). Miano et al. (1988) reported that the synchronous-scan excitation spectra of soil fulvic acid showed a decrease in overall fluorescence intensity, whereas those of river aquatic and peat fulvic acids showed an increase as pH increased from about 5 to 10. These observations could be explained by the fact that river aquatic fulvic acids are perhaps mainly derived from plant and tree residues, which contain more phenolic and lignin-derived organic compounds than those found in soil. Additionally, it has been reported that the intensity and wavelength of each peak in the synchronous spectra are affected differently by pH changes according to the nature and origin of the sample (Miano et al., 1988; Pullin and Cabaniss, 1995). As shown in Fig. 3, the pH dependence of the synchronous fluorescence intensity was more pronounced in the region of ~350–450 nm than in the region of ~470–480 nm. In particular, pH did not appear to significantly influence the peak intensity of the soil HA at ~470 nm. Therefore, together with the changes in peak intensity and red shift, the pH-dependent synchronous-scan excitation spectra provided more information or structural signatures of NOM and NOM fractions than both the emission and excitation spectra alone could have provided. The spectral intensity and signatures in different regions (e.g., 380–450 and 450–480 nm) may be used as an indicator of the multicomponent or heterogeneous nature of NOM or NOM components. For example, the synchronous spectral peak intensity and its red shift in the region (or shoulder) of 450–480 nm could be used to indicate the presence or absence of high molecular weight and polycondensed humic-like organic components. An increased fluorescence intensity and red shift in this region may result from an increased content of high molecular weight humic materials. On the other hand, the presence of large amounts of simple, dissociated phenolic and quinone types of organic compounds may result in increased fluorescence in the region of about 380–430 nm. These properties of synchronous fluorescence spectra could thus potentially be used to discern the various NOM components from different origins and may be suitable for tracer studies of humic substances as suggested by Pullin and Cabaniss (1995).

4. Conclusions

Although the observed fluorescence spectra is the sum of the spectra of many different fluorophores present in NOM, fluorescence spectroscopy was found to be useful to discern not only NOM compounds from various origins but also NOM subcomponents with varying compositions and functional properties. Fluorescence peak positioning and shift, peak intensity, and peak broadening appear to be the most useful observations, and could be correlated to such structural signatures as molecular sizes or polycondensation and contents of aromatics, phenolics, carboxyl and hydroxyl functional groups. Although the fluorescence index was able to discern various fulvic acids from different origins (such as microbially derived fulvic acids and the terrestrially-derived fulvic acids) (McKnight et al., 2001), it did not adequately differentiate the humic acid because of the red shift of its peak excitation fluorescence. The peak emission fluorescence as well as the synchronous-scan excitation of NOM-CH, NOM-PP, and soil HA increased consistently (from ~440, 450 to 502 nm) with an increase in the molecular weight and polycondensation of aromatics and phenolics in these organic materials. In addition, synchronous excitation spectra were found to provide improved peak resolution and structural signatures, and its peak intensity and red shift in the region of about 450–480 nm could be used to differentiate the presence or absence of high molecular weight and polycondensed humic-like organic components or the multicomponent nature of NOM and its subfractions. Results of the present study also indicate that the fractionation of bulk NOM could provide well-defined NOM subcomponents (such as NOM-PP and NOM-CH) with distinct chemical and structural properties. It therefore points to the importance of isolation and improved characterization of various NOM subcomponents in order to better understand the behavior and roles of NOM in the natural environment.

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