

Supporting Information

For

Spectral Characteristics of Dissolved Organic Matter (DOM) in the Middle Reaches
of the Huai River in a Dry Season

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Text S1. UV-Vis spectroscopy and three-dimensional fluorescence spectral parameters.

For the UV-Vis measurements, a N5000PLUS UV-Visible absorption spectrophotometer (YOKE, China) was used with a scanning range of 200-800 nm and a scanning speed of 1 nm. Milli-Q ultrapure water was used as the blank control. The EEM measurements were conducted using an F-7100 three-dimensional fluorescence spectrophotometer (Hitachi, Japan) with a photomultiplier tube voltage of 700 V, a scanning interval of 5 nm, and a scanning speed of 1200 nm/s. The response time was set to automatic, and the excitation (Ex) and emission (Em) wavelength ranges were 220-450 nm and 250-550 nm, respectively.

Text S2. Liquid Chromatography-Organic Carbon Detection

Liquid chromatography-organic carbon detection (LC-OCD) is a precise quantitative technique used to analyze the DOM compositions, including their organic carbon and organic nitrogen contents, as well as detect fluorescent and non-fluorescent compounds. This technique employs a weak cation-exchange column (Polymethacrylate-based, TSK HW 50S, TOSOH, Japan) equipped with three detectors, namely organic carbon (OCD), organic nitrogen (OND), and UV (UVD) detectors, operating at a wavelength of 254 nm. In addition, this method utilizes ChromCALC software (DOC-LABOR, Karlsruhe, Germany) to identify and quantify organic carbon components of DOM. Indeed, the use of these detectors and software provides a comprehensive analysis of DOM compositions, offering insights into the different molecular weight fractions and their effects on water quality(1). The LC-OCD technique can separate DOM components into five main fractions based on size exclusion chromatography (SEC), namely biopolymer (BP), humic (HS), building block (BB), low molecular-weight acid (LMWA), and low molecular-weight neutral (LMWN) compounds(2).

Text S3. Graphics and Data Processing

In this study, Bigemap GIS Office and ArcMap 10.8.1 were employed to reveal the geographical locations of the study area and sampling sites. Data processing, parallel factor (PARAFAC) modeling, and plotting were performed using different software tools, including R (4.2.3), Matlab 2023, Excel, IBM SPSS Statistics 27, and Origin 2024. The LC-OCD data were analyzed using ChromCALC software to identify and quantify the different DOM fractions in the water samples. The results were presented in graphical forms to reveal the distributions of BP, HS, BB, LMWA, and LMWN compounds in the water samples.

The EEMs data of water samples were measured using an F-7100 fluorescence spectrophotometer. The light source was a 150 W ozone-free xenon lamp, and the photomultiplier tube (PMT) voltage was set to 700 V. Both the excitation and emission slit widths were set to 5 nm. The excitation wavelength (Ex) range was 220-450 nm, and the emission wavelength (Em) range was 250-550 nm, with a scanning interval of 5 nm. The scanning speed was 1200 nm/min. A 1 cm quartz cuvette with four optical paths was used for the measurements, and Milli-Q ultrapure water was also measured for EEMs data. The "staRdom" package (version 1.1.21) in R (version 4.4.1) was used for data processing, including blank correction, spectral correction, inner filter effect correction, scattering removal, interpolation smoothing, Raman normalization, PARAFAC model construction, outlier removal, residual analysis, split-half validation, and Tucker's congruence coefficient (TCC) test. The calculation methods for the fluorescence index (FI), humification index (HIX), and biological index (BIX) can be found in the relevant literature(3, 4).

Text S4. Sample Determination

The temperature (T) and electrical conductivity (EC) of the water samples were measured in situ using an EC300 portable conductivity meter (YSI, USA). In addition, the pH values and dissolved oxygen (OD) concentrations were measured in situ using a PhSJ-4F pH meter (INESA, China) and a ProODO dissolved oxygen meter (YSI, USA), respectively. On the other hand, 500 ml of each water sample was filtered through a 0.45 μm glass fiber filter (treated at 400 $^{\circ}\text{C}$) and analyzed for further hydrochemical parameters. The methods used to determine the concentrations of total nitrogen (TN), total phosphorus (TP), chemical oxygen demand (COD_{Cr}), ammonia nitrogen (NH_4^+), nitrate nitrogen (NO_3^-), and chlorophyll a (Chl-a) are reported in Table 1. The DOC concentrations in the water samples were analyzed using a total organic carbon analyzer (Shimadzu TOC-V CPH, Japan). Prior to the measurements, the pH values of the samples were adjusted to remove inorganic carbon. Specifically, the adjustment of pH to acidic levels (typically below pH 4.5) can lead to the conversion of bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions into carbon dioxide (CO_2), which can then be removed as a gas.

Text S5. Calculation formulas for UV-vis and fluorescence parameters and meanings

E2/E3 represents the ratio of absorbance at 254 nm to that at 365 nm. When E2/E3 is less than 3.5, the DOM primarily consists of higher molecular weight organic matter, with humic substances being more abundant than fulvic acids. Conversely, when E2/E3 is greater than 3.5, the DOM is dominated by lower molecular weight organic matter, with fulvic acids being more abundant than humic substances(5).

SR is used to indicate the source of DOM. When $SR > 1$, it suggests that the DOM primarily originates from biological sources, while $SR < 1$ indicates a significant input of high molecular weight, highly aromatic vascular plant-derived terrestrial DOM(6).

FI is the ratio of fluorescence intensity at an emission wavelength of 450 nm to that at 500 nm, with an excitation wavelength of 370 nm, used to describe the source of humic substances in DOM. $FI > 1.9$ indicates that DOM mainly originates from microbial metabolic activity in the water, while $FI < 1.4$ suggests a terrestrial origin(7).

$$FI = \frac{I_{Em}(450nm:370nm)}{I_{Em}(500nm:370nm)}$$

HIX is the ratio of integrated fluorescence intensity at emission wavelengths of 435-480 nm with an excitation wavelength of 225 nm, used to indicate the degree of humification in DOM. When $1.5 < HIX < 3.0$, DOM exhibits low humification, while $HIX > 3.0$ indicates a high degree of humification(8).

$$HIX = \frac{\sum I_{Em}(435\sim 480nm:255nm)}{\sum I_{Em}(300\sim 345nm:255nm)}$$

BIX is the ratio of fluorescence intensity at an emission wavelength of 380 nm to the maximum fluorescence intensity at 420-350 nm, with an excitation wavelength of 310 nm. BIX is used to indicate the proportion of autochthonous DOM. When $0.6 < BIX < 0.7$, there is little recent autochthonous input; when $0.7 < BIX < 0.8$, the input is moderate; when $0.8 < BIX < 1.0$, the input is high; and when $BIX > 1.0$, DOM is primarily of autochthonous origin(9).

$$BIX = \frac{I_{Em}(380nm:310nm)}{\max(I_{Em}(420\sim 435nm:310nm))}$$

Table S1. Measurement methods for water quality indicators

Parameters	Experimental method
TN	Alkaline potassium persulfate digestion UV spectrophotometric method (HJ 636-2012)
TP	Ammonium molybdate spectrophotometric method (GB 11893-89)
COD _{cr}	Determination of the chemical oxygen demand-Dichromate Method (HJ 828-2017)
Chla	Determination of chlorophyll a -Spectrophotometric method (HJ 897- 2017)
NH ₄ ⁺	Determination of ammonia nitrogen-Nessler's reagent Spectrophotometry (HJ 535-2009)
NO ₃ ⁻	Determination of nitrate-nitrogen-Ultraviolet Spectrophotometry (HJ/T 346-2007)

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