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Fluorescence analysis for water characterization: measurement processes, influencing factors, and data analysis

Zi-Bo Jing, Wen-Long Wang*, Yu-Jia Nong, Ping Zhu, Yao Lu and Qian-Yuan Wu

State Environmental Protection Key Laboratory of Microorganism Application and Risk Control (SMARC), Guangdong Provincial Engineering Research Center for Urban Water Recycling and Environmental Safety, Institute of Environment and Ecology, Tsinghua Shenzhen International Graduate School, Tsinghua University, Shenzhen 518055, China

*Corresponding author. E-mail: wwl20@sz.tsinghua.edu.cn

ABSTRACT

Fluorescence analysis is a sensitive and selective method that provides abundant information and does not result in sample destruction. This technology is widely used in the detection of dissolved organic matter in the environment. Some challenges with fluorescence analysis are its higher sensitivity so that it is sensitive to background signals, the difficulty of extracting useful information, and the complexity and diversity of analytical methods. This review summarizes recent applications of fluorescence analysis in water research for the characterization of pollutants, evaluation of water treatment processes, and monitoring of emerging contaminants such as drugs, disinfection by-products, and toxicity. Two-dimensional fluorescence and excitation–emission matrix fluorescence analysis methods are discussed, along with their advantages and disadvantages, and application scope. Methods for sample processing, instrument calibration, and data analysis are proposed. This review is an important source of information for the application of fluorescence technology in water research such as the analysis of emerging contaminants.

Key words: analysis, DOM, fluorescence, measurement

HIGHLIGHTS

- The application of fluorescence technology in water environments is summarized.
- The analytical methods and application scopes of two-dimensional fluorescence and EEM are discussed.
- A more accurate fluorescence analysis flow to control measurement and analysis errors is proposed.

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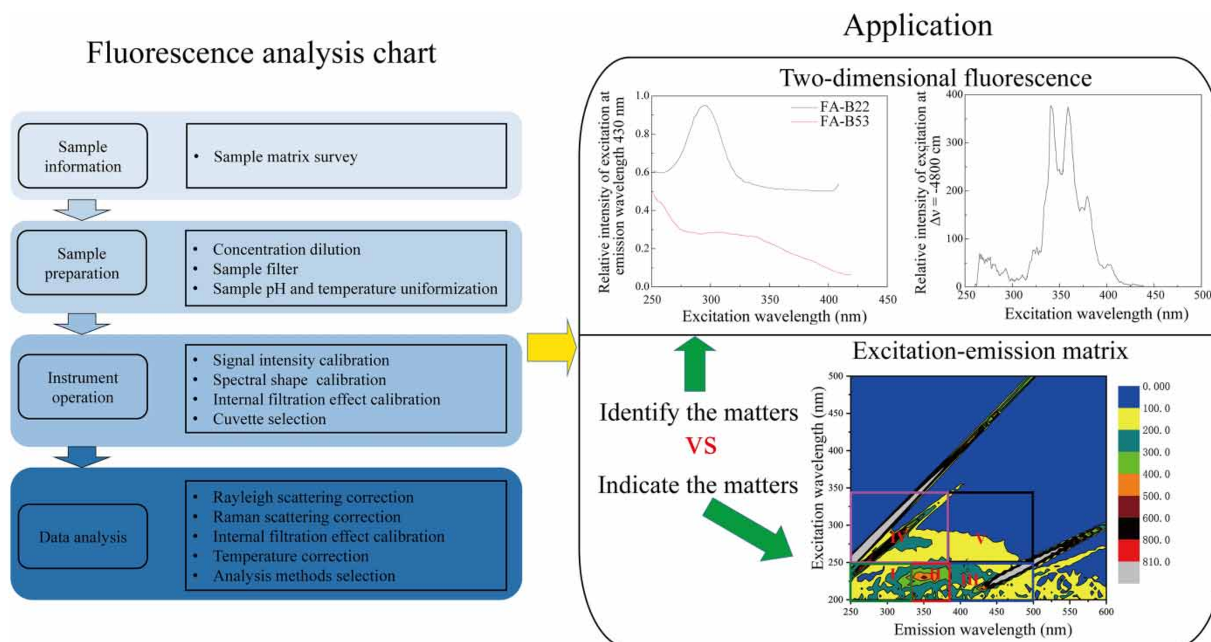
GRAPHICAL ABSTRACT



1. INTRODUCTION

Dissolved organic matter (DOM) is a complex mixture of humic acid, carbohydrates, amino acids, proteins, other natural organic matter (NOM), and synthetic organic compounds, and is widely found in water systems (Berg *et al.* 2019; Shi *et al.* 2021). In wastewater treatment systems, DOM causes membrane fouling and the disinfection by-products (DBPs) are precursors that affect the process operation and have potential health risks, respectively (Yang *et al.* 2015b; Li *et al.* 2020; Bai *et al.* 2021; Liu *et al.* 2021; Ren *et al.* 2021). Water quality indexes such as the chemical oxygen demand (COD) and biochemical oxygen demand (BOD) are used to assess water quality (Sgroi *et al.* 2017; Li *et al.* 2020; Xu *et al.* 2020; Shi *et al.* 2021). However, these analyses require the pretreatment of samples and have long detection times, which means they are not suitable for the rapid characterization of organic compounds in water.

Rapid and convenient analytical methods to measure DOM in aquatic environments are required. Especially in recent years, emerging contaminants put forward higher requirements for the detection of organic pollutants in water. Optical spectroscopy methods, including ultraviolet–visible absorption spectrometry (Zhang *et al.* 2020b), infrared spectroscopy (Yang *et al.* 2015b), and fluorescence spectroscopy (Yang *et al.* 2015a), have been used to analyze DOM. Fluorescence analysis has good selectivity and high sensitivity, provides abundant information and does not involve the destruction of the samples. Consequently, fluorescence analysis methods are frequently used to analyze DOM in water systems (Westerhoff *et al.* 2001; Li *et al.* 2014). These methods can be used to analyze the type and concentration of DOM in water (Lee *et al.* 2015; Ji *et al.* 2018), and the conversion of DOM in the water treatment process (Rodriguez-Vidal *et al.* 2020). They can also be used to evaluate the water treatment efficiency, such as the removal rate of trace organic pollutants (Sgroi *et al.* 2017), and indicate toxicity changes and disinfection by-product variations in water (Shen *et al.* 2018; Chen *et al.* 2020; Wang *et al.* 2021; Xu *et al.* 2021; Huang *et al.* 2022).

Traditional two-dimensional (2D) fluorescence analysis techniques, such as fluorescence excitation spectroscopy, fluorescence emission spectroscopy, and synchronous fluorescence spectroscopy, are mostly used to identify and characterize one or more substances (Bruckman *et al.* 2012; Foudeil *et al.* 2015). Excitation–emission matrix (EEM) analysis methods, including the fluorescence index (FI), fluorescence regional integration (FRI), and parallel factor analysis (PARAFAC) (Sgroi *et al.* 2017; Li *et al.* 2020), can be used to trace the sources of pollutants, evaluate the effects of water treatment processes, and monitor pollutants. These methods have been applied to water quality analysis of rivers and lakes (Patel-Sorrentino *et al.* 2002; Zhang *et al.* 2020a), groundwater (Vera *et al.* 2017), drinking water (Xu *et al.* 2021), municipal

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wastewater (Li *et al.* 2014; Mao *et al.* 2021), reclaimed water, and industrial wastewater (Rodriguez-Vidal *et al.* 2020; Islam *et al.* 2021). However, there are still many uncertainties about the process of fluorescence data analysis. High sample concentrations can exceed the analytical range, and extraction of useful information from complex multi-dimensional fluorescence data is difficult. In addition, water quality parameters and interactions between different components in the samples can affect fluorescence analysis.

In this paper, we aimed to (1) introduce the mechanism of fluorescence methods and the development of its application in water systems, (2) review 2D fluorescence and excitation–emission fluorescence methods and the application scope, and (3) identify and analyze the errors in fluorescence analysis to establish a rigorous fluorescence analysis process.

2. FLUORESCENCE APPLICATION

Fluorescence is a photoluminescence phenomenon and its general principles can be illustrated using a Jablonski diagram (Figure 1) (Noomnarm & Clegg 2009; Khan *et al.* 2022). Molecules in the ground state absorb excitation light (high energy photons, xenon light), photon energy is transferred to the molecules, and they enter an excited state. The excited molecules return to the ground state by non-radiative transitions (emission of heat or kinetic energy) and photoluminescence (emission of fluorescence and phosphorescence). The ground state (S_0) absorbs lower wavelengths of light (λ_1) and enters into the second excited singlet state (S_2). Alternatively, S_0 absorbs higher wavelengths of light (λ_2) and enters into the first excited singlet state (S_1). The higher energy second excited singlet state (S_2) can enter into the lower energy first excited singlet state (S_1) by internal conversion, and the first excited singlet state (S_1) can then return to the ground state by emission of fluorescence (λ_3). The first excited singlet state (S_1) may also enter the first excited triplet state (T_1) by intersystem crossing and then return to the ground state by emission of phosphorescence (λ_4). Vibrational relaxation occurs in the same electron energy level and transitions from high vibrational energy to low vibrational energy using energy exchange. According to their energies, the wavelengths are in order $\lambda_1 > \lambda_2 > \lambda_3 > \lambda_4$.

A search on the Web of Science for articles published between 2012 and 2021 with 'fluorescence' as the topic and 'environmental sciences ecology, water resources, and geochemistry geophysics' as the research interests gave 13,782 hits. A large number of articles illustrates how fluorescence has attracted much interest over the past 10 years, especially the past 4 years (Figure 2). The co-occurrence of certain keywords with 'fluorescence' from 2012 to 2021 was explored by bibliometric analysis (Figure 3) (van Eck & Waltman 2010). Fluorescence, DOM, water quality, drinking water, wastewater, and PARAFAC are frequently encountered as keywords with fluorescence, and this shows that fluorescence technology is often used for DOM analysis in aqueous environments. The colors (purple, green, and yellow) of the dots in Figure 3 represent

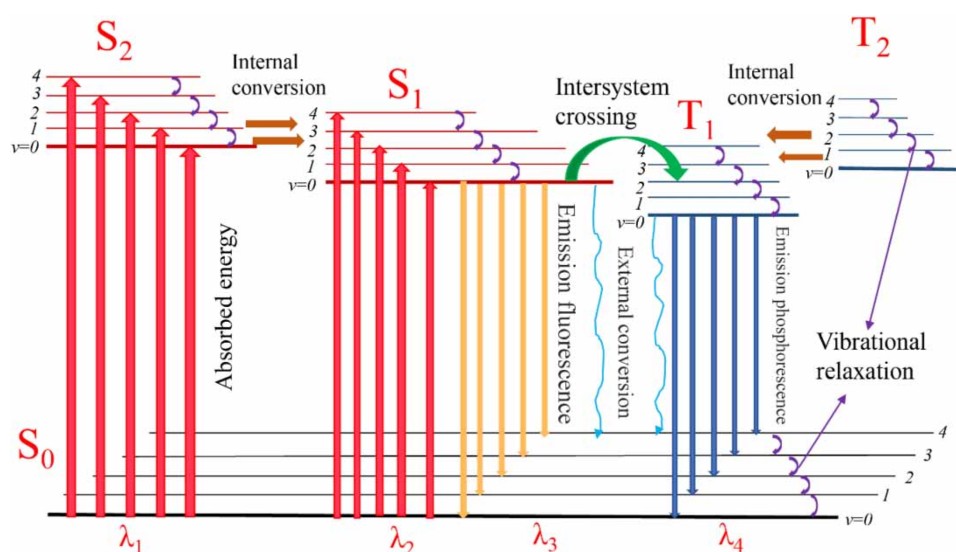

Figure 1 | Jablonski diagram showing energy transduction of different energy levels and fluorescence emission. S_0: ground state, S_1: first excited singlet state, S_2: second excited singlet state, T_1: first excited triplet state, T_2: second excited triplet state, λ_1: lower wavelength light, λ_2: higher wavelength light, λ_3: emission fluorescence, λ_4: emission phosphorescence (Noomnarm & Clegg 2009).

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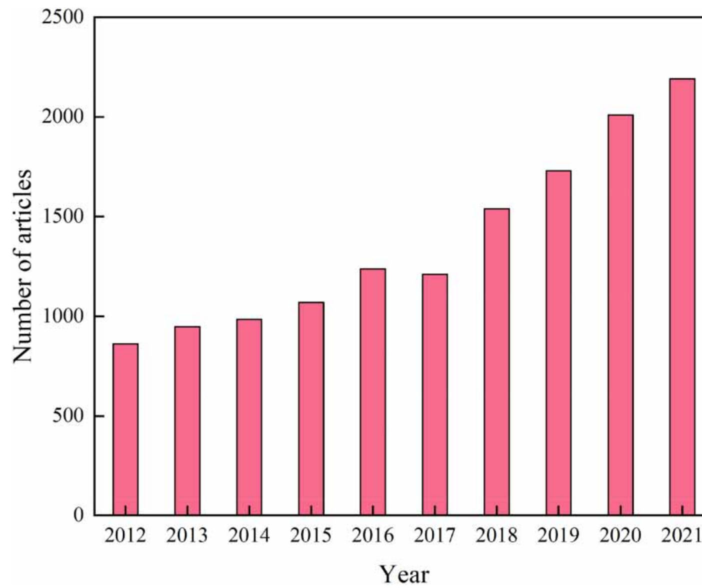

Figure 2 | Web of Science search of the number of articles about the topic of 'fluorescence' published between 2012 and 2021.

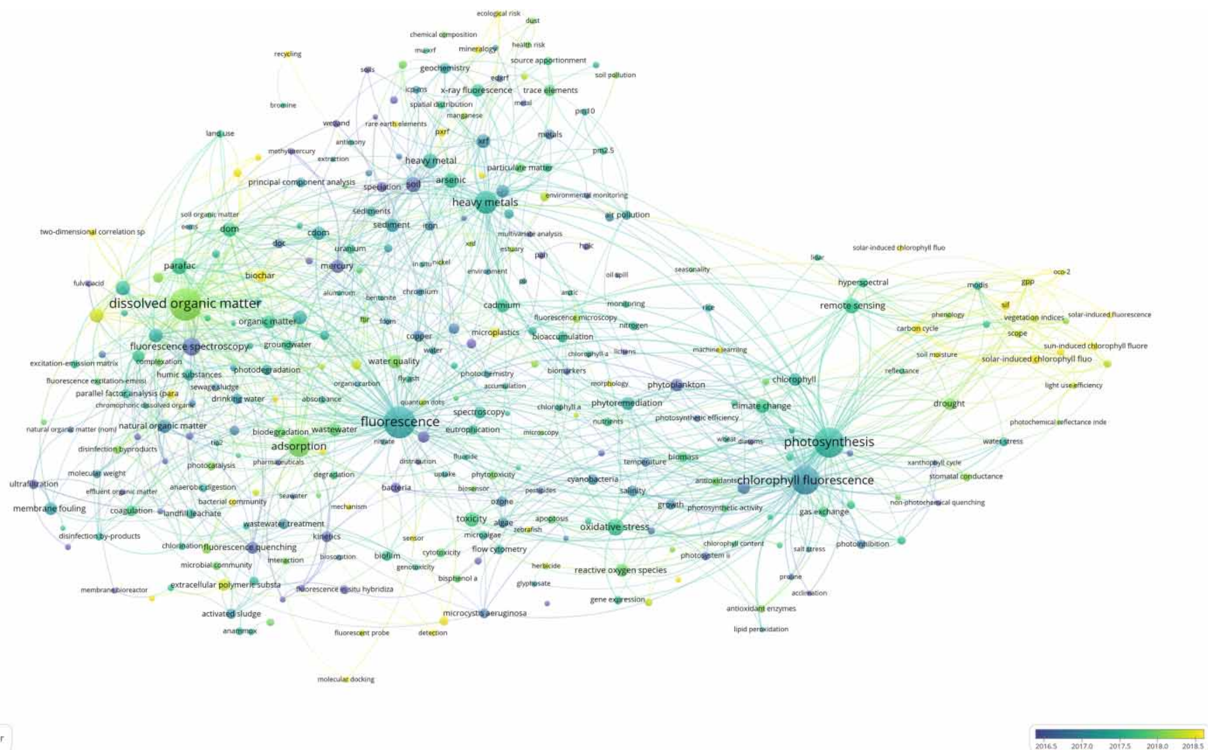

Figure 3 | Co-occurrence analysis of keywords related to fluorescence.

the order in which the keywords appear. DOM received more attention in recent years, and groundwater, drinking water, wastewater, EEM, humic substances, extracellular polymeric substances, and PARAFAC are closely related to DOM. PARAFAC is an important three-dimensional (3D) fluorescence analysis method. Fluorescence is often used to characterize DOM properties such as pollutant traceability and for water quality analysis and algal release analysis (Hanh *et al.* 2009;

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Sgroi *et al.* 2017). It is also used to evaluate efficiency in drinking water treatment and sewage treatment (Li *et al.* 2020; Islam *et al.* 2021; Shi *et al.* 2021; Chen *et al.* 2022). Additionally, fluorescence can be used to indicate the emerging contaminants in water, such as trace organic contaminants, micropollutants, and DBPs (Yang *et al.* 2015b; Sgroi *et al.* 2017; Yadav *et al.* 2019; Fan *et al.* 2020).

3. 2D FLUORESCENCE SPECTRA

A 2D fluorescence spectrum represents the relationship between the fluorescence intensity and excitation wavelength or emission wavelength. 2D spectra are simple and intuitive with the x-axis representing the excitation wavelength or emission wavelength, and the y-axis representing the relative fluorescence intensity (Figure 4). From a 2D fluorescence spectrum, we can intuitively obtain the number and position of fluorescence peaks. These peaks can then be used to identify fluorescent substances or as the basis for selecting appropriate excitation and emission wavelengths for the fluorescence analysis of substances.

3.1. Fluorescence excitation and emission spectra

A fluorescence excitation spectrum is a plot of the excitation wavelength versus the fluorescence intensity obtained with a fixed emission wavelength (Figure 4(a)). Fluorescence excitation spectra show the fluorescence quantum yield at different excitation wavelengths. The peak position can be selected according to the sample's excitation spectrum when determining the sample's concentration or composition (Wakebe & Van Keuren 1999; Ma *et al.* 2011; Bruckman *et al.* 2012). The fluorescence excitation spectrum can be used to analyze the fluorescence characteristics of specific substances such as xanthene dyes (Wakebe & Van Keuren 1999), erythrosine (Ma *et al.* 2011), NOM (Abbt-Braun & Frimmel 1999), and fulvic acid from groundwater (Kumke *et al.* 1999). The process of absorption of energy by fluorescent substances is an excitation process, which means that the excitation and absorption spectra will have similar shapes, but the fluorescence spectrum is not completely equivalent to the absorption spectrum.

For the fluorescence emission spectrum, the fluorescence intensity is scanned under different emission wavelengths at a fixed excitation wavelength to obtain the relationship between fluorescence intensity and the emission wavelength (Figure 4(b)). Substances can be identified using fluorescence emission spectra. The emission spectrum of a fluorescent substance will show a Stokes shift (Reynolds 2003), where the emission and absorption spectra have the same shape but the emission wavelength will be greater than the excitation wavelength. This occurs because the molecule loses some energy through internal conversion and vibration relaxation when it reaches the first excited singlet state (or second excited singlet state) and returns to the ground state. The spacing of vibrational energy levels in the ground state is related to the shape of the emission spectrum. The ground state molecules will be in different vibrational energy levels after excitation, which causes the

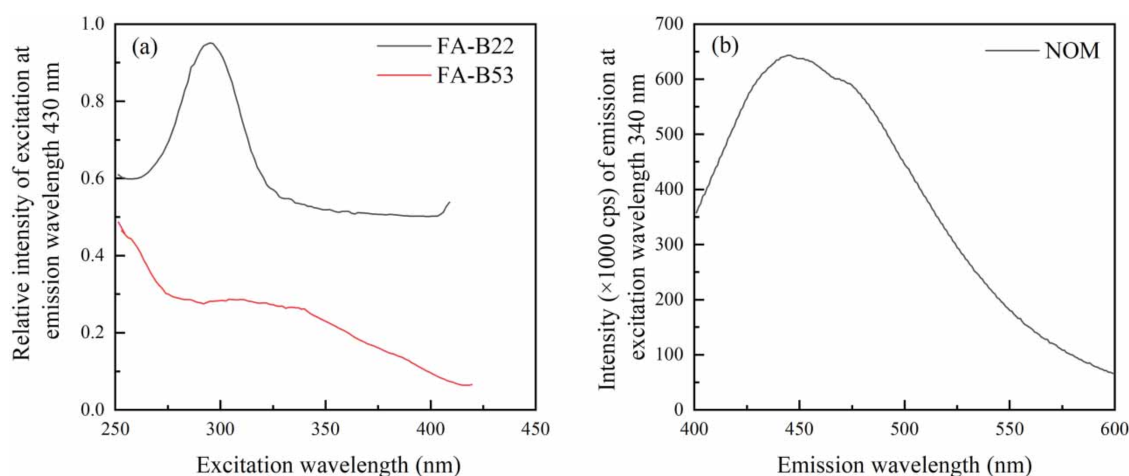

Figure 4 | Fluorescence excitation and emission spectra of (a) B22 fulvic acid and B53 fulvic at an emission wavelength of 430 nm with normalization of the spectra at an emission wavelength of 295 nm (Kumke *et al.* 1999), and (b) aquatic natural organic matter (NOM) at a fixed excitation wavelength of 340 nm (Chen *et al.* 2003a).

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first absorption state in the absorption spectrum, and the ground state is always at the lowest vibrational energy level. Therefore, the shape of the first absorption band is related to the distribution of vibrational energy levels after excitation. According to the Franck–Condon principle (Stuhlmann *et al.* 2014), the probability of reaching a certain vibrational level is high during the excitation transition, and the probability of reaching the same vibrational level is also high during the radiative transition. Therefore, the fluorescence emission spectrum and absorption spectrum are mirror images. The independence of the emission spectrum from the excitation wavelength is mainly determined by the energy difference between the lowest vibrational energy level of the first excited singlet state and each vibrational energy level of the ground state.

Fluorescence emission spectra have been used to identify substances (Li *et al.* 1996; Zsolnay *et al.* 1999), analyze the fluorescence of NOM (Chen *et al.* 2003a) and DOM (Lombardi & Jardim 1999; Wei *et al.* 2005), and study the interactions of other factors with organic matter in the environment (Lu & Jaffe 2001; Gadad & Nanny 2008).

3.2. Synchronous fluorescence spectrum

For complex mixtures, such as sewage containing a variety of pollutants, the spectrum obtained by conventional fluorescence spectroscopy (emission and excitation spectroscopy) will be a combination of individual molecular peaks, and this will result in featureless spectral bands (Samokhvalov 2020). Synchronous fluorescence spectroscopy (SFS) is based on synchronous scanning technology proposed by Lloyd (1971). This method has been used in the identification and quantitation of complex mixtures (Vo-Dinh 1978). In SFS, the excitation and emission wavelengths are scanned at the same time, and a spectrogram is constructed from the measured fluorescence intensity and excitation wavelength (emission wavelength) (Figure 5).

The main types of SFS are constant-wavelength SFS (CWSFS), constant-energy SFS (CESFS), variable-angle SFS, and matrix isopotential SFS. In CWSFS, the excitation wavelength and emission wavelength are kept at a constant interval

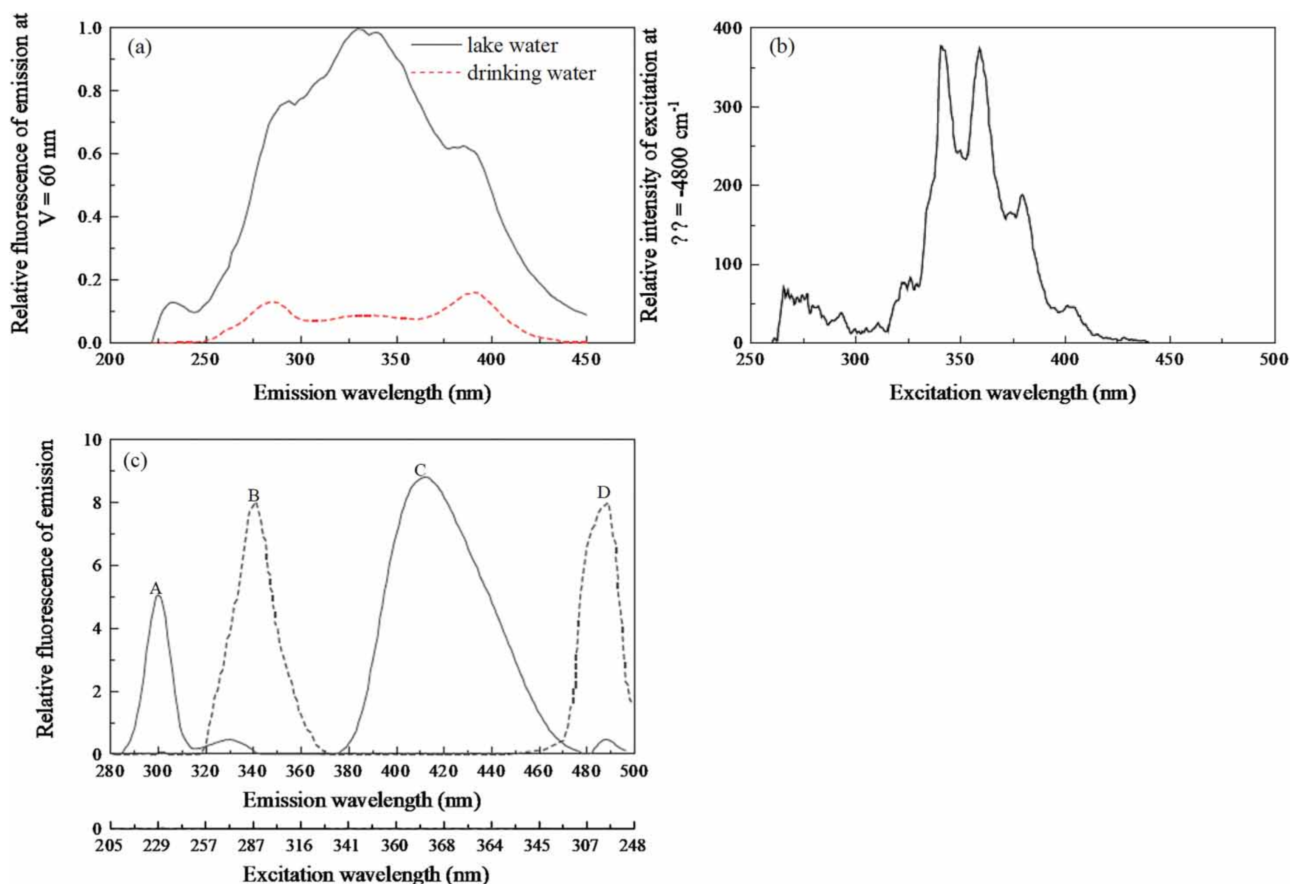

Figure 5 | (a) Constant-wavelength synchronous fluorescence spectra ($\Delta\lambda = 60$ nm) of lake water and drinking water (Reynolds 2003). (b) Constant-energy synchronous fluorescence spectra ($\Delta\nu = -4{,}800$ cm^{-1}) of a mixture of 16 PAHs (Yang *et al.* 2008). (c) Non-linear variable-angle synchronous fluorescence spectra for atenolol (A), propranolol (B), amiloride (C) and dipyridamole (D).

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($\Delta\lambda = \lambda_{em} - \lambda_{ex}$) during fluorescence scanning (Figure 5(a)) (Lloyd 1971). CESFS was proposed by Inman & Winefordner (1982) as a method to keep a constant excitation energy and emission monochromator during wavelength scanning (Figure 5(b)). CESFS is based on the specific vibration energy of the molecule. If the selected constant energy difference is equal to the vibrational energy difference, and the excitation energy and emission energy are within the vibrational energy difference, a synchronous spectrum with the maximum intensity is generated. Compared with CWSFS, CESFS can effectively overcome the Raman scattering and improve the analytical sensitivity (Andrade-Eiroa *et al.* 2010). Compared with variable angle SFS, constant energy, and constant wavelength scans are performed with constant separation between emission and excitation beams (Figure 5(c)). The spectrum will be a straight line with a slope of one and the axes in nanometers or reciprocal centimeters. The scan path of variable angle SFS is a straight line with varying slopes (Andrade-Eiroa *et al.* 2010). The nonlinear scan path is a broken line or curve in the EEM diagram. Cabaniss (1991) distinguished variable angle SFS by the slope and intercept, which are defined by wavelength and energy. Matrix isopotential SFS was proposed by Pulgarin & Molina (1994) as a nonlinear variable angle SFS method. In this method, the fluorescence intensity line of the matrix can be used to eliminate the influence of the matrix.

SFS is a well-known and well-established technology that has been widely used for the analysis of water and wastewater samples (Pullin & Cabaniss 1995; Reynolds 2003; Andrade-Eiroa *et al.* 2010). It has been applied to the detection of organic substances such as polycyclic aromatic hydrocarbons (PAHs) and pesticides in water (Rodriguez & Sanz 2000; Foudeil *et al.* 2015), the determination of humic and fulvic acids (Peuravuori *et al.* 2002), wastewater fingerprinting (Wu *et al.* 2006), and analysis of water toxicity (Hanh *et al.* 2009). Although SFS has been widely used in various fields, its application is limited by the internal filtration effect, fluorescence quenching, Raman and Rayleigh scattering, and the strong superposition effect of fluorescence signals from multi-contaminant matrix samples.

4. EEM

Compared with 2D fluorescence, the main difference with EEM is that changes in the fluorescence intensity information for the excitation and emission can be obtained simultaneously. Contour fluorescence spectrograms or 3D projections are commonly used to display EEM data. Coble *et al.* (1990) reported the first application of an EEM to analyze the fluorescence characteristics of DOM in the Black Sea. Currently, EEM is the main method used to characterize the source, composition, and other information of DOM. However, EEMs containing multi-dimensional information are often difficult to analyze to achieve the research objectives (Sgroi *et al.* 2017; Li *et al.* 2020). The three simplified methods for EEM data analysis are the FI, FRI, and PARAFAC.

4.1. FI

The FI is the ratio of fluorescence intensities at emission wavelengths of 470 and 520 nm ($FI = F_{470}/F_{520}$) when the excitation wavelength is 370 nm. This method can be used to distinguish the source of DOM because an $FI > 1.9$ indicates strong microbial action, an $FI < 1.4$ indicates the DOM is from terrestrial or soil sources, and an FI of 1.4–1.9 indicates that the DOM is from a combination of terrestrial and autogenous sources (McKnight *et al.* 2001).

The biological index (BIX) and humification index (HIX) have also been developed as indicators of DOM (Rodriguez-Vidal *et al.* 2020). The BIX is calculated as the ratio of fluorescence intensities with emission wavelengths of 380 and 430 nm ($BIX = F_{380}/F_{430}$) at an excitation wavelength of 245 nm. A $BIX > 1$ indicates that biological sources are mainly influenced by organisms, and a BIX of 0.6–0.7 indicates that terrestrial input or human activities greatly affect biological sources (Huguet *et al.* 2009). The HIX is the ratio of the average light intensity in the range of 435–480 nm and 300–345 nm when the excitation wavelength is 245 nm. The humification degree increases with the HIX (Morling *et al.* 2017).

The FI method has been widely used to characterize organic matter sources in water research (Table 1). The FI, BIX, and HIX have been used to distinguish the sources of DOM (microbial or terrestrial) (Rodriguez-Vidal *et al.* 2020), colored DOM in wastewater (Clark *et al.* 2020), and DOM in lakes (Carstea *et al.* 2014; Zhang *et al.* 2020b). The FI has also been used to evaluate the water quality indicators of COD, 5-day BOD, total nitrogen, and ammoniacal nitrogen (Zhang *et al.* 2020a). Moreover, it has been shown that the FI can be used to evaluate emerging contaminants, such as DBPs, and their potential for formation (Yang *et al.* 2015b; Shen *et al.* 2018; Xu *et al.* 2021).

4.2. FRI

An EEM contains tens of thousands of excitation–emission wavelength-dependent fluorescence intensity dots. The volume of data makes analysis difficult. To address this, Chen *et al.* (2003b) proposed dividing the EEM into five EEM regions using a

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Table 1 | Summary of the application of EEM fluorescence analysis methods

Analysis methods	Water type and treatment process	Excitation/Emission	Indication	Reference
FI	Drinking water	BIX	Soluble microbial products and disinfection by-products formation potentials	Shen *et al.* (2018)
	Lake	FI	Origin of chromophoric dissolved organic matters as terrestrial humic-like substances	Carstea *et al.* (2014)
	Drinking water treatment plant	BIX, HIX	Total trichloromethane formation potentials	Yang *et al.* (2015b)
	Drinking water	FI, BIX and HIX	Dissolved organic matters content and its disinfection by-products formation potential	Xu *et al.* (2021)
	Lake	FI, BIX and HIX	The concentration of chemical oxygen demand, biochemical oxygen demand, total nitrogen and ammonia nitrogen	Zhang *et al.* (2020a)
	Wastewater	FI, BIX and HIX	Origin of dissolved organic matters	Rodriguez-Vidal *et al.* (2020)
	Wastewater treatment plant	FI, BIX	Origin of chromophoric dissolved organic matters	Clark *et al.* (2020)
	Rainwater	HIX	Humification degree	Yang *et al.* (2019)
	Lake	FI, HIX	Origin of dissolved organic matters	Zhang *et al.* (2020b)
FRI	Drinking water	Regions III, V	Disinfection by-products and disinfection by-products formation potentials	Trueman *et al.* (2016)
	Drinking water treatment plant	Regions III, VI, V	Disinfection by-products	Fan *et al.* (2020)
	Drinking water	Region IV	Soluble microbial products and disinfection by-products formation potentials	Shen *et al.* (2018)
	River	Regions II, IV	Nitrogenous disinfection byproduct	Tan *et al.* (2017)
	Drinking water treatment plant	Regions II, IV	Nitrogenous disinfection byproduct	Lin *et al.* (2019)
	Wastewater treatment plan	Regions IV, V	Disinfection by-products	Liu *et al.* (2016)
	Wastewater	Regions II, III	Toxicity evolution during UV-driven oxidation	Huang *et al.* (2022)
	Wastewater	Regions I, II, IV	Toxicity evolution during up-flow anaerobic sludge blanket	Chen *et al.* (2020)
	Wastewater	250 ~ 300 nm/ > 320 nm	The concentration of chemical oxygen demand	Wang *et al.* (2022)
	Lake	Regions III, VI, V	The concentration of chromophoric dissolved organic matters	Ji *et al.* (2018)
	Wastewater treatment plan	Regions III, V	The concentration of chromophoric dissolved organic matters	Islam *et al.* (2021)
	Wastewater treatment plan	Regions V	Evaluation of emerging trace organic compounds removal	Sgroi *et al.* (2017)
	Landfill leachate	Regions II, V	The contamination of groundwater by landfill leachate	He & Fan (2016)
	Wastewater treatment plan	Regions II	Evaluation of antibiotic removal	Yadav *et al.* (2019)
PARAFAC	Wastewater treatment plan	C2 275 /340 nm	Evaluation of antibiotic removal	Yadav *et al.* (2019)
	Wastewater treatment plan	245, 350/450 nm; < 240, 315/380	Evaluation of emerging trace organic compounds removal	Sgroi *et al.* (2017)
	Drinking water treatment plant	C1 245/414 nm C2 230 (280)/328 nm	Nitrosamine	Maqbool *et al.* (2020)
	Drinking water treatment plant	C2 355/454.5 nm C3 250, 280/354.5 nm	Trichloromethane and para nitroso dimethylaniline formation potentials	Yang *et al.* (2015b)
	Bench-scale experiment	C1 260/455 nm C2 220/435 nm C3 225, 270/390 nm	The removal of humic substances by coagulation/flocculation process	Aftab & Hur (2017)

(*Continued.*)

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Table 1 | Continued

Analysis methods	Water type and treatment process	Excitation/Emission	Indication	Reference
	Drinking water	C1 250(310)/425 nm C2 260(380)/480 nm C4 260/440 nm	Disinfection by-products formation potentials	Xu *et al.* (2021)
	Drinking water	C1 224 (314)/398 nm C2 344/466 nm C3 289/344 nm C4 279/294 nm	The performance of coagulation-filtration process	Sanchez *et al.* (2013)
	Lake	C1 260/430 nm C2 280 (430)/555 nm C3 240 (390)/480 nm C4 250/515 nm	The concentration of chemical oxygen demand, biochemical oxygen demand, total nitrogen and ammonia nitrogen	Zhang *et al.* (2020a)
	Wastewater	C1 240/421 nm C2 305/415 nm	The specific fingerprint of the wastewater	Rodriguez-Vidal *et al.* (2020)
	Rainwater	C1 345/437 nm C2 300/408 nm C3 (250,330)/456 nm C4 275/311 nm	The concentration of chromophoric dissolved organic matters	Yang *et al.* (2019)

method named FRI. FRI uses vertical or horizontal lines to divide the EEM fluorescence contour spectra into the following regions: I, aromatic proteins class I (tyrosine-like substances); II, aromatic proteins class II (tryptophan-like substances); III, fulvic acid-like substances; IV, soluble microbial by-product-like substances; and V, humic acid-like substances. It is worth noting that the EEM regions determined by the FRI method are constant, and the material itself or external conditions only slightly affect the DOM position (Li *et al.* 2020). As a semi-quantitative method, the fluorescence flux of different regions can be calculated by the volume integral to obtaining two indexes. These indexes are the volume ($\Phi_{i,\ n}$ for each region, $\Phi_{T,\ n}$ for the whole region) and percent fluorescence response ($P_{i,\ n}$), and can be used to evaluate differences between samples or the change for a substance within a single sample (Li *et al.* 2020).

Because FRI analysis is simple and stable, it has been widely applied to investigate the composition and variation of DOM in water and wastewater (Table 1). FRI is often used to track and determine the fate of organic matter during water treatment (Sgroi *et al.* 2017). FRI has also been used to evaluate the performance of processes at treatment facilities, such as ultrafiltration, biological activated carbon, ultraviolet advanced oxidation, and membrane bioreactors (Vera *et al.* 2017; Shen *et al.* 2018; Lin *et al.* 2019; Huang *et al.* 2022). Similar to the FI method, FRI can be used to indicate emerging contaminants (Sgroi *et al.* 2017) such as pharmaceuticals and personal care products (Yadav *et al.* 2019), DBPs (Trueman *et al.* 2016; Shen *et al.* 2018; Fan *et al.* 2020), nitrogenous DBPs (Tan *et al.* 2017; Lin *et al.* 2019), and toxic compounds (Chen *et al.* 2020; Huang *et al.* 2022) in addition to detecting conventional indicators of water quality (Wang *et al.* 2022). Compared with the FI method, FRI is beneficial for application to substances with similar structures.

4.3. PARAFAC

For EEM with complex data structures, multi-way data analysis methods (chemometrics) are required. PARAFAC is the main chemometrics method used to process EEM data and has been applied to the analysis of DOM in water and wastewater (Sgroi *et al.* 2017; Li *et al.* 2020). PARAFAC is a quantitative and qualitative analysis method. It decomposes complex EEM fluorescence data into individual fluorescence phenomena and has become the most commonly used EEM analysis method. The three-way dataset is decomposed into three loading matrices by the PARAFAC model as shown in Equation (1) (Bro 1997; Stedmon & Bro 2008).

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

where x_{ijk} is one element of a three-way data array with dimensions I, J, and K. In the analysis of EEMs, x_{ijk} is the fluorescence intensity of sample i measured at emission wavelength j, and excitation wavelength k. The final term ε_{ijk} represents the

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unexplained signal (residuals containing noise and other unmodeled variations). The outcomes of the model are the parameters *a*, *b*, and *c*. Ideally, *a*, *b*, and *c* represent the concentration, emission spectra, and excitation spectra of the underlying fluorophores, respectively. The stages of PARAFAC analysis are data consideration, preliminary data treatment, exploration of the dataset, validation of the model, and interpretation of the results (Stedmon & Bro 2008). For the data consideration stage, approximately 20–100 samples are required for EEM PARAFAC analysis and established models cannot be extrapolated to other data for analysis. Instrument calibration and removal of Raman and Rayleigh scattering are the main steps for preliminary data treatment. The drEEM toolbox in MATLAB can be used to decompose the dataset, validate the model, and interpret the results (Hambly *et al.* 2015).

Several representative components are usually obtained by EEM PARAFAC analysis of samples in aquatic environments (Table 1). These components can be used instead of conventional water quality indicators, such as the COD, BOD_5, ammoniacal nitrogen, and total phosphorus (Zhang *et al.* 2020a), and emerging contaminants, such as DBPs, nitrogenous DBPs, *N*-nitrosamines, and pharmaceuticals and personal care products (Yang *et al.* 2015b; Sgroi *et al.* 2017; Yadav *et al.* 2019; Maqbool *et al.* 2020; Xu *et al.* 2021). These components can also be used to assess the effectiveness of water treatment technologies and the fate of organic matter in water treatment processes (Sanchez *et al.* 2013; Aftab & Hur 2017; Sgroi *et al.* 2017; Rodriguez-Vidal *et al.* 2020).

5. FACTORS INFLUENCING THE FLUORESCENCE SPECTRUM MEASUREMENT AND ANALYSIS

The fluorescence produced by a substance is related to the substance's structure; however, equipment, operational parameters, and environmental factors can affect the shape and size of the fluorescence spectrum. Therefore, the sensitivity and selectivity of fluorescence analysis can be improved by selecting appropriate operating conditions.

5.1. Influence of operational parameters

In all fluorescence measurement methods, instrument calibration is a necessary step to correct instrument biases. Instrument calibration includes the signal intensity and spectral shape. For example, for DOM measurement, the fluorescent molecules or fluorophores emitting fluorescence are unknown, so it is necessary to calibrate the signal intensity to compare different samples. The most common calibration method involves measuring quinine sulfate at an excitation wavelength of 350 nm and emission wavelength of 450 nm (Coble *et al.* 1993). The Raman signal of pure water can also be used for correction (Determann *et al.* 1994; Stedmon *et al.* 2003). Spectral correction takes into account the spectral output deviation of the instrument light source and biases of the instrument light transmission components. With the development of instrument technology, most currently available fluorescence measuring devices have a built-in reference detector to correct the optical signal for the light source spectrum. Residual deviations can be corrected periodically with rhodamine-B or rhodamine-101 (Karstens & Kobs 1980). Emission spectra also need to be corrected, but they generally show little variation and correction can generally be performed by changing the instrument operation manually.

Scattered light, such as that from Rayleigh and Raman scattering, has a large influence on fluorescence measurement. Molecular absorption of low photon energy can only excite electrons in the molecule to other higher vibrational levels of the ground state, but not to a higher excited state. In Rayleigh scattering, an excited electron that loses no energy quickly returns to its original ground state and emits light in which the radiation wavelength is the same as the excitation wavelength. In other vibrational levels of the ground state, the electrons in the molecule do not return to the initial ground state, but return to a higher or lower level than the original vibrational level. At this time, the wavelength of radiated light is longer or shorter than the excitation wavelength, and the radiated light is Raman scattered. Therefore, the occurrence of Rayleigh and Raman scattering in fluorescence measurements limits the sensitivity and reliability of fluorescence analysis. Emission gratings with a double monochromator and a cut-off filter in the measuring instrument can attenuate the effect of scattered light (Murphy *et al.* 2013). Raman scattering can be removed by deducting the pure water spectrum from the sample spectrum (Stedmon & Bro 2008). In the Rayleigh scatter-affected region, scattered signals are often treated as missing data to remove the effects of scattered light (Christensen *et al.* 2003; Stedmon & Bro 2008), and a blank fluorescence spectrum is used for subsequent fluorescence modeling and analysis. The above problems can be avoided by inserting zeros outside of the data region (Rinnan *et al.* 2005). The *smootheem* functions in the drEEM toolbox in MATLAB can be used to remove scattered signals or interpolate to remove Rayleigh and Raman scattering (Murphy *et al.* 2013).

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5.2. Influence of water parameters

Because of the absorption of excited or emitted fluorescent photons by the sample matrix, the inner filter effect (IFE) reduces the fluorescence yield. The main reason for the IFE is that some chromophores in the sample absorb photons at the same wavelength as the target molecule (Chen *et al.* 2018). There are two types of IFE: primary IFE involves the absorption of excited photons by the fluorophores and chromophores; and secondary IFE involves the absorption of photons emitted by fluorophores and chromophores (Ohno 2002). IFE will distort the fluorescence spectrum and adversely affect the fluorescence analysis of substances. Generally, analysis of samples with low concentrations is considered to have a low IFE. However, the IFE should be corrected for accurate analysis of samples at high concentrations (Panigrahi & Mishra 2019). The IFE calibration methods include instrument calibration, parameter correction, and mathematical correction (Khan *et al.* 2022). Instrument calibration is accomplished by instrument theory, parameter correction uses absorbance, optical density, and other parameters for correction, and mathematical correction is used for subsequent fluorescence data analysis (Ohno 2002; Chen *et al.* 2018).

The emission fluorescence characteristics of some molecules with acidic or fluorophores can be considered as two types. A variation in pH will change the proportions of two fluorescent molecules, which will affect the position and shape of the fluorescence spectrum and the fluorescence intensity. For example, a study of the fluorescence spectra of humic substances from the International Humic Substances Society showed that the fluorescence spectra of samples redshifted with an increase in pH (Pullin & Cabaniss 1995). This shift was attributed to a change in the fluorescence characteristics of acidic functional groups in humic substances (Mobed *et al.* 1996). Moreover, it has been verified that the fluorescence intensity increases with increasing pH. This has been observed in investigations of organic matter in river water and the detection of extracellular organic matter under different pH conditions (Patel-Sorrentino *et al.* 2002; Sheng & Yu 2006). When the pH changes from neutral to alkaline, the fluorescence intensity of treated wastewater decreases by 30–40% (Westerhoff *et al.* 2001). Similarly, salinity can also change the position and shape of the fluorescence spectrum and the fluorescence intensity by changing the molecular characteristics of fluorescent molecules (Khan *et al.* 2022). For example, one study found that the fluorescence intensity increased by approximately 10% when the pH was increased from 7.0 to 8.5 and the salinity was doubled (Esteves *et al.* 1999).

Increases in the temperature have an adverse effect on fluorescence because the probability of collisions between molecules increases, which increases the probability of de-excitation (Carstea *et al.* 2014; Lee *et al.* 2015). The fluorescence intensities of humic substances from the International Humic Substances Society, tryptophan standard, river water, and sewage were quenched when the temperature was increased from 10 °C to 45 °C (Baker 2005). Elliott *et al.* (2006) observed that the fluorescence decreased by more than 40% as the temperature increased. The degree of thermal quenching depends on the type of water because the fluorophores in water come from different sources. Terrestrial humic-like components in rural water samples cause higher quenching than those in urban water samples (Carstea *et al.* 2014). Thus, it is necessary to correct the influence of temperature on the fluorescence spectrum in DOM fluorescence analysis. Temperature correction tools based on sequential mathematical correction methodology have been used to address the effect of temperature on fluorescence measurements (Watras *et al.* 2011; Goffin *et al.* 2020).

Metal ions can chelate with DOM or precipitate DOM to enhance or quench fluorescence. There are two types of DOM fluorescence quenching by metal ions. The first, which is called dynamic quenching, involves the formation of stable non-fluorescent complexes between metal ions and DOM fluorescence binding sites. The second, static quenching, involves the formation of metal complexes that have only partial fluorescence because of the inherent chemical heterogeneity of DOM (i.e., different chemical structures and/or similar chemical structures in different environments) (da Silva *et al.* 1998; Yamashita & Jaffe 2008). Static quenching decreases with temperature increases, whereas dynamic quenching increases with increases in temperature (Khan *et al.* 2022). In dynamic quenching, non-fluorescent metal complexes deactivate excited molecules through intermolecular or intramolecular collisions (Lakowicz 2006). As the temperature increases, it is difficult for the molecules in the ground state to enter the excited state, which weakens the fluorescence signal. Paramagnetic metal ions (Cu^{2+}, Fe^{3+}, Hg^{2+}, Ni^{2+}, and Zn^{2+}) can quench the fluorescence of humic-like substances but not protein-like substances (Provenzano *et al.* 2004; Henderson *et al.* 2009). Diamagnetic metal ions (Al^{3+}, Mg^{2+}, Ca^{2+}, and Cd^{2+}) show different effects on fluorescence (i.e., enhancement, quenching, or minimal effect) (Elkins & Nelson 2002). Tryptophan-like fluorescence is quenched by Cu^{2+}, Ni^{2+}, Fe^{3+}, and Mo^{3+}, but not by Mn^{2+}, Co^{2+}, Ca^{2+}, Zn^{3+}, Cr^{3+}, and Na^{+} (Henderson *et al.* 2009). Different concentrations of metal ions and fluorophores, pH values, and temperatures in various systems lead to different levels of

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fluorescence quenching (Henderson *et al.* 2009; Pan *et al.* 2012). Therefore, for fluorescence measurements in complex matrices such as sewage, the influence of different components should be considered.

The solvent effect is another important factor affecting fluorescence analysis (Mataga *et al.* 1955). Under the influence of the dielectric constant and refractive index of a solution, the fluorescence intensity and maximum wavelength of the fluorescence spectrum are generally affected by the solvent. Hydrogen bonding between the solvent and fluorescent molecules can also cause the solvent effect (Siqintuya *et al.* 2005). The polarity of the solvent is the main contributor to the solvent effect on the fluorescence properties of a substance (Xie *et al.* 2004; Qiu *et al.* 2010). The complexation of ground and excited

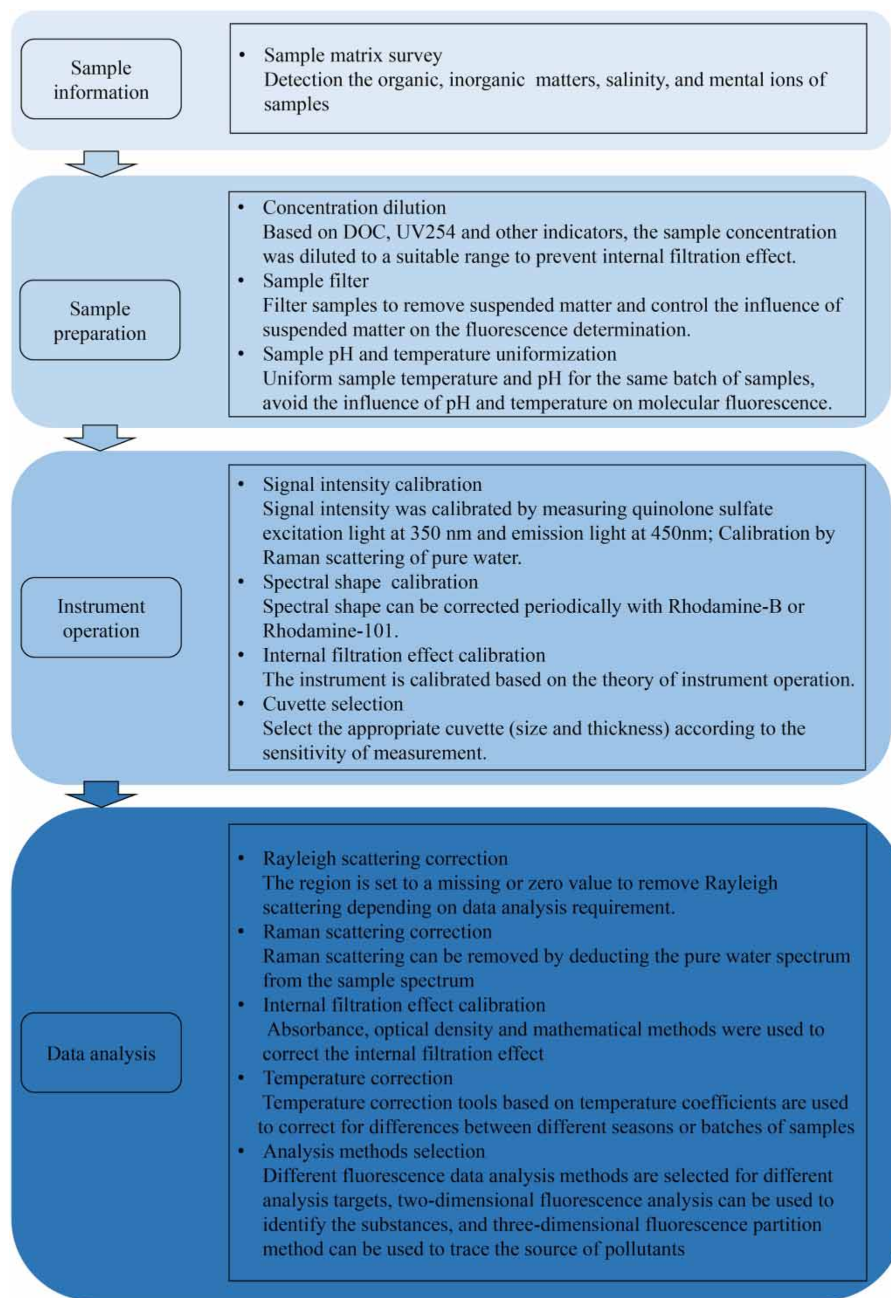

Figure 6 | Flow chart of fluorescence measurement data analysis. Derived from the literature: (Karstens & Kobs 1980; Coble *et al.* 1993; Pullin & Cabaniss 1995; Ohno 2002; Christensen *et al.* 2003; Stedmon *et al.* 2003; Baker 2005; Stedmon & Bro 2008; Watras *et al.* 2011; Chen *et al.* 2018; Panigrahi & Mishra 2019; Khan *et al.* 2022).

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molecules and polar molecules enhances fluorescence (Rechthaler & Kohler 1994). The fluorescence spectra of quinolone antibiotics in different solvents showed that the dielectric effect of the solvent was stronger than that of specific hydrogen bonding (Park *et al.* 2002). Therefore, the solvent effect should be considered when fluorescence technology is used to identify certain substances or analyze the removal of characteristic pollutants.

5.3. Flow diagram of fluorescence measurement and data analysis

The accuracy, stability, and sensitivity of fluorescence measurement and data analysis can be affected by various factors. Consequently, calibration is required during experimental and analytical processes. For the measurement and analysis of EEM fluorescence data as an example, we developed a flow diagram to represent considerations in fluorescence measurement and analysis (Figure 6). First, the organic and inorganic components, salinity, and ionic strength of the samples should be investigated and analyzed, along with their effects on the target substances. Second, for sample preparation before measurement, the pH and temperature should be adjusted. In addition, the sample concentration should be diluted to a suitable level to avoid IFE. Especially, solvent effects should be considered when using 2D spectroscopy for the identification of substances or in laboratory-scale removal studies for specific contaminants. Third, instrument calibration should be performed to calibrate the signal intensity, spectral shape, and IFE during measurement. Finally, for analysis of the data after fluorescence measurement, the IFE should be removed by mathematical analysis, the temperature correction coefficient should be used to correct the temperature effect (e.g., for comparison of samples from different seasons), and Raman and Rayleigh scattering should be corrected. There are many other environmental factors or operating conditions that could affect the fluorescence spectrum, and these still need to be elucidated and solved.

6. CONCLUSIONS

Fluorescence analysis has been widely used to characterize DOM in water and wastewater and used to trace pollutants, assess the performance of water treatment technologies, and indicator characteristic pollutants including emerging contaminants (e.g., drugs and DBPs).

There are two main categories of fluorescence analysis methods: 2D methods, such as fluorescence excitation and emission spectroscopy and synchronous fluorescence spectroscopy; and EEM methods of the FI, FRI, and PARAFAC. The 2D analysis methods are mainly limited to the identification of one or several pollutants. By contrast, EEM analysis methods provide more complex fluorescence information and can be used to analyze a variety of pollutants. Consequently, EEM analysis has become the most widely used technology.

Moreover, this review evaluated a variety of methods to reduce the error in fluorescence analysis and developed a flow chart for fluorescence analysis. The error in fluorescence analysis is affected by two types of parameters: operation parameters, including the instrument calibration, cuvette selection, and removal of Raman and Rayleigh scattering; and water quality parameters, including the IFE, pH, temperature, metal ions, and solvent effect. This analysis flow chart will support future research on improving the accuracy of fluorescence analysis.

7. RECOMMENDATIONS FOR FURTHER STUDIES

Although fluorescence technology has been widely used in water environments, the following issues are required to be further studied and analyzed in the future:

1. There is a need to conduct a series of studies to explore the relationship of emerging contaminants with fluorescence. With the continuous discovery of emerging pollutants, there is an increasing emphasis on their rapid characterization. Therefore, the exploration of fluorescence characteristics of pollutants is conducive to the rapid and convenient analysis of water quality.
2. Further studies are needed to analyze the influence limits of temperature, pH, metal ions, internal filtration effect, and other environmental factors, and to develop a more complete and standard fluorescence analysis process to obtain accurate fluorescence data.
3. Online water quality detection has become an important form of water quality analysis and display for drinking water treatment and supply, and wastewater treatment and discharge. Fluorescence has great advantages as a fast and sensitive method for the detection of organic matter. Furthermore, the correlation of fluorescence signals and conventional indicators such as COD and BOD, and emerging contaminants such as antibiotics, endocrine disruptors, and DBPs.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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