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RESEARCH ARTICLE

Three-dimensional fluorescence spectral characterization of soil dissolved organic matters in the fluctuating water-level zone of Kai County, Three Gorges Reservoir

Fang FANG, Yan YANG, Jinsong GUO (✉), Hong ZHOU, Chuan FU, Zhe LI

Key Laboratory of Three Gorges Reservoir Region's Eco-Environment of Ministry of Education, Chongqing University, Chongqing 400045, China

Abstract Three-dimensional fluorescence spectroscopy was used to investigate the fluorescent properties of soil dissolved organic matter (DOM) in the water-level-fluctuation zone (WLFZ) of Kai County, Three Gorges Reservoir (TGR). Most of the soil DOM analyzed in this study was found to contain four fluorescence peaks. Peaks A and C represent humic-like fluorescence, whereas peaks B and D represent tryptophan-like fluorescence. Peaks E and F, which represent tyrosine-like fluorescence, only appeared in certain soils. Soil humus was the main source of DOM in soil, and higher concentration of soil DOM was found in the exposed soil than submerged soil. Compared to the peaks A and B, the fluorescence intensities of peaks C and D were strongly influenced by the fluctuating water level. Analysis of fluorescence intensities of different peaks in soil DOM showed that WLFZ soil was not contaminated significantly. Soil DOM contained at least two types of humic-like fluorescence groups and two types of protein-like fluorescence groups. The proportion of the content of peak A in soil organic matter was quite stable. The soil DOM in exposed soil had relatively high humification and aromaticity, and periodic submerging and exposure of soil had an impact on the humification of soil DOM.

Keywords water-level-fluctuation zone (WLFZ), soil, dissolved organic matter (DOM), three-dimensional fluorescence spectra

1 Introduction

A water-level-fluctuation zone (WLFZ) is a zone in which the surface alternates between wet and dry. Since rivers, lakes, reservoirs and other water bodies fluctuate seasonally, parts of their land are periodically either covered by water or exposed [1,2]. The WLFZ in the Three Gorges Reservoir (TGR) is the zone between the water level of 145 m for flood control and the normal water level of 175 m for impoundment. It covers a total (surface) area of 348.93 km^2. As the zone where the transition between land and water converges, the WLFZ has a sensitive and fragile ecosystem. This could result in a series of ecological and environmental problems in the WLFZ as well as the TGR [1,3].

Currently, research into the environment of the WLFZ has focused on the impact of the fluctuating water levels on the water and soil environments, as well as the ecological environment. The effects of water-level fluctuations on the adsorption and release of soil N and P in the WLFZ have been studied [2,4,5]. However, there are very few studies on soil dissolved organic matter (DOM) in the WLFZ. Soil DOM plays an important role in the migration and transformation of pollutants [2]. DOM may interact with soil nutrients such as N and P, and trace heavy metal ions, influencing their migration and transformation at the land/water interface, their toxicity and their bio-availability [4,5]. Soil DOM can also be significantly impacted by changes in the environmental conditions of an ecosystem [2]. Since soil in the WLFZ is periodically submerged and exposed, it is likely that its DOM is different from that of ordinary soil. Therefore, it is necessary to study the physical and chemical properties of soil DOM in the WLFZ.

In natural ecosystems, soil DOM mainly comes from plant root exudates and litter, soil organic matter itself, as well as soil animals and the products of microbial metabolism. In agro-ecological systems, the use of exogenous organic matter such as straw, all kinds of manure, urban sewage sludge and other organic waste is another important source of DOM [6,7].

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E-mail: guo0768@126.com

DOM contains humic acid, fulvic acid, and various hydrophilic organic matters (including fatty acids, amino acids, some carbohydrates, etc.) in addition to other unknown complex organic components. Application of fluorescence spectroscopy to the study of DOM is based on the fact that DOM contains large quantities of aromatic structures and unsaturated fatty chains with various kinds of functional groups [8]. Three-dimensional fluorescence spectroscopy, developed in recent years, has the advantages of high sensitivity, high selectivity, and high information content, and does not damage the structure of samples. So it is widely used to study the physical and chemical characteristics of DOM from soil, surface water and seawater in terms of source determination, compositions and macro-structures [9–13].

The fluorophores in DOM can be divided into two major categories, humic-like fluorophores and protein-like fluorophores [9], while protein-like fluorophores can be subdivided into tryptophan-like one and tyrosine-like one [14]. In general, peaks A and C reflect the fluorescence intensities of fulvic acid and humic acid, which are related to the carbonyl and carboxyl groups in the structure of humus [11]. Peaks B, D, E and F are related to the aromatic amino acid structures in DOM, which reflect fluorescence due to biologically degraded tryptophan and tyrosine [14]. Fluorescent matters and the corresponding peak positions of DOM are shown in Table 1.

In this study, three-dimensional fluorescence spectroscopy was used to investigate the fluorescence spectral characteristics of DOM in the WLFZ of Kai County in the TGR. The DOM was classified, and its sources were analyzed, with the objective of providing evidence for an in-depth theoretical study on soil DOM and the migration and transformation of pollutants in the WLFZ.

2 Materials and methods

2.1 Sample area

Kai County is located in the north-east of Chongqing City, with Bashan Mountain to the north and the Yangtze River to the south. It is in the center of the TGR area. The old town area in Kai County is located in a valley and an area of low hills, which makes the county the most liable to be flooded in the TGR. The (surface) area of the WLFZ in Kai County is 42.78 km^2. Urban land, rural towns and farmlands were the main ways to land utilization of WLFZ in Kai County, and the typical regions included Hanfeng town (E108°28′, N31°10′) and Fengle town (E108°24′, N31°11′), Zhuxi town (E108°17′, N31°07′) and Linjiang town (E108°13′, N31°05′), and Houba town (E108°30′, N31°12′) and Zhenan town (E108°18′,N31°09′, respectively. This study selected these towns as sample areas, and the sampling points are shown in Fig. 1.

2.2 Soil sampling

Samples were collected on January 10th and January 11th 2009 when the water level was 171 m, and the weather was sunny. A total of 24 samples were collected. Nine of the total soil samples were collected below a sampling elevation of 171 m. Three samples were sediments, namely, Fengle1, Fengle2 and Hanfeng1, and the remaining 6 were floodplain soil. Fifteen soil samples were collected between sampling elevations of 171 and 175 m. These corresponded to exposed soil of the WLFZ. Each sample was a mixture of 4 equivalent volumes of the subsample taken at depths of 0–20 cm within 1 m × 1 m. Collected samples were kept in polyethylene bags, which were freeze-dried after transportation to the laboratory. The latitude and longitude of sampling points were recorded by GPS.

2.3 Data testing

Soil organic matter (SOM) is a variety of carbon compounds which exist as the various forms and status in soil. It is an important indicator of the carbon content in the soil. The SOM was analyzed using the potassium dichromate method.

The three-dimensional fluorescence spectra of soil DOM were obtained by using a HITACHI F-4500 fluorescence spectrometer. The extraction of soil DOM and the testing of fluorescence spectroscopy are as follows:

1) Extraction of soil DOM

Extraction of soil DOM was carried out as described in the literature [12]. Freeze-dried soil samples were milled

Table 1 Fluorescent matters and the corresponding peak positions in DOM

fluorescence peak	Ex(excitation wavelength)/Em(emission wavelength)/nm	type	references
A	310–360/410–480	visible humic-like	[9–11]
B	270–290/320–350	high excitation wavelength tryptophan-like	[9,11]
C	240–270/370–480	UV humic-like	[9–11]
D	220–230/320–350	low excitation wavelength tryptophan-like	[9,11]
E	270–290/300–320	high excitation wavelength tyrosine-like	[9,11]
F	220–230/300–320	low excitation wavelength tyrosine-like	[9]

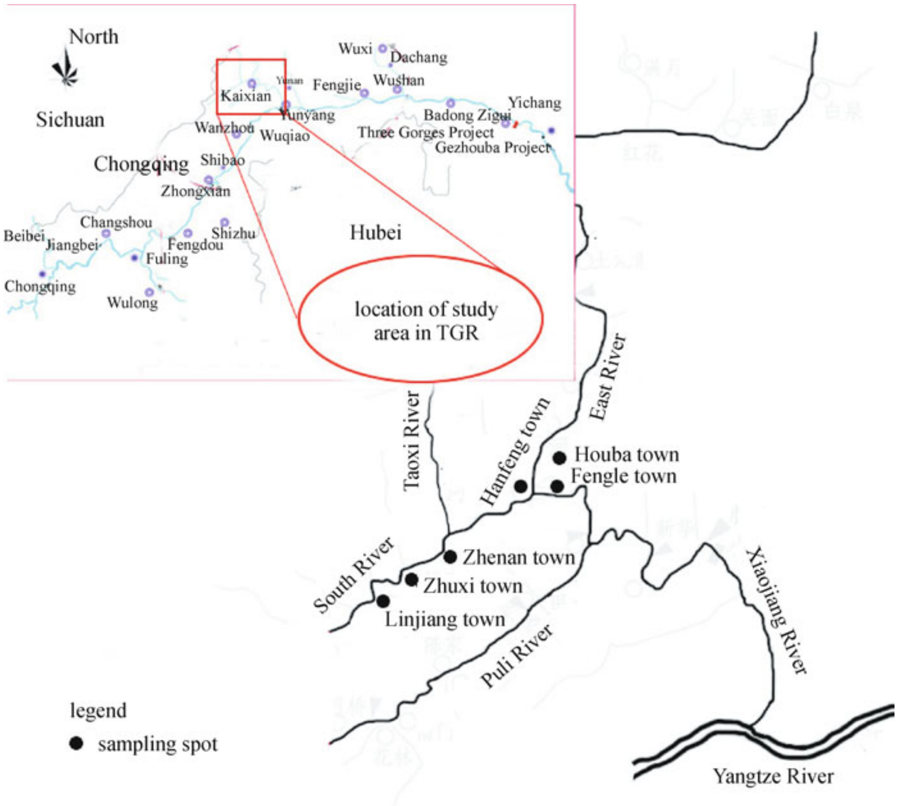


Fig. 1 Map of sampling locations in research area

by ceramic grinding cymbals and were passed through a stainless-steel sieve of 100 meshes. After sieving, deionized water was added to some of the samples at a ratio of 1g: 2 mL, and samples were shaken for 16 h (200 r·min^{-1}) at room temperature (20°C). Samples were then centrifuged for 30 min at 4000 r·min^{-1}, filtered through a 0.45 μm filter, and kept in a refrigerator at 4°C. Analyses were completed within 1 week.

2) Testing of fluorescence spectroscopy

A Hitachi F-4500 fluorescence spectrophotometer was used. The samples were kept at a constant temperature before the measurement (constant temperature water-bath: 20±1°C). The blank sample in this experiment was MilliQ water. The F-4500 system program was employed for data processing with an excitation range of 200 to 450 nm and an emission range of 250 to 500 nm. Instrumental parameters were as follows: excitation slit, 5 nm, emission slit, 10 nm; scan speed 1200 nm·min^{-1}; excitation light source, 150 W xenon lamp; photomultiplier tube voltage, 700 V; Signal/Noise Ratio: larger (greater) than 110; response time, automatic; scanning spectrum, automatic instrumental correction. The emission spectra were obtained by using an excitation wavelength of 254 nm, and the emission was recorded from (between) 280 to 500 nm. Blank water scans were run every 10 analyses using a sealed MilliQ water cell. The test results would deduct the data from three-dimensional fluorescence spectra and the emission fluorescence spectra measuring by MilliQ water under the same conditions.

2.4 Data analysis

SigmaPlot 9.0 software was used to map the three-dimensional fluorescence spectra, and SPSS16.0 software was used for data correlation analysis.

The ratio of the fluorescence intensity, $r(M, N)$, can be obtained from the following Eq. (1):

$$r(M,N) = \frac{I_m}{I_n}, \tag{1}$$

where I_m is the fluorescence intensity of peak M, and I_n is the fluorescence intensity of peak N.

The value of $r(M, N)$ is commonly used to study the nature of DOM. Usually there are r(B, A), r(C, A) as well as r(D, B). r(B, A) is commonly used to distinguish the degree of pollution [11], and the value of r(B, A) from contaminated river DOM is generally greater than 1.5 [11]. r(C, A) is the ratio of the UV humic-like fluorescence intensity to the visible humic-like fluorescence intensity, which is an indicator associated with organic structure and maturity [9,11]. r(C, A) is related to the molecular size of organic matter, the solution pH, etc. [9,11]. When the value of r(C, A) is constant, it indicates that there is only one type of humic-like fluorophore in DOM. When the value of

r(C, A) varies, it explains that there are at least two types of humic-like fluorophore in DOM [9,13]. Another study indicates that r(D, B), the ratio of the fluorescence intensities of protein-like peaks D and B, has a similar indicator function to r(C, A). This means that changes in r(D, B) also indicate the presence of at least two types of protein-like fluorophore in DOM [11].

The results showed that the humification index (HIX) values were sensitive to the DOM concentration of the solution and were linear with respect to the transmittance of the solution at the 254 nm used as the excitation wavelength [15–17]. *HIX* can be expressed by the following equation:

$$HIX = \frac{\sum I_{435\to480}}{\sum I_{300\to345}}, \tag{2}$$

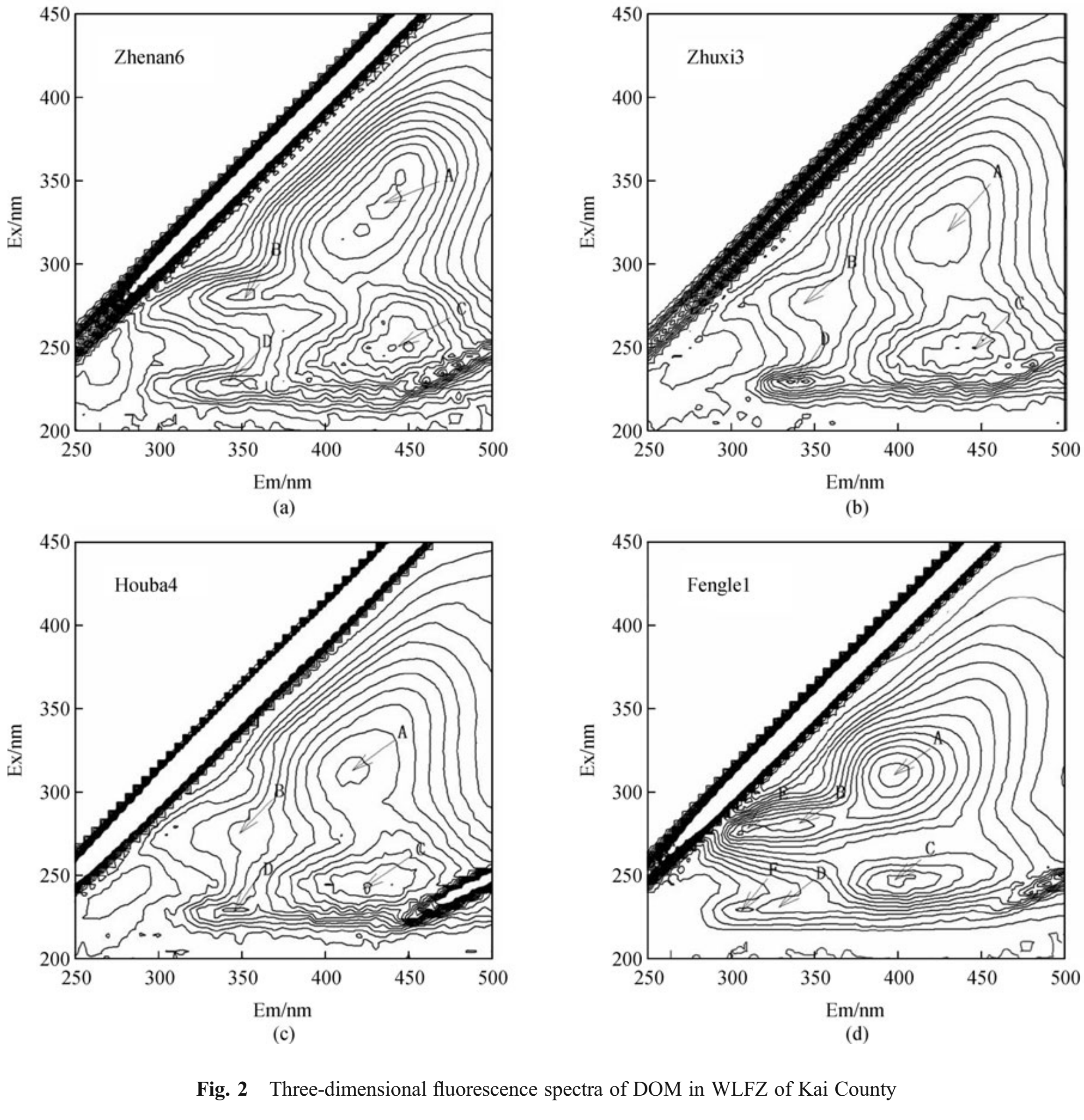
where *HIX* is the degree of soil DOM humification, aromaticity, adsorption, the molecular weight or other relevant parameters, $I_{435\to480}$ is the sum of the fluorescence intensities from the emission wavelength of 300 nm to 345 nm, and $I_{300\to345}$ is the sum of the fluorescence intensities from the emission wavelength of 435 nm to 480 nm.

3 Results and discussion

3.1 Fluorescence peak types of soil DOM

Three-dimensional fluorescence spectra of DOM from the typical WLFZ of Kai County are shown in Fig. 2. Fluorescence intensities of soil DOM, soil organic matter in the WLFZ of Kai County and other data are presented in Table 2.

In Table 2, it can be seen that there were four significant fluorescence peaks in most three-dimensional fluorescence



(a) (b) (c) (d)

Fig. 2 Three-dimensional fluorescence spectra of DOM in WLFZ of Kai County

Table 2 Fluorescence intensities and ratios of DOM and soil organic matter in WLFZ

	DOM sample	sample elevation/m	fluorescence intensity/arb						*r*(C, A)	*r*(D, B)	*r*(B, A)	*HIX*	soil organic matter/%
			Flu A	Flu B	Flu C	Flu D	Flu E	Flu F					
submerged area	Fengle 1	154–155	728.8	532.0	570.7	237.0	627.8	211.5	0.78	0.45	0.73	2.40	2.46
	Fengle 2	154–156	385.5	220.5	473.6	205.7	—	162.2	1.23	0.93	0.57	3.16	1.41
	Linjiang 1	169–171	1351	1281	748.1	—	1129	422.9	0.55	—	0.95	2.12	2.69
	Linjiang 2	170–171	166.4	—	285.7	217.1	—	151.2	1.72	—	—	2.92	1.06
	Zhuxi 1	170–171	376.1	274.2	345.0	133.4	—	—	0.92	0.49	0.73	2.40	1.61
	Hanfeng 1	162–163	319.1	133.8	416.3	191.9	—	—	1.30	1.43	0.42	5.34	0.52
	Hanfeng 2	170–171	150.1	113.3	243.4	125.7	—	—	1.62	1.11	0.75	3.00	0.63
	Hanfeng 3	170–171	331.7	211.9	429.2	312.4	—	—	1.29	1.47	0.64	3.23	0.78
	Hanfeng 4	169–170	176.6	—	269.1	127.5	—	—	1.52	—	—	3.50	0.63
exposed area	Linjiang 3	173–174	663.7	425.2	858.2	1041	—	—	1.29	2.45	0.64	4.33	1.98
	Zhuxi 2	172–173	192.2	252.0	339.6	239.5	—	—	1.77	0.95	1.31	4.39	0.90
	Zhuxi 3	173–174	466.1	251.9	757.3	829.3	—	—	1.62	3.29	0.54	4.56	1.09
	Zhuxi 4	174–175	513.6	265.7	663.8	287.1	—	—	1.29	1.08	0.52	5.09	1.51
	Zhuxi 5	173–174	762.5	390.8	783.8	300.2	—	—	1.03	0.77	0.51	4.64	2.73
	Zhenan 1	174–175	314.4	233.1	554.6	740.8	—	—	1.76	3.18	0.74	4.17	0.91
	Zhenan 2	174–175	317.2	351.9	432.3	318.3	—	—	1.36	0.90	1.11	7.04	0.70
	Zhenan 3	173–174	448.6	—	652.3	281.3	—	—	1.45	—	—	5.14	1.18
	Zhenan 4	173–174	449.7	462.4	652.2	666.7	—	—	1.45	1.44	1.03	3.22	1.21
	Zhenan 5	173–174	372.8	273.9	583.9	441.4	—	—	1.57	1.61	0.73	3.41	0.95
	Zhenan 6	173–174	593.6	453.1	769.4	636.9	—	—	1.30	1.41	0.76	3.75	1.52
	Houba 1	172–173	526.4	514.0	570.4	317.4	—	—	1.08	0.62	0.98	3.16	1.88
	Houba 2	174–175	494.2	—	679.1	230.6	—	—	1.37	—	—	5.36	1.01
	Houba 3	173–174	414.2	—	605.6	281.4	—	—	1.46	—	—	5.94	1.08
	Houba 4	173–174	408.3	—	427.7	582.3	—	—	1.05	—	—	6.10	1.12

Note: water level was 171 m

spectra of soil DOM (Figs. 2(a)–(c)). According to the locations of the fluorescence peaks, the classes were the visible humic-like peak (peak A located at Ex/Em = 300–359 nm/395–450 nm), the UV humic-like peak (peak C located at Ex/Em = 235–260 nm/395–445 nm), the high excitation wavelength tryptophan-like peak (peak B at Ex/Em = 275–280 nm/335–350 nm) and the low excitation wavelength tryptophan-like peak (peak D at Ex/Em = 225–231 nm/335–360 nm). Soil samples from Fengle and Linjiang towns showed another two tyrosine-like peaks (Fig. 2(d)): a high excitation wavelength tyrosine-like peak (peak E at Ex/Em = 280 nm /310 nm) and a low excitation wavelength tyrosine-like peak (peak F at Ex/Em = 225–230 nm/300–325 nm).

It can be found from the spectrometry of the 24 soil samples that the three-dimensional fluorescence spectra of six samples showed only three fluorescence peaks, and there was no evident protein-like peak B. However, the fluorescence intensities around peak B exhibited a gradually increasing trend from low to high wavelength, indicating there was a trend of forming the fluorescence peak B. This may be due to the relatively low content of tryptophan-like material in the soil DOM, resulting in peak B being covered up (obscured) by other fluorescence peaks with higher intensities. Some studies have found a similar phenomenon for water DOM in three-dimensional fluorescence spectra [11,18]. In addition, peaks E and F can only be found in the soil from Fengle town and Linjiang town. Probably, the complexity of this DOM was the result of human activities in the two towns near the sampling location.

3.2 Fluorescence peak intensities of soil DOM

The average fluorescence intensities of different peaks of soil DOM from submerged and exposed areas of Kai County are shown in Fig. 3.

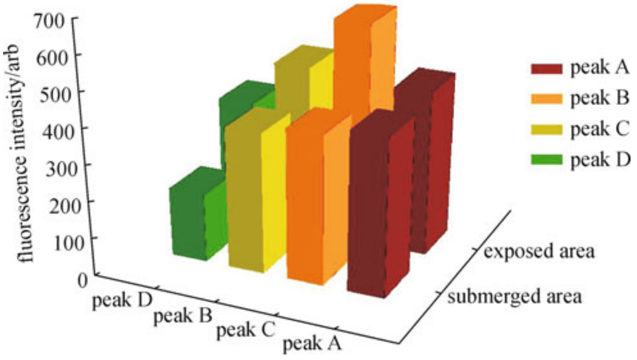


Fig. 3 Average fluorescence intensities of different peaks of soil DOM in WLFZ of Kai County

The order of the average fluorescence intensities of the different peaks from soil DOM in the submerged area was $A > C > B > D$. Clearly, the average fluorescence intensity due to the humic-like material was greater than that due to the protein-like material. This was similar to the conclusions of Baker's study of river DOM [19]. Analysis of variance between the two groups, humic-like fluorescence peaks A and C, and protein-like fluorescence peaks B and D, showed more obvious differences (T Test, $\text{sig} = 0 < 0.01$). The fluorescence intensity of humic-like materials was greater than that of protein-like materials, and the fluorescence efficiency of protein-like materials was larger than the humic-like materials [20], indicating that soil humus was the main source of submerged soil DOM. This conclusion was the same as that drawn by McDowell [7] in a study of forest soil. There were no obvious differences between the two humic-like fluorescence peaks A and C nor between the two protein-like fluorescence peaks B and D, in the submerged area.

The order of average fluorescence intensities of the different peaks from soil DOM in the exposed area was $C > B > A > D$. It can be seen from Fig. 3 that there are no differences between the average intensities of peaks A and B, but the intensity of peak C is significantly higher than D in the exposed area. Overall, the humic-like fluorescence intensity was greater than the protein-like fluorescence intensity, and the analysis of variance between the two groups showed a significant difference (T Test, $\text{sig} = 0.009 < 0.01$). This indicated that soil humus was also the main source of DOM in exposed soil. However, compared to the submerged area, the intensities of the protein-like fluorescence peaks B and D were very high in the exposed areas, indicating that protein-like materials produced by human activities which can contaminate the exposed soil, as well as that produced by plants, animals and microbial activity in the soil, were also important sources of DOM. There were obvious differences between the humic-like fluorescence peaks A and C (ANOVA, $\text{sig} = 0.005 < 0.01$), the mean values of which were 462.50±142.72 arb and 622.01±144.23 arb, respectively. Previous studies [14] pointed out that the main composition of the humic-like peak A was fulvic acid, while that of peak C was humic acid. This suggested that the humic-like material in the exposed soil DOM consisted of mainly humic substances of large molecular weight. The protein-like fluorescence peaks B and D from soil DOM in the exposed area did not demonstrate obvious differences.

A comparison of the average intensities of submerged and exposed soil DOM showed that the average intensity of fluorescence peak C was the highest in the exposed soil, while that of peak D was the lowest in the submerged soil. The fluorescence intensities of humic-like and protein-like peaks in the exposed area were higher than those in the submerged area, indicating that higher concentration of soil DOM was found in the exposed soil than submerged soil of the WLFZ. On the one hand, this may be due to an increase in litter and root exudates, which could be caused by long-term planting of crops and growth of weeds in exposed soil. Exposed soil has good light conditions, adequate oxygen and suitable water, which may enhance plant and animal growth and increase microbiological metabolic activity. The exposed area is also much closer to the zone of human activity, and organic pollutants may only reach the submerged area after interception by the exposed area. As a result, the exposed area may retain most of the pollutants in its soil. On the other hand, protein-like substances released by microorganisms and soil organic matter reduction by mineralization would cause a reduction of DOM in the submerged soil. In addition, analysis of variance of the average intensities of submerged and exposed soil DOM showed that there were obvious differences between peaks C of DOM from submerged and exposed soil (ANOVA, sig = $0.004 < 0.01$), as did the peaks D (ANOVA, sig = $0.005 < 0.01$). But peaks A and the peaks B had no significant differences. These results indicated that the fluorescence intensities of peaks C and D were greatly influenced by water level fluctuations.

3.3 Discussion

3.3.1 Ratios of fluorescence intensities

The ratios of soil DOM in the WLFZ of Kai County can be seen in Table 3.

Table 3 Ratios of soil DOM in WLFZ of Kai County

	r(B, A)	*r*(C, A)	*r*(D, B)
submerged area average	0.68±0.06	1.21±0.13	0.98±0.44
distribution	0.42–0.95	0.55–1.72	0.45–1.47
exposed area average	0.81±0.08	1.39±0.06	1.61±0.95
distribution	0.51–1.31	1.03–1.77	0.62–3.29

Analysis of variance between the two groups indicated that there were no obvious differences in the r(B, A) of soil DOM in the submerged and exposed areas. Some studies [11] showed that the value of r(B, A) of contaminated river DOM was generally greater than 1.5, and the average values in this study were less than 1.5, indicating that the soil in the WLFZ was not contaminated significantly.

The values of *r*(C, A) and *r*(D, B) were significantly different, indicating that DOM contained at least two types of humic-like and protein-like fluorophores. Compared with the related literature [9,11], the distributions of *r*(C, A) and *r*(D, B) in the WLFZ soil were very wide, indicating that the contamination degrees for the soil from different locations were not consistent. Studies by Coble [9] showed that the average value of *r*(C, A) in sediment pore water was 0.77, and the *r*(C, A) value in sediment pore water from Cuicui Lake was 1.26. It was also reported that the distribution of *r*(C, A) in sediment pore water of Erhai varied between 0.94 and 1.79, and that of *r*(D, B) between 0.90 and 1.75 [11].

The distributions of *r*(C, A) and *r*(D, B) differed between submerged and exposed soil. As shown in Table 2 and Fig. 3, the average fluorescence intensity of peak C was quite large in exposed soil and that of peak D was quite low in submerged soil. Some scholars pointed out that the soil moisture content could affect the components of soil humus [21,22]. In the study on slope farmland soil using ^{137}Cs tracer technique, Chen [21] found that humic acids accumulated significantly in the relative well-drained sites, while only a small amount was obtained in the sites with poor drainage. Dou [22] pointed out that flooding conditions were conducive to the accumulation of fulvic acids in soil, while dry conditions favored the accumulation of humic acids. These findings might support the conclusion that fluorescence intensities of soil humic acids in the submerged area were lower than the exposed area indirectly. Tyrosine was insoluble in water, and tryptophan was slightly soluble in water [23]. Soil in the WLFZ was submerged over time, promoting the dissolution of tryptophan, which may be one of the reasons why the average intensity of tryptophan fluorescence peak D was weak in the submerged soil.

3.3.2 Correlations and analyses of the different fluorescence peak intensities and their relationship to SOM

The correlations between fluorescence intensities of soil DOM and the correlations between fluorescence intensities and SOM in the WLFZ of Kai County can be seen in Table 4.

In general, SOM is the most important component of soil DOM [19]. There was no significant difference between the amount of SOM in submerged and exposed areas, the means of which were 1.31±0.27 and 1.32±0.14. Good correlations were observed between SOM and the visible humic-like fluorescence peak A, the UV humic-like fluorescence peak C, and the high excitation wavelength tryptophan-like fluorescence peak B, as shown in Table 4. However, the correlation between SOM and the low excitation wavelength tryptophan-like fluorescence peak D was poor. The best correlation between SOM and a fluorescence peak was that between SOM and peak A, and this correlation indicated that the proportion of fluorescent visible humic-like content in the SOM was relatively stable. In contrast, the worst correlation ($r = 0.156$), that between SOM and peak D, indicated that the proportion of low excitation wavelength tryptophan-like content in the SOM was unstable. This may be associated with the nature of peak D, which was sensitive to soil moisture, concentrations of oxygen and other environmental conditions.

The DOM of different soil samples in the WLFZ had different *r*(C, A) values, and the distribution was quite wide. However, the humic-like fluorescence peaks A and C continued to display a good correlation. Probably, the main components of peak C – humic acid, and that of peak A,

Table 4 Correlations between fluorescence intensities of soil DOM and correlations between fluorescence intensities and soil organic matter in WLFZ of Kai County

type		soil organic matter	peak A	peak B	peak C	peak D
peak A	correlation coefficient *r*	0.857**	1.000			
	sample number *n*	23	24			
peak B	correlation coefficient *r*	0.711**	0.913**	1.000		
	sample number *n*	17	18	18		
peak C	correlation coefficient *r*	0.590**	0.714**	0.492*	1.000	
	sample number *n*	24	24	18	24	
peak D	correlation coefficient *r*	0.156	0.373	0.310	0.641**	1.000
	sample number *n*	23	23	17	23	23

Notes: * Correlation is significant at 0.05 level, ** Correlation is significant at 0.01 level

fulvic acid, have similar sources, migration, transformation, etc. This result agrees with Fu [11] and Baker [24], but to date, there is still no reasonable explanation for the spectroscopic behavior of DOM. The tryptophan-like fluorescence peaks B and D have a poor correlation. Peaks B and D can be affected by different soil sources and environment and microbial activity, which may cause this poor correlation.

The correlation between the high excitation wavelength tryptophan-like fluorescence peak B and the humic-like fluorescence peaks A and C is good. The low excitation wavelength tryptophan-like fluorescence peak D and the UV humic-like fluorescence peak C also have a good correlation. However, peak D is poorly related to the visible humic-like fluorescence peak A. Generally, the good correlation indicates that the two have similar sources or both of the chemical structures have some connection in the soil DOM of the WLFZ [11]. Studies [18] showed that protein-like fluorescence peaks B and D were weakly correlated to humic-like fluorescence peaks A and C, which was inconsistent with the results from this study. Related research still should be conducted in-depth in the future.

3.3.3 HIX

Usually, the larger the HIX values, the greater the humification, aromaticity and adsorption as well as the molecular weight of the soil DOM. Ohno showed that the HIX values in soil DOM from the Bear Brook Watershed in Maine ranged from 1.15 to 5.38 [12]. In this study, the HIX values ranged from 2.12 to 7.04 in soil DOM in the WLFZ of Kai County, and most values were in the range from 3.00 to 5.00 (Table 2), which was close to the values in Ohno's report. The HIX values showed obvious differences between the submerged and exposed areas (ANOVA, sig = $0.002 < 0.01$). The mean HIX value in the submerged area was 3.11±0.32, and that in the exposed area was 4.69±0.29, indicating that periodic submerging and exposure of soil had an impact on the humification of DOM. Compared with the submerged area, the soil DOM in the exposed area had a higher degree of humification and aromaticity; the molecular weight distribution of DOM was based on the humic acid of the large molecular weight, and the content of fulvic acid of small molecular weight was relatively small in the exposed area. This result confirmed findings in section 3.2 that the fluorescence intensity of peak C was much greater than that of peak A in the exposed area.

4 Conclusions

Three-dimensional fluorescence spectroscopy was used to investigate the fluorescent properties of DOM in the WLFZ of Kai County, and the following conclusions were reached:

1) Most of the soil DOM was found to contain four fluorescence peaks: peaks A and C, which represent humic-like fluorescence, and peaks B and D, which represent tryptophan-like fluorescence. Two additional peaks, E and F, which represent tyrosine-like fluorescence, only appeared in certain soils.

2) Analysis of soil DOM fluorescence intensities showed that humus was the main source of soil DOM, and higher concentration of soil DOM was found in the exposed soil than the submerged soil of the WLFZ. The fluorescence intensities of peaks C and D were greatly influenced by the water level fluctuations.

3) According to the analysis of fluorescence intensity ratios of soil DOM in the WLFZ, WLFZ soil was not contaminated significantly, and the contamination degrees for the soil from different locations were not consistent. The soil DOM contained at least two types of humic-like fluorescence groups and two types of protein-like fluorescence groups.

4) Analysis of the correlations between soil DOM fluorescence peak intensities and their relationship to SOM indicated that the proportion of the content of peak A in SOM was quite stable, and the fluorescence intensities of peaks A and C had a good correlation in the soil DOM of the WLFZ.

5) Analysis of the HIX values in submerged and exposed soil DOM indicated that the soil DOM in exposed areas had a higher degree of humification and aromaticity, and periodic submerging and exposure of soil had an impact on the humification of DOM.

6) Current research results indicated fluctuating water levels would significantly impact fluorescence properties of DOM in the fluctuating water-level zone of the Three Gorges Reservoir, but how fluctuating levels affect DOM is not fully known. It needs a long-term study on how the fluctuating water levels impact DOM composition, nature, migration and transformation.

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