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Three-dimensional fluorescence spectral characteristics of dissolved organic carbon in cave drip waters and their responses to environment changes: Four cave systems as an example in Guizhou Province, China

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Understanding the responses of fluorescence spectral characteristics of cave drip waters to modern environment and climate changes is key to the reconstructions of environmental and climatic changes using fluorescence spectral characteristics of speleothems. The fluorescence spectral characteristics of dissolved organic carbon (DOC) in four active cave systems were analyzed with a three-dimensional (3D) fluorescence spectral analysis method. We found that the fluorescence types of DOC were mainly of fulvic-like and protein-like fluorescences, both in soil waters and cave drip waters. The intensity of fulvic-like fluorescence was positively correlated with the concentrations of DOC, suggesting that the DOC of cave drip waters was derived from the overlying soil layer of a cave system. Compared with the other cave systems, the variation range of the excitation and emission wavelengths for fulvic-like fluorescence of cave drip waters in Liangfeng cave system that had forest vegetation was smaller and the excitation wavelength was longer, while its fluorescence intensity varied significantly. By contrast, the excitation and emission wavelengths and fluorescence intensity for that in Jiangjun cave system that had a scrub and tussock vegetation showed the most significant variation, while its excitation wavelength was shorter. This implies that the variation of vegetation overlying a cave appears to be a factor affecting the fluorescence spectral characteristics of cave drip waters.

cave drip water, three-dimensional fluorescence spectral characteristics, fulvic-like fluorescence, vegetation type, soil layer type

It is necessary to understand how the fluorescence spectral characteristics of microlayers of speleothems in cave systems respond to modern environment and climate changes, if we want to use these as proxy indicators in reconstructing of paleoenvironmental and paleoclimatic changes[1−3]. Paleoclimatic researchers paid attention to the fluorescence spectral characteristics of speleothems, because the ages of speleothems could be precisely determined by uranium series mass spectrometry dating method[4−7]. Shopov et al. found that the fluorescence intensity (FI) of speleothems had oscillations at time-scales ranging from days to tens of thousand years, suggesting that the FI was controlled by climate factors[8]. And Bakers et al. found that the fluorescence excitation and emission wavelengths were correlated with the humification degree of soils and annual average precipitation[2]. Recent studies demonstrated that FI was corre-

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lated with climate changes at annual timescale[9]. However, FI was affected by many factors, leading to the different responses of fluorescence variation to surface environment changes at different sampling sites even within a cave system[10−13]. Moreover, the main component of organic matters in speleothems was found to be fulvic acids (FA)[14]; the concentrations of organic matters in speleothems with a darker color were higher than that of a lighter one, but the former had a lower FI due to their self-absorption in fluorescence[15]; the changes of fluorescent wavelength in speleothems could reflect a long timescale (in decades or centennial timescale) variation of new or old carbon components[16]. Therefore it is necessary to calibrate the fluorescence signals when interpreting paleoenvironmental and paleoclimatic changes by fluorescence spectra of speleothems. Some researches in reconstruction of paleoenvironmental and paleoclimatic changes by using fluorescence spectra of speleothems had been carried out in China, but still remain as an outstanding issue, especially in the researches of responses of dissolved organic carbon (DOC) fluorescence spectra to environment and climate changes in active karst cave systems[17−20]. In this paper, we present the results of the responses of DOC fluorescence spectra to environment change in four active karst cave systems located in Guizhou Province, southwest China, aiming to providing information on fluorescence spectra in interpreting paleoenvironmental and paleoclimatic changes encoded in speleothems.

1 Study areas and methods

1.1 Study areas

The following four cave systems located in Guizhou Province of the Pearl River watershed were chosen, namely, Liangfeng cave (LFC) in Dongtang district of Libo County, Qixing cave (QXC) in Kaikou district of Dunyun City, Jiangjun cave (JJC) in Qiyanqiao district of Anshun City and Xiniu cave (XNC) in Chengguan district of Zhenning County. Basic information of them is listed in Table 1, and sample numbers were the same as in ref. [21].

1.2 Methods

The methods of sampling and filtrating are referred to refs. [21, 22]. Hitachi F-4500 fluorescence spectrometer was used to measure the 3D fluorescence spectra, using the following specific settings: 150W xenon arc lamp as a excitation source; photomultiplier tube voltage set at 700 V; the excitation wavelengths scanned from 240 to 400 nm at 5 nm steps, and emission wavelengths scanned from 250 to 550 nm at 10 nm steps; response time set at a automatic way; scanning speed for 1200 nm/min; the calibration of the apparatus set at a automatic way. Fluorescence samples were sent into 1 cm quartz cuvette, and maintained at a constant temperature (20 ± 1℃) before measuring. The 3D fluorescence spectra data were plotted by SigmaPlot software[23]. The repeated measurement deviation of FI was less than 5%. The measurement method of DOC is referred to ref. [22].

Table 1 Basic information of the four cave systems

Cave	Liangfeng cave	Qixing cave	Jiangjun cave	Xiniu cave
Location	108°02′29″E 25°16′21″N	107°16′09″E 25°59′47″N	106°03′54″E 26°17′03″N	105°46′35″E 26°03′27″N
Entrance elevation (m a.s.l.)	620	1020	1360	1300
Type of rock	carboniferous biogenic limestone	carboniferous limestone and dolomitic-limestone	triassic dolomite	triassic dolomite
Type of vegetation	primary karst forest	brushwood	scrub and tussock	shorn and tussock
Continuity of Soil cover	discontinuity	better continuity	discontinuity	discontinuity
Type of Landform	peak cluster-shallow depression	peak cluster-shallow depression	peak forest- valleys	karst hills-valley
Thickness of cave roof (m)	80−140	50−90	50−60	20−60
Mean annual temperature (℃)	15.3	15.9	14−16	14.4−19.7
Range for temperature change in cave (℃)	14.5−16	10.5−15	12.5−15	15−17
Mean annual rainfall (mm)	1752	1445	1360	1200

2 Results

The peak wavelengths of FI of the DOC for the four cave systems could be divided into four fluorescence centres (shown in Figure 1) on the basis of current researches on 3D fluorescence spectra of natural organic carbon[12,23].

(i) The first excitation-emission wavelength pairs at 345—360/436—476 nm, and they present in both cave drip waters and soil waters from four cave systems. The variation ranges for four cave systems are, 345—360/436—476 nm for LFC, 345—360/458—476 nm for QXC and 350—355/460—476 nm for JJC, respectively. The variation ranges of excitation wavelength for LFC and QXC are wider than that for JJC, while the variation ranges of emission wavelength for LFC are wider than that for QXC and JJC. However, the excitation-emission wavelength pairs of drip waters from XNC only occurred at a monitoring site in June 2003, as 355/444 nm. The excitation–emission wavelength pairs of the four cave systems may be attributed to humic acid (HA) fluorescence by comparing with the previous studies, though there is no obvious peak center. Moreover, maximum peaks of FI occurred in both drip waters and soil waters within these distribution ranges of the wavelength pairs, though not in all samples. According to their 3D fluorescence spectra, they probably are a tail in the fluorescence peaks of FA with a low molecular weight extending to longer excitation and emission wavelengths[12]. Therefore this excitation-emission pairs could be attributed to fulvic-like fluorescence.

(ii) The second excitation–emission wavelength pairs at 285—340/380—458 nm. The variation ranges of excitation wavelengths for the four cave systems are similar: 290—340 nm for LFC and 285—340 nm for three others. As to emission wavelength, the variation ranges are different (Figure 1): 392—454 nm for LFC, 380—458 nm for QXC, 400—442 nm for JJC and 396—444 nm for XNC, respectively; and the most significant variation occurs at QXC which amounts to 78 nm. This excitation-emission wavelength pairs present in both cave drip water and soil water samples, and the excitation and emission wavelengths for LFC exhibit a red shift trend, while it trends to be evenly distributed for JJC. Compared with the previous research results[12], this pairs would be attributed to be fulvic-like fluorescence.

(iii) The third excitation and emission wavelength pairs at 260—285/328—384 nm, and they present in all the four cave systems. The variation of excitation wavelength for JJC ranges from 260 nm to 285 nm, while it is from 265 nm to 285 nm for the three others. The variations range of emission wavelength for the four cave systems are similar, in detail, 330—364 nm for LFC, 328—380 nm for QXC, 330—380 nm for JJC and 330—370 nm for XNC, respectively. Thereby this pairs would be attributed to protein-like fluorescence.

(iv) The last excitation and emission wavelength pairs at 240—280/290—448 nm. The excitation and

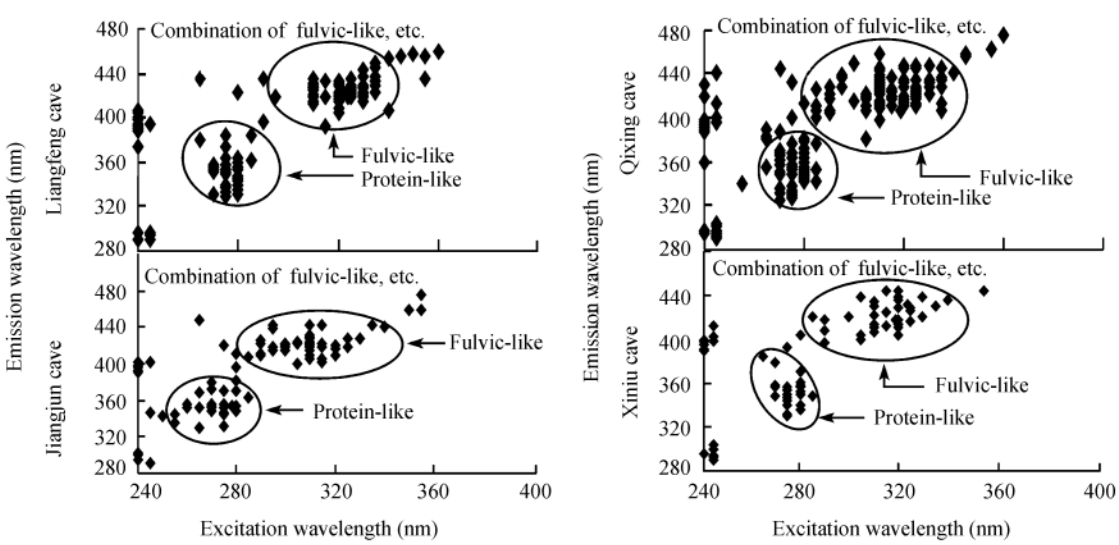

Figure 1 Types of fluorescence spectra of the four cave systems.

emission wavelengths vary at a wide range in this wavelength pairs, and the fluorescence peak of QXC occurs at a longer wavelength when compared with the three others. The FI maxima of this pairs are the most significant one among these four excitation-emission wavelength pairs. As shown in Figure 1, we still could be able to divide this pairs into four fluorescence peak regions as follows: ①240－245/290－304 nm; ② 240－255/336－360 nm; ③240－245/374－440 nm; ④265－280/382－448 nm, only a few samples with maximum FI occur at this wavelength pairs, and fluorescence peaks for some samples are not clear due to effect by Raman scattering of waters. According to the previous studies by Zepp et al.[24] on ocean organic matter and results of others[25,26], we could categorize ① and ② as protein-like fluorescence, which namely are tyrosine-like and tryptophan-like fluorescence respectively, and ③ and ④ as humic-like fluorescence. Although Researches by Baker et al. found that a range of emission wavelengths excited by a fixed excitation wavelength at 225－245 nm suggested the presence of a relatively simple, single fluoro-phore group such as a protein[12]; Coble et al. also reported the presence of a peak at 250 nm[27], hence study on the peak of 240－280/ 290－448 nm excitation-emission pairs is yet not enough. The peak wavelength of this pairs is deformed for it is close to that of Raman scattering of waters, meanwhile, as a fluorescence material at high energy level, the variation of characteristics of organic molecule is still unclear. Hence further research into this peak of fluorescence spectra is still needed.

3 Discussion

3.1 Relationship between fulvic-like FI and DOC

Aforementioned analysis demonstrated that the main fluorescence peaks are fulvic-like and protein-like fluorescences, hence we chose fulvic-like fluorescence in cave systems to study the correlation between FI and DOC. It is found that as for both soil waters and cave drip waters, there was a good linear relationship between FI and DOC (statistically significant at a 90% confidence level, see Figure 2), and the correlation coefficient for XNC even reaches to 0.99, least for JJC at 0.6. This implied that the main fluorescence matter in cave systems was DOC, and DOC in cave drip waters was derived from that of waters in the overlying soil layer. Moreover, there were different functional groups in organic matter that produced fluorescence, and the difference of these functional groups would affect the intensity of fluorescence, thus causing the DOC with the same concentrations would produce fluorescences with dif- ferent intensity.

3.2 The responses of fulvic-like fluorescence to vegetation types

From Table 2, we found that the excitation and emission wavelengths of cave drip waters in LFC with an overlying forest varied insignificantly, and its excitation wavelength was longer than that of drip waters in the other three caves. The variation of excitation wavelength would reflect the change in the rate of soil humification, and the humification rate of organic matter in cave drip waters in LFC was higher than that of the three others; then significant variation of FI in LFC maybe illustrates

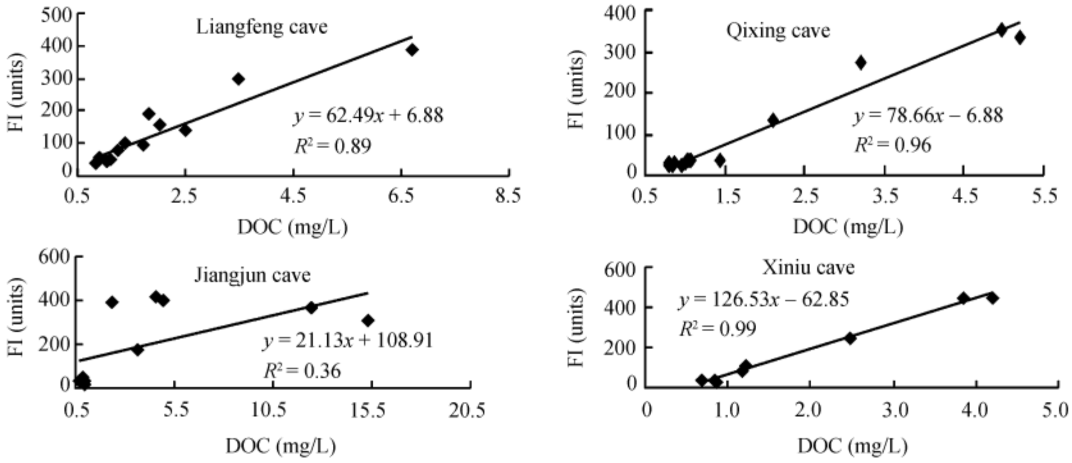

Figure 2 The correlation of DOC concentrations and the FI of FA fluorescence for the four cave systems.

Table 2 The variations of the excitation and emission wavelengths and FI of cave drip waters

Cave	Excitation wavelength (nm)	Standard deviation (nm)	Emission wavelength (nm)	Standard deviation (nm)	FI (units)	Standard deviation (units)
LFC	319.8	8.4	419.2	8.0	47.24	18.84
QXC	314.0	12.6	419.6	9.5	28.89	8.95
JJC	308.5	13.9	421.3	11.4	32.76	20.29
XNC	314.9	12.2	416.0	11.3	36.70	16.64

the seasonal changes of the concentrations of FA. By contrast, JJC with an overlying of scrub and tussock showed the most significant variation both for the excitation and emission wavelengths and FI, while shorter in excitation wavelength, thus reflecting the joining of fluorescence matter with a low humification rate into cave drip waters; and the variation of FI could also reflect the change of FA in concentrations. Moreover, the variations of excitation and emission wavelengths of cave drip waters of XNC and QXC were in the middle of the above two cave systems, thus demonstrating the variations of excitation and emission wavelengths and FI could reflect the differences of overlying vegetations at the four cave systems. There were sparse distributions of overlying soil layers for LFC, JJC and XNC, then some soil organic carbon at different humification ratio would transport into cave systems via waters, hence probably caused significant variations of FI. As for QXC, there was a continuously distributed overlying soil layer and would provide a stable source of organic materials for cave drip waters, then the variation of FI was insignificant, and that was affected less by the change of season; therefore the different type of overlying soil layer could affect the variation of FI. These results agree with those of Baker and Genty [12], which were carried out in four cave systems with different vegetation and soil layer types in Britain and France. Hence, the types of vegetation and soil layer under paleoenvironmental and paleoclimate conditions could be deduced from the fluorescence of speleothems in karst cave systems.

4 Conclusions

The main types of DOC fluorescence of soil waters in overlying soil layer and drip waters in cave systems are fulvic-like and protein-like fluorescences, secondary types are the combination fluorescence of fulvic-like and protein-like fluorescences, and the tail of fulvic-like fluorescence.

For the four cave systems, the main fluorescence matter is DOC, and both fulvic-like and DOC in cave drip waters are derived from that of overlying soil layer.

The excitation and emission wavelengths of fulvic-like fluorescence for LFC with a overlying forest varies insignificantly, and its excitation wavelength is longer than that of the three other cave systems, and the FI varies significantly; on the contrary, the excitation and emission wavelengths of fulvic-like fluorescence and FI for JJC with a overlying scrub and tussock show a maximum variation, while its excitation wavelength is shorter. Hence the variations of FI and wavelengths could reflect the changes of overlying vegetation.

Recent researches reveal that the fluorescence characteristics are affected by many factors, and there are many uncertain factors as to how these characteristics response to environment changes. Further studies are still needed to fully understand the responses of DOC fluorescence to environment changes in cave systems.

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