The structure and origin of dissolved organic matter studied by UV-vis spectroscopy and fluorescence spectroscopy in lake in arid and semi-arid region
Guo Xu-jing, Xi Bei-dou, Yu Hui-bin, Ma Wen-chao and He Xiao-song

ABSTRACT
To develop a proper indicator which could predict water quality and trace pollution sources is critically important for the management of sustainable aquatic ecosystem. In our study, seven water samples collected from Wuliangsuhai Lake in Inner Mongolia were used. UV-visible spectra and synchronous fluorescence spectra were applied to investigate the humification degree and aromatic structure of dissolved organic matter (DOM) extracted from water samples. The results showed that both samples from W1 site and W3 site display lower humification degree and less aromatic structure, where industrial wastewater and domestic sewage, and reclaimed water of farmland irrigation, were accepted respectively. After computing the values of SUVA254, A280/A250, A283/203 and A226–400, we reached the conclusion that they have a consistent trend (W4 > W6 > W5 > W2 > W7 > W1 > W3). Fluorescence index (f450/500) was always utilised to interpret the origin of organic matter in a complex aquatic environment system. Values of f450/500 are closer to 1.60, indicating that humic substances derived from terrestrial sources and biological sources. Our study demonstrated that reclaimed water of farmland irrigation, industrial wastewater and domestic sewage will definitely influence the humification degree and amount of the aromatic structure of DOM.

Key words | aromatic structure, dissolved organic matter, fluorescence index, fluorescence spectroscopy, humification degree, UV-vis spectroscopy

INTRODUCTION
During the past years, the study of dissolved organic matter (DOM) either occurring in terrestrial or aquatic environments became more and more popular. DOM participates in mobilisation and transportation of acidity, colloids, nutrients, metals/minerals, and pollutants and serves as a substrate for microbial growth. These manifold interactions with its environment result in complex components of substances in DOM with varying size and chemical structure (Dilling & Kaiser 2002). The organic matter, in water environment, can come from plants, soil organic matter (SOM), micro-organisms or the decomposition of algae in water. Its formation process is extremely complicated and changeable.

Consequently, the composition of DOM has been investigated by numerous methods including chemical characterisation and spectroscopic measurements, mostly in combination. The occurrence of the aromatic moieties offers the possibility to estimate the proportion of dissolved organic carbon in DOM using UV-vis spectra because the UV absorption of organic solutes is directly proportional to their content of aromatic compounds (Traina et al. 1990; Chin et al. 1994). Dissolved organic carbon absorbs light in a broad range of wavelengths (λ), in contrast with inorganic compounds, which are more common in natural water, that practically do not absorb light above 230 nm. Thus, wavelength of light absorption in natural waters is a semi-quantitative indicator of the DOM concentration in water. DOM was constituted of aromatic groups, with various degrees and types of substitution, including monosubstituted and polysubstituted phenols,
and diverse aromatic acids. Most of aromatic groups are the chromophores that absorb energy below 400 nm (Korshin et al. 1997).

Fluorescence spectroscopy was developed in the past two decades, as a new fluorescence analysis technique, which can describe the information of fluorescence intensity when the excitation wavelength ($\lambda_{ex}$) and emission wavelength ($\lambda_{em}$) were set at a certain range. Three-dimensional excitation emission matrix fluorescence spectroscopy (due to its sensitivity, non-destructivity, and simplicity) has been used to provide qualitative and quantitative information on the interaction between natural dissolved organic matter and metal ions since it was introduced in the early 1980s (Ryan & Weber 1982a, b). Many researchers employed three-dimensional fluorescence spectroscopy as an important analytical tool for the study of DOM (Stedmon et al. 2003; Robert et al. 2004). The synchronous fluorescence spectra can give more information about function and structure. Humification index (HIX) has been proposed to estimate the degree of the humification of DOM based upon the general observation that the emission spectra of fluorescent organic matter tend to shift to a longer wavelength with the condensation of the molecules (Kalbitz et al. 1999; Milori et al. 2002; Patalsorrentino et al. 2002).

The objectives of this research are 1) to investigate the structure, composition and humification degree of DOM with UV-vis spectroscopy and fluorescence spectroscopy and 2) to explain the origin of organic matter (OM) under a complex environmental system by using fluorescence index ($f_{450/500}$), 3) to reveal the relationships between different pollution sources with the variety of aromatic structure and the humification degree of DOM.

**STUDY AREA**

Wuliangsuhai Lake (108°43′~108°57′E, 40°27′~40°03′N) is located in the end of the Yellow River in Inner Mongolia section, China (Figure 1). Wuliangsuhai Lake is typical of grass-type eutrophic lakes in arid and semi-arid regions, and its existing water area is 293 km².

There is a large agricultural irrigation and drainage network at the upstream area of Wuliangsuhai Lake, which is the primary origin of water source. It does not only accept the drainage of agriculture, but also accepts the upstream industrial wastewater and domestic sewage; the former accounts for 96%, the rest 4%. For this study, seven typical water samples were chosen, whose locations are listed in Figure 1.
MATERIALS AND METHODS

Sample collection

The samples were collected from Wuliangsuhai Lake surface waters with pre-washed polyethylene bottles. Water samples were collected from the half of water depth. The water samples were stored on the top of ice before going back to the laboratory. Once upon return to the laboratory, water samples were immediately filtered through pre-combusted (450 °C for 5 h) 0.45 μm Whatman GF/F glass fibre filters. The container used in the experiment was soaked in dilute nitric acid overnight, and washed with ultrapure water. All chemicals used in this study were of AR grade.

Chemical analysis

The pH values of the water samples were measured with a pH meter (Sartorius PB-10). DOC, TC, and TN concentrations were measured with a total organic carbon (TOC) analyser (muti N/C 2100, Analytikjena, Germany). The relative inaccuracy of the DOC analysis was less than 5% as determined by repeated measurements. Electric conductivity (EC) was measured in saturation paste extracts using an EC meter (FE30, Mettler Toledo). DO concentrations were obtained by iodimetry. Table 1 lists basic parameters of water quality.

Spectral analysis

Ultra-violet and visible spectra measurements were carried out with 1 cm quartz UV-visible cells at room temperature (~20 °C), using a Shimadzu UV-visible double beam spectrophotometer (UV-1700). UV-vis absorption spectrum of DOM samples were obtained at wavelength 200 to 500 nm.

Excitation-emission-matrices (EEM) were measured in 1 cm quartz fluorescence cells at temperature (~20 °C), using a fluorescence spectrophotometer (F-7000, Hitachi, Japan) equipped with a 150-W Xenon arc lamp as the light source. The slit widths were 5 nm for excitation and emission monochromators, and the scan speed was set at 1,200 nm-min⁻¹. EEM spectra were obtained by setting the emission (Em) wavelength range from 280 to 550 nm in 5 nm steps, while the excitation (Ex) wavelength was increased from 200 to 450 nm in 5 nm steps. EEM contour maps were obtained in which each different fluorophore was characterised by an Ex/Em wavelength pair. The fluorescence response to a blank solution (Milli-Q water) was subtracted from the spectra of each sample (McKnight et al. 2001; Chen et al. 2003).

Synchronous fluorescence spectra were applied with the samples prepared with a constant offset (Δλ = 55 nm) between excitation and emission wavelengths and 5 nm slit widths. The scan speed, spectral range and response were set to 240 nm min⁻¹, 260–500 nm and 0.5 s respectively.

RESULTS AND DISCUSSION

UV-vis spectrum analysis

The UV-vis absorption spectra obtained are similar to those reported by Averett et al. (1989) for a sample of standard fulvic acid of 10 mg.dm⁻³. We found a clear absorption plateau displayed in UV-vis spectra between 230 nm and 280 nm. According to extant experience, the absorption plateau near 280 nm was generally caused by the sulfonic acid of lignin, while its ramification was originated from humic substances. Molar absorption intensity was enhanced with the increase of aromatic humus and unsaturated conjugated double bonds in the structure of DOM (Peuravuori & Pihlaja 1997). Absorption values of per unit concentration and the ratio of two absorbances at a specific wavelength, such as A280, A250/365, A233/203 and A226–400, were calculated in our study to reflect the humification degree, the diversity of aromatic structure and the distribution of DOM (see Figure 2).

SUVA254 index (SUVA254 = 100 • A254/CDOC) is derived when UV absorbance in the units of inverse metres (m⁻¹) at 254 nm is divided by organic carbon concentration measured in the units of milligrams per litre (mg.L⁻¹) (Leenheer & Croué 2003; Weishaar et al. 2005). This index is an ‘average’ absorptivity and is used as an indicator for the aromaticity of DOM samples were obtained at wavelength 200 to 500 nm.
the DOM (Weishaar et al. 2003; Schnitzler et al. 2007). Nishijima & Speitel (2004) suggested that UV absorbance at 254 nm could be detected with the existence of unsaturated carbon bonds, including aromatic compounds, which are generally recalcitrant for biodegradation. In our study, SUVA254 values range from 0.436 to 0.782, listed in Table 2. W3 sample mainly received drainage from the sites in the eastern lake. Since the reed acts as a natural filter, the impacts of contaminations on the water samples are relatively less than others. Consequently, W4, W5 and W6 have much higher values of SUVA254. UV absorbances of seven water samples could be arranged in descending order, which is W4 > W6 > W5 > W2 > W7 > W1 > W3.

The wavelength λ = 280 nm may not represent the maximum absorbance for all aromatic structures present in the DOM; however, it was normally chosen because π-π* electron transition occurs in this UV region for phenolic amines, benzoic acids, aniline derivatives, polyenes, and polycyclic aromatic hydrocarbons with two or more rings (Peuravuori & Pihlaja 1997). A280 indicates the concentration of aromatic structure of organic matter. The higher the value is, the more aromatic structures are dissolved in the water (Fan et al. 2006). Chin et al. (1994) proposed that A280 may provide some information on the humification degree, molecular weight and aromaticity of DOM. Wang et al. (2009) have verified it later. The UV absorbance at 280 nm and the molecular size of the organic matter were positively related. As can be seen from Table 2, the aromatic degree ranges from 0.153 to 0.328, in which the aromatic degree of W3 is also the least, and that of W4 is the greatest.

Korshin et al. (1997) pointed out that the value of A253/203 can reveal the substituent species in an aromatic system. In general, the substituents in aromatic rings are largely aliphatic chains. A253/203 value keeps low in unsubstituted aromatic ring structures, and increases when the aromatic ring is highly modified with hydroxyl, carbonyl, ester and carboxylic groups. Table 2 summarises the values of A253/203. The A253/203 values of W1 and W3 samples were significantly lower than those of the other five samples, demonstrating that W1 and W3 samples contain more aliphatic chains in the substituents of aromatic ring while the other samples may carry more carbonyl, carboxyl, hydroxyl and ester substituents of the aromatic ring. Inferring from order of A253/203 values (W4 > W6 > W5 > W2 > W7 > W1 > W3), we could draw a conclusion that the substituent species transited from hydroxyl, carbonyl, ester and carboxylic groups to unsubstituted aromatic ring structures.

A226–400 denotes the absorbance curve of area integration from 226 nm to 400 nm. UV absorption spectra of dissolved organic matter in the 226 nm to 400 nm range basically reflect the characteristics of absorption spectra. Generally, absorption band at 226 nm–250 nm was generated from unsaturated bond π-π*. Absorption band at the vicinity of 226 nm–400 nm arises from multiple conjugated benzene ring structure (HAO et al. 2006). Therefore, A226–400 could illustrate the variance of organic compounds containing

Table 2 | the values of SUVA254, A280, A253/203, A226–400 and A250/365 for seven sample sites

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>SUVA254</th>
<th>A280</th>
<th>A253/203</th>
<th>A226–400</th>
<th>A250/365</th>
</tr>
</thead>
<tbody>
<tr>
<td>W1</td>
<td>0.494</td>
<td>0.202</td>
<td>0.135</td>
<td>28.952</td>
<td>3.145</td>
</tr>
<tr>
<td>W2</td>
<td>0.581</td>
<td>0.273</td>
<td>0.239</td>
<td>36.738</td>
<td>4.225</td>
</tr>
<tr>
<td>W3</td>
<td>0.436</td>
<td>0.153</td>
<td>0.115</td>
<td>22.370</td>
<td>3.060</td>
</tr>
<tr>
<td>W4</td>
<td>0.782</td>
<td>0.328</td>
<td>0.285</td>
<td>43.750</td>
<td>4.876</td>
</tr>
<tr>
<td>W5</td>
<td>0.665</td>
<td>0.284</td>
<td>0.266</td>
<td>38.037</td>
<td>4.483</td>
</tr>
<tr>
<td>W6</td>
<td>0.730</td>
<td>0.315</td>
<td>0.293</td>
<td>41.997</td>
<td>4.656</td>
</tr>
<tr>
<td>W7</td>
<td>0.511</td>
<td>0.255</td>
<td>0.223</td>
<td>34.584</td>
<td>4.105</td>
</tr>
</tbody>
</table>
benzene ring in aqueous solution. In common sense, the more organic molecules contain benzene ring structures, the greater their stability will be and the more difficult to degrade them. The results showed that the maximum value of $A_{226-400}$ is 43.750 for W4 sample, indicating that DOM of W4 sample has a higher stability.

Another UV-vis ratio is important to elucidate the water carbon origin. The $A_{250/365}$ ratios close to 4.0 were assumed as fulvic acid in strongly coloured waters (Stedmon et al. 2003). Minero et al. (2007) pointed out that the ratio ($A_{250/365}$) less than 3.5 represents the origin of humic acids and more than 3.5 stands for the origin of fulvic acids. The $A_{250/365}$ ratios in our study range from 3.060 to 4.876, as shown in Table 2. The $A_{250/365}$ ratios of all our DOM samples carry the values close to 4 and more than 3.5, which indicates there are the fulvic acids in the dissolved organic carbon.

We are happy to find significant positive correlation of the SUVA$_{254}$, $A_{280}$, $A_{253/203}$, $A_{226-400}$ and $A_{250/365}$. Humification degree and aromaticity of DOM in W1 and W3 samples are lower than those of others. However, the humification of W3 sample is lower than that of W1 sample. A possible reason is that microbial degradation results in the increase of humic substances in W3 sample. From Table 1, the contents of DO are 0.5 mg.L$^{-1}$ and 0.6 mg.L$^{-1}$ for W1 and W3 respectively, which are lower than those of others. Furthermore, W1 and W3 samples obtain higher TN content. After Wuliangsuhai Lake continually received reclaimed water of farmland irrigation, domestic sewage and industrial wastewater, the water quality is likely to deteriorate and the humification degrees of DOM decrease as well. On the contrary, W7 is with higher humification degree extracted from the lake outlet area, largely due to a purification process which degrades some contaminants. W2 was obtained from the eastern part of Nature Reserve, whose humification degree of DOM is considerably higher than those of W1 and W3. W4, W5 and W6 were from the eastern lakes; with no industry firm or dense residential area in their neighbourhood, water qualities over there are much better. Therefore, these three samples have the highest level of humification.

**Fluorescence spectroscopy analysis**

Fluorescence properties of DOM are other important parameters for its chemical characterisation and source assessment (Coble 1996). 3DEEM can detect fluorescence peaks of diverse types, such as fulvic-like and humic acid. The study on humic substances with fluorescence spectroscopy is based on its structure containing a large number of functional groups, for instance with a variety of aromatic ring structures or unsaturated aliphatic chain or both. Due to its advantages of high sensitivity, good selectivity and not undermining matter structure, fluorescence spectroscopy well suites a study of chemical and physical properties with humic substances. Humic substances contain a number of different fluorophores, which provide the information on DOM’s structure, functional groups, types, heterogeneity, and dynamics characteristics between intramolecular and intermolecular, and the information of fluorescence intensity.

In 3DEEM spectra, four defined peaks could be identified (Figure 3a). The first peak was present at the wavelength Ex/Em = 230~260/390~450 nm, which was assigned to peak A for this study. It was previously reported that the peak is associated with fluorescent fulvic-like (Wu et al. 2001; Yamashita & Tanoue 2003). The second peak (peak C) appeared as a shoulder and was found near the wavelengths Ex/Em = 290~350/390~450 nm, which appear to relate to humic acid. The third peak (peak B) and the fourth peak (peak D) are associated with the presence of proteins at wavelength Ex/Em = 225/310~340 nm, Ex/Em = 275/310~340 nm respectively. Peak B and peak D mostly appeared as a shoulder except for W1 sample (Figure 3b).
W1 site accepted the upstream industrial wastewater and domestic sewage containing more protein materials, resulting in the presence of strong protein-like peaks. The value of fluorescence intensity (FI) is very high in DOM sample (FI = 5.027.0, FI is the sum of protein-like peaks), demonstrating that the degree of pollution is also more serious. W3 site accepted return water of farmland irrigation; the fluorescence intensity (FI = 823.9) of protein-like peak is obviously lower than that of W1 site. W2 site was also subjected to the influence of industrial wastewater and domestic sewage from the total drain; its fluorescence intensity of protein-like peak is higher than those other sites except for W1 site. In the other samples in a relatively clean region, protein-like peak is also relatively weak (FI = 1.030.5–1,534.3).

McKnight et al. (2001) 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 \( I_{450/500} \)), at an excitation wavelength of 370 nm. 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. When the pH values were adjusted to 8.0, 8.5 and 9.0, the \( I_{450/500} \) values are almost unchanged, indicating that the pH value barely affects the values of \( I_{450/500} \). The results are consistent with previous investigations by McKnight et al. (2001). The values of \( I_{450/500} \) for seven samples range from 1.594 to 1.746. In W1 and W3 sites, the values of \( I_{450/500} \) are 1.730 and 1.746 respectively, explaining that the two obtain a low aromaticity. There is a significant positive relationship between the degree of humification and fluorescence index; the lower the fluorescence index value, the stronger the degree of humification in heavily polluted areas in Wuliangsuhai Lake, showing that the humification of DOM decreases with increasing aromaticity. DOM has a low aromaticity with a high fluorescence index value (McKnight et al. 2001), and similarly a high humification degree with a low fluorescence index value.

### Correlation analysis

Weishaar et al. (2005) showed that UV absorption at 254 nm, when normalised to dissolved organic carbon (DOC) concentration, a parameter called specific UV absorbance (SUVA254), correlated strongly (R2 = 0.97) with DOM aromaticity. There is a significant positive relationship between SUVA254 and HIX (R2 = 0.90, p < 0.001) for water DOM of Wuliangsuhai Lake, showing that the humification of DOM increases with increasing aromaticity. DOM has a low aromaticity with a high fluorescence index value (McKnight et al. 2001), and similarly a high humification degree with a low fluorescence index value.

This study analysed the correlation of the SUVA254, A280, A226–400, A250/365 and \( I_{450/500} \). It can be demonstrated from Table 3 that there are significant correlations with each other.

The result of correlation analysis shows that there is a significant negative correlation between the degree of humification and fluorescence index; the lower the fluorescence index, the stronger the degree of humification. In heavily polluted areas in Wuliangsuhai, a lower fluorescence index

### Table 3

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<tr>
<td>( I_{450/500} )</td>
<td>( R )</td>
<td>(-0.759)</td>
<td>(-0.927)</td>
<td>(-0.958)</td>
<td>(-0.914)</td>
<td>(-0.954)</td>
<td></td>
</tr>
<tr>
<td>( P )</td>
<td>0.048</td>
<td>0.003</td>
<td>0.001</td>
<td>0.004</td>
<td>0.001</td>
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Jardim 1999). The humification index was calculated using the ratio intensity of peak III/peak II (HIX). The lowest HIX value is W3 sample, which was influenced by return water of farmland irrigation. Due to the impact of industrial wastewater and domestic sewage from the total drain, W1 sample has a lower HIX value, indicating a poor humification.
$f_{450/500}$ shows the source of DOM was suited to terrestrial sources, with increased degree of humification. With increased fluorescence index $f_{450/500}$, the source of DOM changes from terrestrial source to microbial source, accompanying the decreased degree of humification. W1 and W3 sites were polluted by upstream industrial sewage and agricultural non-point source respectively, which have relatively lower degree of humification, but obtained obviously higher values of $f_{450/500}$. Inversely, in relatively clean W2, W4, W5, W6 and W7 sites, when the degree of humification was higher, the value of $f_{450/500}$ was lower.

CONCLUSIONS

The present study aimed to demonstrate the use of UV-visible spectra and fluorescence spectroscopy to monitor the structure and origin of dissolved organic matter. A simple, practical and inexpensive experimental procedure using the UV-vis ratios was demonstrated to interpret the humification degree and molecular structure of DOM. This study was undertaken to apply the fluorescence technique to further characterise the structural and functional properties of DOM collected from Wuliangsuhai Lake. Fluorescence index ($f_{450/500}$) was used to explain the organic matter (OM) origin in a complex environmental system. The correlation between the UV-vis ratios and fluorescence index explains how different origins of DOM affect its humification degree. The study provides a scientific basis for monitoring lake pollution in arid and semi-arid regions.

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