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Fluorescence wavelength and intensity variations of cave waters

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Abstract

The fluorescence properties of groundwaters percolating into four cave systems have been monitored over the period 1997–1998. Fluorescence was excited between 220 and 400 nm and the emission measured from 300 to 500 nm using a fluorescence spectrophotometer. Three fluorescence centres were observed; one at the excitation–emission pair of 290–340:395–430 nm, (humic-like, probably fulvic acid), one at 265–280:300–370 nm (protein like) and a less defined region of high fluorescence at 230–280:310–420 nm (humic and/or protein like). The most consistent fluorescence intensity was observed in the excitation–emission pair of 290–340:395–430 nm, attributed to a fulvic acid source. Subtle differences ($\pm$ 5%) in the fluorescence excitation and emission wavelength of this fluorescence peak in the groundwater were observed between the four sites, and the fluorescence intensity varied considerably ($\times$ 60) between the four sites. Both the wavelength and the intensity variations in fluorescence are caused by the differences in the vegetation cover, soil type and humification. Data from the most intensely monitored site (Brown's Folly Mine, England; 9 sample stations, 10–20 days frequency sampling) revealed no spatial variability in the 290–340:395–430 nm (fulvic acid) fluorescence; in contrast time-series analysis suggests that the seasonal variations do occur, with a decrease in the emission wavelength correlating with the first (autumn) peak in fluorescence intensity, and a decrease in the excitation wavelength correlating with a second (winter) fluorescence intensity peak. Results demonstrate the potential of utilising fluorescence wavelength variations in sourcing karst groundwaters, and as a possible palaeoenvironmental proxy of the overlying soil conditions if trapped within the cave speleothems.

Keywords: Fluorescence; Luminescence; Karst; Groundwaters; Speleothems

1. Introduction

Fluorescence occurs when molecules which, having been previously excited by a high-energy light source that raised the energy levels of the electrons within the molecule, release energy in the form of light. The emitted fluorescent light is at a longer wavelength than the excitation; the most common form of fluorescence is emission in the long wave ultra-violet and blue wavelengths (350–500 nm) after excitation by UV light (200–400 nm). Fluorescence in the natural waters is predominantly generated by the organic acids (humic and fulvic) and the amino-acid groups within proteins, which predominantly derive from decomposed plant material in the overlying soil (Senesi et al., 1991), although protein luminescence may derive from both floral and faunal organic material (Burstein and Emelyanenko, 1996), and inorganic minerals may occasionally generate fluorescence (e.g. UO^{2+}, Mn^{2+}, Pedone et al., 1990;

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Shopov et al., 1994). Several studies have investigated the fluorescence of organic matter extracts from soils and dissolved organic matter in rivers and marine waters (Coble et al., 1990; Senesi et al., 1991; De Souza Sierra et al., 1994; Coble, 1996; Luster et al., 1996; Mobed et al., 1996; Erich and Trusty, 1997; Ohno and Cronan, 1997). These studies have revealed the presence of several fluorescence intensity peaks at excitation and emission wavelength pairs of 250–260:380–460 nm, 300–340:410–480 nm, 270–280:300–360 nm and 250–260:300–360 nm. The first two have been ascribed to an organic acid (humic or fulvic acid) source, the latter two to amino acid groupings within proteins which are also products of vegetation and animal decay products. Previous studies have suggested that organic acids from different sources (e.g. marine vs. terrestrial; Coble, 1996) as well as from different soil types and molecular weight (Senesi et al., 1991; Barancíková et al., 1997; Miano et al., 1988; Rivero et al., 1998) may be differentiated by their fluorescence properties. The latter studies suggest that humic acids (HA) have a higher excitation and emission wavelength of fluorescence than fulvic acids (FA) (Senesi et al., 1991; Miano et al., 1988), because of an increase in the degree of aromacity, the content of carboxylic groups and polycondensed aromatic and conjugated structures within the HA. Thus, it might be expected that the organic matter derived from soils with a higher proportion of HA to FA would have a higher wavelength of fluorescence excitation and emission that those with a low HA/FA ratio.

Many other factors may also influence the fluorescence intensity and wavelength of dissolved organic matter in soil extracts and natural waters. These include changes in:

(a) pH. Increased fluorescence intensity has been observed with increasing pH (Miano et al., 1988; Senesi et al., 1991). Miano et al., (1988); Senesi et al., (1991) and Mobed et al., (1996) observed a red shift in fluorescence wavelength of an excitation–emission pair of 400:460 nm (HA), and both a red and blue shift of the excitation–emission pair 300–340:410–480 nm for soil and aquatic derived samples, respectively (the latter from a mean of 300:410 nm to 300:430 nm for a shift from pH 6.0 to 10.0). pH effects on fluorescence are due to changes in both the fluorescence characteristics of the acidic functional groups (phenols and phenolates) in the organic molecules and/or changes in the conformations of the organic molecules which could expose different functional groups to the bulk aqueous solvent.

(b) Metal-ion interactions. Many researchers have identified a quenching effect of fluorescence with metal-ion interactions with humic and FA. Early studies (for example Shotyk and Sposito, 1988; Cabaniss, 1992; Seritti et al., 1994; Cook and Langford, 1995) were technologically limited to the use of synchronous scan fluorescence techniques, and identified a quenching of fluorescence intensity which preferentially occurred at longer wavelengths. However, Luster et al. (1996), using excitation emission fluorescence techniques, demonstrated that both enhanced fluorescence and shifts in fluorescence wavelength may occur, a phenomenon mostly missed by earlier studies, because of the necessity to use fixed fluorescence wavelengths. Erich and Trusty (1997), using excitation–emission fluorescence, observed a 12 nm decrease in excitation wavelength and 6 nm decrease in emission wavelength of the 300–340:410–480 nm pair after liming the soil.

(c) Climate. Changes in the rate of soil humification, which increases with increasing temperature and soil moisture for many soil types (Meentmeyer, 1978; Heal and French 1983), may be reflected in changes in the composition (molecular weight or degree of aromaticity) of the soil dissolved organic acids (Zech et al., 1992; 1997), and thus, their fluorescence properties. Although climatic variations influence humification rate, this effect is moderated by the length of time organic materials are held within the soil organic matter (SOM) reservoir, which in turn may be vegetation dependent, and in the groundwater storage. For example, Sanger et al. (1997) demonstrated that SOM decomposition rate increases from spruce woodland to ash woodland to grassland. Tegen and Dorr, 1996 demonstrated that the soil carbon reservoir can be modelled as a mixture of fast (lifetime $\backsim$ one y component) and slow (lifetime $\backsim$ 100 y) components. Genty et al. (1998) demonstrate, by using bomb ^{14}C as a tracer, than a 5–10 y lag occurs

Table 1
Groundwater excitation and emission wavelength of maximum fluorescence, with site soil, vegetation and climate data

Site	Lat/Long	Vegetation Cover	Soil Type	Mean Annual		Water sampling programme	Number of sample sites	Fluorescence Properties			Groundwater geochemistry		
				Rainfall (mm)[a]	T (°C)			Ex (nm)	Em (nm)	Int (units)	pH	Ca mmol l^{-1}	Mg mmol l^{-1}
UAT Tartair, Sutherland Scotland	58°8′N 4°56′W	Blanket Bog	Raw Peat	1900	7	1997–1998, every 2 months	5, plus 7 point samples	306.1 ± 4.7	414.6 ± 3.3	23.4 ± 8.4	8.0–8.7	0.6–1.0	0.5–0.8
PC, Derbyshire, England	53°12′N 1°56′W	Deciduous Woodland	Brown Ranker	1300	9	1997–1998, every month	1, plus 6 point samples	301.0 ± 11.4	406.2 ± 2.7	255 ± 335	11–13	0.8–1.5	< 0.02
VIL, Grotte de Villars, Dordogne, France	45°25′N 0°45′E	Dry woodland	Brown Rendzina	870	11	1997–1998, every month	5	295.5 ± 1.0	406.5 ± 0.4	432.0 ± 149.8	7.3–7.9	2.4–3.6	0.05–0.15
BFM, Wiltshire, England	51°23′N 2°22’W	Deciduous Woodland	Brown Rendzina	800	10	1997–1998, every 10–20 days	11	324.7 ± 1.7	407.9 ± 5.0	27.9 ± 6.6	7.5–8.3	1.4–2.7	0.14–0.60

[a] Data rounded to nearest 10 mm when meteorological station is within 30 km, other wise data is interpolated from nearby stations and quoted to nearest 50 mm. Temperature data to nearest °C.

from SOM formation to deposition within cave speleothems.

Despite the aforementioned studies into soil extracts, and marine and river waters, very little research has investigated the fluorescence properties of groundwater. Those studies that were undertaken have concentrated on karst groundwaters feeding cave speleothem deposits. These waters are likely to have fewer variables affecting fluorescence; with pH typically between 7.0 and 8.5 they are not likely to experience significant quenching or fluorescence wavelength changes; and have calcium ion concentrations $> 1\ \text{mmol}\ \text{l}^{-1}$, suggesting that any metal–organic interactions will be dominated by this ion. Baker et al. (1997) and Baker and Barnes (1998) have investigated the variations of fluorescence intensity of groundwater feeding stalagmites and flowstones at a karst site in S.W. England. These studies have suggested that the fluorescence intensity varied on a seasonal basis, with an increased flux of organic matter in winter caused by the following:

1. high discharge at these time periods which can transport the high molecular weight organic acids;
2. the availability of a dissolved organic matter source in the overlying soil;
3. the soil moisture deficit being eliminated in autumn/winter which permits a connection between soil and groundwater zones.

However, both the studies utilised a fixed excitation wavelength (HeCd laser at 325 nm), which prevented the investigation of variations in the wavelength of groundwater fluorescence excitation and emission. Research into the fluorescence intensity variations of karst groundwaters were driven by the recognition of annual and long term (10^2–10^3 y) fluorescence intensity variations preserved in cave secondary calcite deposits (speleothems such as stalagmites and stalactites) (Baker et al., 1993; Shopov et al., 1994; Genty et al., 1997; Tan et al., 1997). These fluorescence variations are derived from SOM transported via the groundwater system and which becomes trapped within the speleothem calcite during calcite deposition due to the high zero point charge of calcite and the negative charge of the organic matter in solution (Ramseyer et al., 1997). The presence of annual fluorescence intensity variations, forming laminations within speleothem calcite, has important implications for the reconstruction of past climate, as they can provide a precise chronology with which to constrain palaeoclimate proxies contained within speleothem calcite (Baker et al., 1993). In addition, if the winter variations of fluorescence intensity correlate with the variations in climate, then this may also provide a high-resolution record of past climate change.

Recent research has also demonstrated the presence of variations in the fluorescence emission wavelength of organic materials; both seasonal variations of groundwater feeding a flowstone deposit (Baker and Barnes, 1998), and 10^1–10^3 y variations within speleothem calcite (Baker et al., 1998a). The precise cause of such variations is as yet unknown, although changes in soil development, soil humification due to climate change, and changes in overlying vegetation are all likely causal factors over the decadal to the millennial timescale. In order to understand better the records contained in speleothem fluorescence wavelength variations, a more detailed understanding of both contemporary spatial and temporal patterns and the processes generating groundwater fluorescence intensity and wavelength variations is needed.

2. Site descriptions

Four sites were chosen for the analysis of groundwater fluorescence properties, based on their differing vegetation and soil cover, groundwater geochemistry, and climate, as well as their accessibility for frequent sampling trips. Site details are listed in Table 1. Basic groundwater geochemistry data (drawn from Baker et al., 1998b and unpublished data) demonstrates that all sites have groundwaters that are dominated by Ca or Ca–Mg ions. pH at all sites is between the usual range of 7.0–8.5, except at Poole's Cavern (PC). This site was chosen to investigate the importance of pH on groundwater fluorescence properties, because although covered by one vegetation type since 1820 AD (deciduous woodland), the surface had previously been covered by waste from lime-workings which still influence the geochemistry of the groundwaters today, with the chosen sample sites having hyper-alkaline chemistry (Baker et al., 1998c) with pH > 11.0. Grotte de Villars (VIL) and Uamh an Tartair (UAT) sites were chosen for their widely contrasting soil

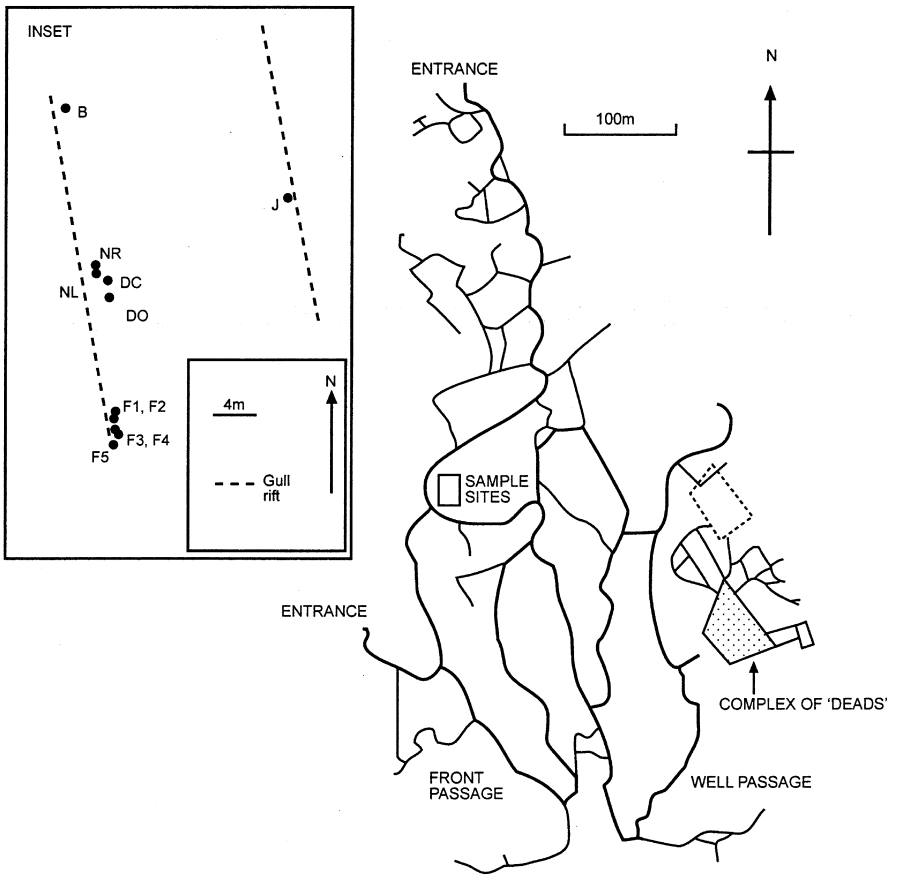

Fig. 1. Location of sampling stations at BFM, England. The locations are at the St. Pauls/Steps region of the mine and overlain by secondary deciduous woodland.

types and wide range of climate. Comparison of Brown's Folly Mine (BFM) and VIL groundwaters permits the comparison of sites nearly identical in soil type and vegetation cover, but with a slightly warmer climate at VIL and a shorter period of soil development ($<$ 100 y) at BFM due to mining activity at the site.

Each of the sites were visited on multiple occasions, depending on the accessibility. The highest temporal and spatial resolution sampling was achieved at BFM. Nine water-sampling sites were analysed over the course of the period 1997–1998 at 10–20 days sampling interval. The locations of these samples are shown in Fig. 1, together with the position of gull rifts (formed by cambering of the limestone) and joints that determine the hydrological routing through the Jurassic oolitic limestone at the site. Water samples were obtained at five sites within the VIL at monthly sampling intervals, and at one site at PC, Derbyshire, at monthly intervals, together with six point samples. Water from UAT, Sutherland, was sampled at six weekly intervals during the summer, with flooding of the cave preventing access in winter. The groundwaters were feeding stalagmite samples at PC, VIL and UAM, and a mixture of speleothem types at BFM. Site details, including descriptions and surveys can be found in Pitty, 1966 (PC); Lawson (1988) (UAT) and Baker et al. (1998b) (BFM, VIL).

3. Methods

Drip water samples were collected in 30–125 ml

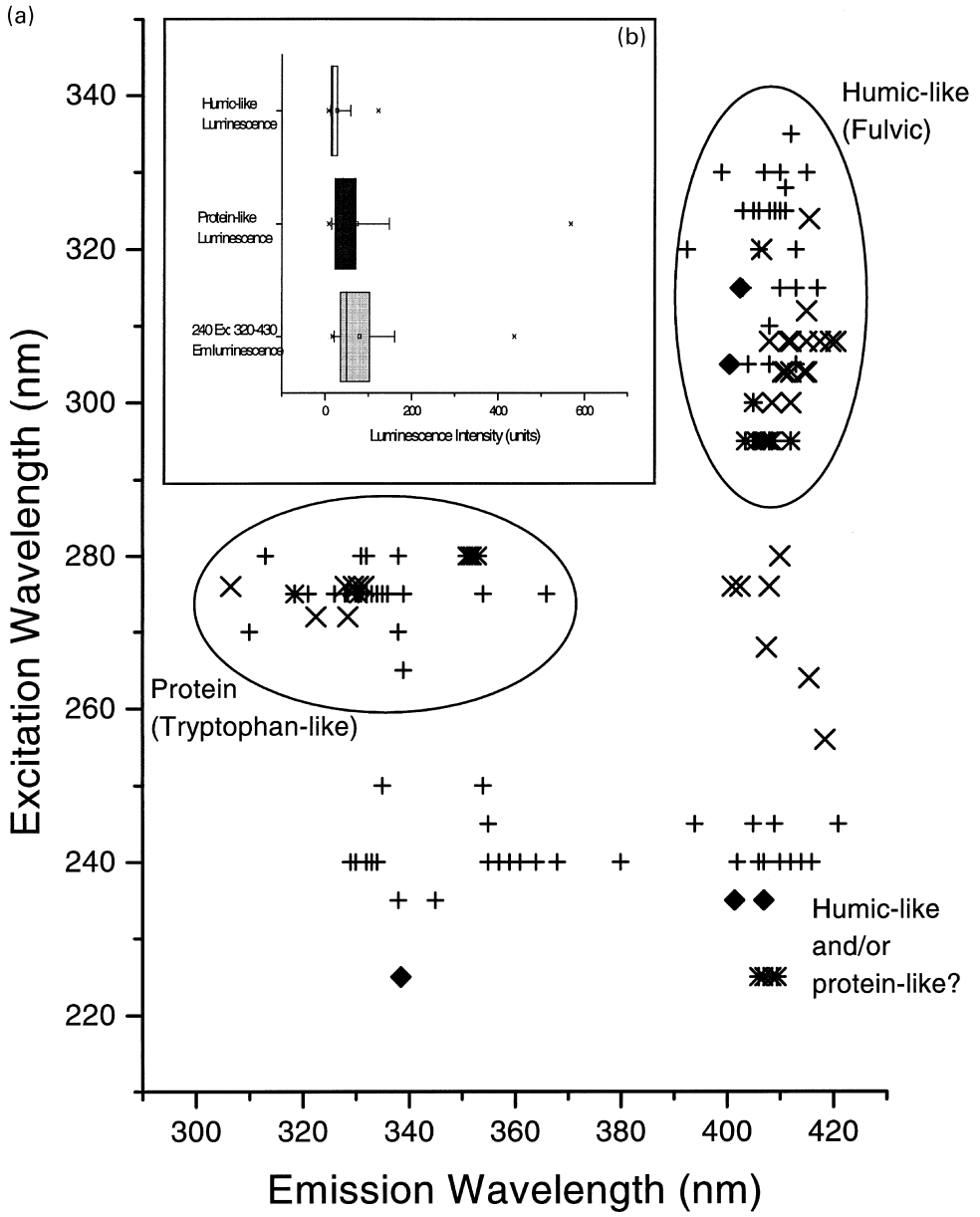

Fig. 2. (a) Excitation and emission wavelengths for the three main fluorescence peaks observed in BFM (pluses), VIL (crosses), PC (stars) and UAT (diamonds) groundwaters. (b) Inset: fluorescence intensity variations of the three fluorescence peaks at BFM (range, interquartile range, median, mean and outliers).

glass bottles that had been precleaned in dilute HCl, non-fluorescent detergent and deionised water prior to sample collection. Water samples were filtered with Whatman GF/C 0.45 μm glass microfibre filter papers (pre-ashed to 400°C) on return to the laboratory, and split into two fractions. One fraction was preserved with 1–3 drops of concentrated HNO_3 for trace element and major ion analysis (Table 1 and unpublished data); the

other was frozen in glass bottles for between one week and one year prior to fluorescence analysis.

A soil water sample was also obtained from a sampling pump installed above the BFM site. The sample was extracted using plastic tubing and a syringe and immediately transferred to a glass bottle. Three attempts to extract soil water occurred between the period 10/97 and 4/98, but on two occasions no sample was obtained.

The fluorescence intensity of the groundwater samples was determined using a Perkin–Elmer luminescence spectrophotometer LS-50B. The spectrophotometer had a xenon excitation source, and slits were set at 5 nm for both excitation and emission. Two ranges of excitation and emission wavelengths were utilised.

1. To assess the number and location of fluorescence centres, excitation wavelengths were incremented from 220 to 400 nm at 5 nm steps; for each excitation wavelength, fluorescence emission was detected from 300 to 500 nm at 0.5 nm steps.
2. For high resolution analysis of just the fulvic-acid fluorescence peak (290–340 nm excitation, 395–430 nm emission wavelength pair), excitation wavelengths were scanned from 280 to 340 nm at between 2 and 5 nm steps, with emission wavelengths scanned from 395 to 430 nm at 0.5 nm steps.

For each water sample, the fluorescence was measured as the maximum intensity at an excitation–emission wavelength pair. Analyses were performed at a constant temperature of 22 ± 2°C. Blank water scans were run every 5–15 analyses using distilled water; with the Raman peak of water at an excitation of 348 nm used as a test for machine stability. Raman emission at 395 nm averaged 14.9 ± 0.5 intensity units ($n = 35$), with no drift during the analytical period (January–March 1998). Random duplicate samples were also run throughout the analysis period; the wavelength of peak fluorescence was reproduced within ± 3 nm for all analyses and the intensity of fluorescence within ± 10%. In addition, stability of the Raman peak was assessed for a 5 min period at the start of each day of data collection, and sample collection occurred only when the signal: noise ratio of the spectrophotometer was greater than 500:1.

4. Results and discussion

4.1. Wavelengths of fluorescence peaks in karst groundwaters

Fluorescence analyses over the range of excitation wavelengths from 220 to 400 nm and emission wavelengths 300–500 nm were analysed for groundwaters from three sampling occasions for the BFM site, and for samples at PC, UAT, and VIL randomly chosen throughout the sampling programmes. Fluorescence wavelength variations are presented in Fig. 2(a) and intensities in Fig. 2(b). Results demonstrate the presence of three regions of fluorescence, two of which form clear peaks.

1. A 290–340:380–430 nm wavelength pair, present in groundwaters from all four sites, which can be attributed to humic-like material, most probably FA (Erich and Trusty, 1997).
2. Another peak at 265–280:300–370 nm, again present in groundwaters from all four sites, which can be attributed to amino-acid fluorescence within proteins, which may derive from decomposed plant matter, as well as soil bacteria and decomposed animals. The most likely source is tryptophan, which accounts for most protein fluorescence and exhibits peaks in the same region (Lakowicz, 1983). Both of these fluorescence peaks have been reported elsewhere for soils, fluvial and marine waters (Coble, 1996; Luster et al., 1996; Mobed et al., 1996; Erich and Trusty, 1997). The presence of protein-like fluorescence in karst groundwaters confirms the results by Rousseau et al. (1992), which demonstrated the presence of protein derivatives in cave speleothems, and suggests that a significant carbon-flux from the soil to the groundwater system may be in this form of dissolved organic carbon.
3. A third region of fluorescence, at 230–280:310–420 nm, demonstrates significant differences between sites, with UAT exhibiting fluorescence at a higher excitation wavelength than the other sites, and PC and VIL at a lower excitation wavelength. These fluorescence centres may also have a protein source; a range of emission wavelengths excited by a fixed excitation wavelength suggests the presence of a relatively simple, single fluoro-

phore such as a protein, and may explain the linear fluorescence trend at excitation 225–245 nm. However, previous studies have not usually extended to such high energy levels and so fluorescence at this wavelength is poorly understood. Coble (1996); Erich and Trusty (1997) and Ohno and Cronan (1997) all report a peak at ∽ 250 nm but this is at the limit of their scans and may really represent a peak at shorter wavelengths. De Souza Sierra et al. (1994) report peaks at 250:300 nm and 250:440 nm, attributed to a protein and a possible humic-like fluorophore, respectively. Indeed it is likely that the fluorescence observed here (Fig. 2(a)) may be divided into two fluorescence sources at emissions 320–370 and 380–420 nm, and further research is needed in this optical region.

The relative fluorescence intensities of the three peaks are presented in Fig. 2(b), and demonstrate a higher intensity of fluorescence with shorter fluorescence wavelength, although with a wide range of values which reflect seasonal variations in fluorescence intensity (see next section (Section 4.2). Increasing the fluorescence at shorter wavelengths does not necessarily imply a greater concentration of the protein-like material in the groundwater compared to humic and fulvic substances as fluorescence intensity typically increases with decreased molecular weight.

The range of fluorescence excitation and emission wavelengths observed here is in good agreement with other studies of leaf litter extract and dissolved organic matter (Erich and Trusty, 1997; Luster et al., 1996 and references therein). However, fluorescence at longer (UV–blue) wavelengths, representative of HA, was not observed in any of our groundwater samples, except as a weak tail of the fulvic-like fluorescence peak at UAT. A UV–blue fluorescence peak has been observed elsewhere (e.g. soil extracts, Senesi et al., 1991; Rivero et al, 1998; humic acid standards, Mobed et al., 1996). Three possible effects, acting singly or in combination, may prevent long wavelength fluorescence:

1. The relatively hydrophobic nature of the longer molecular weight HA in comparison to FA, prevents the HA from being transported from the soil zone. However, from the limited evidence of the one soil water sample at BFM, which has a fluorescence wavelength only slightly higher than the cave water samples, suggests this is not a significant factor.
2. The HA are present in the groundwaters, but their fluorescence is being quenched by calcium and other metals in solution. However, as detailed earlier, although synchronous scan fluorescence studies would suggest a quenching of the long wavelength fluorescence peak by metal ions, studies of liming effects on soil fulvic acid have demonstrated only a limited effect (Erich and Trusty, 1997).
3. The high Ca-soils overlying the sites provide a significant source of calcium in solution which flocculates the organic acids in the soil. Romkens and Dolfing (1998) have demonstrated this effect preferentially flocculates high molecular weight organic acids (humics) as opposed to short length acids (fulvics). Thus, the HA may remain as flocs in the overlying soil whereas the FA is transported into the groundwater. The results from UAT, which is overlain by a 70 cm thick, low-Ca peat, and which has groundwaters with a tail of fluorescence within the region expected for HA, suggests that flocculation is a likely explanation for the observed fluorescence patterns at the four sites.

4.2. Fluorescence properties of the 290–340:395–430 nm wavelength pair

Fluorescence wavelength variations for groundwaters at the four sites are presented in Fig. 3. Subtle differences are apparent between sites, with a variability of ± 20 nm in the excitation and emission wavelengths of this fulvic-like fluorescence peak. The groundwater at UAT exhibits the highest emission wavelengths. This site, overlain by blanket peat, may be expected to have higher emission wavelengths due to the higher degree of aromacity, increased content of carboxylic groups and polycondensed aromatic and conjugated structures in this soil type (Senesi et al., 1991). Indeed, waters from this site were the only ones that exhibited a significant tail in the fluorescence peak extending to higher excitation and emission wavelengths (> 360 nm excitation and > 450 nm emission), which have been widely observed in HA from soil extracts. The optical

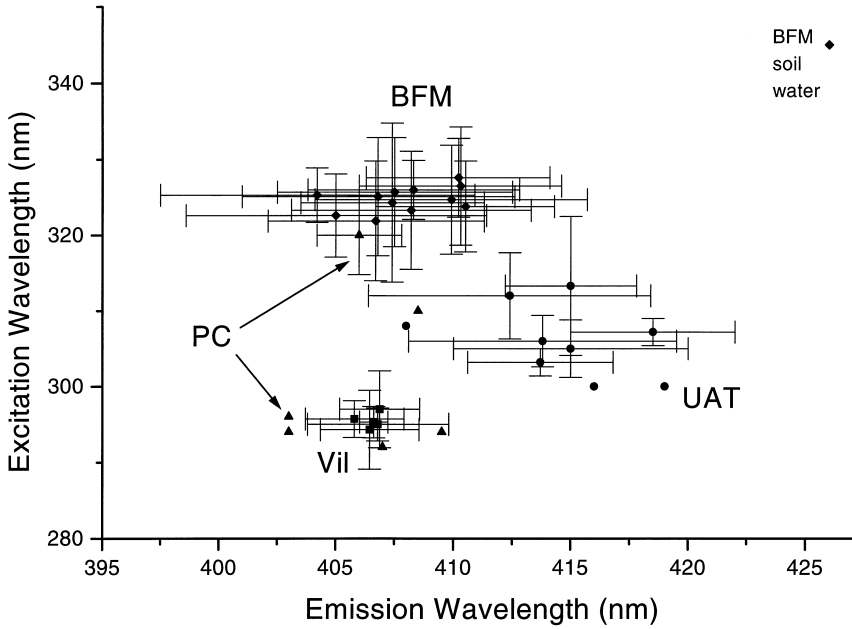

Fig. 3. Excitation and emission wavelength variations of the groundwater samples from the four sites. Error bars are the 1σ range for sampling locations where multiple samples were obtained. Diamonds = BFM, triangle = PC, light squares = VIL, circles = UAT. One BFM soil water analysis is also shown.

properties of the other sites are less easy to distinguish from their wavelength variations, with significant overlap between PC, BFM and VIL sites, despite their widely differing climate and geochemistry, suggesting that soil and vegetation type may be the dominant control of fluorescence wavelength. BFM and VIL groundwater sites are tightly clustered, whereas those from PC scatter over a wide range. The cause of this variation may be supposed to be due to local differences in the overlying soil at the latter site (between relatively undisturbed areas and those affected by lime waste) and vegetation cover (woodland glades, predominantly in areas of former lime waste, and ash/elm woodland); woodland cover at VIL and BFM is more uniform.

Variations in mean fluorescence intensity between the sites are significantly greater than that of the fluorescence wavelength. Therefore, when both fluorescence intensity and wavelength results are plotted, further differentiation of the groundwaters in terms of their optical properties becomes possible. Fig. 4 presents data for the four sites (error bars are omitted for clarity). All four sites are now better differentiated in terms of their fluorescence wavelength and intensity variations, with VIL and PC having significantly higher luminescence intensities than BFM and UAT. Explaining the causes of the high fluorescence intensity at VIL and PC compared to BFM is difficult due to the many factors affecting fluorescence properties. Both VIL and PC have markedly contrasting climate but well developed soils with deciduous woodland vegetation, which may provide substantially more fluorescent materials than at BFM and UAT. At BFM, soil thickness is < 20 cm and has developed since the date of mine abandonment in AD 1904, which may explain the relatively low fluorescence intensity. The low fluorescence at UAT cannot be due to source limitations of organic matter as it is overlain by blanket bog, and is likely to be due to the higher aromacity of the dissolved organic material as previously observed in peat extracts (Senesi et al., 1991).

4.3. Spatial variability of groundwater fluorescence at BFM

Detailed analysis of data from BFM (Table 2 and Fig. 1) demonstrate no spatial trends in either the

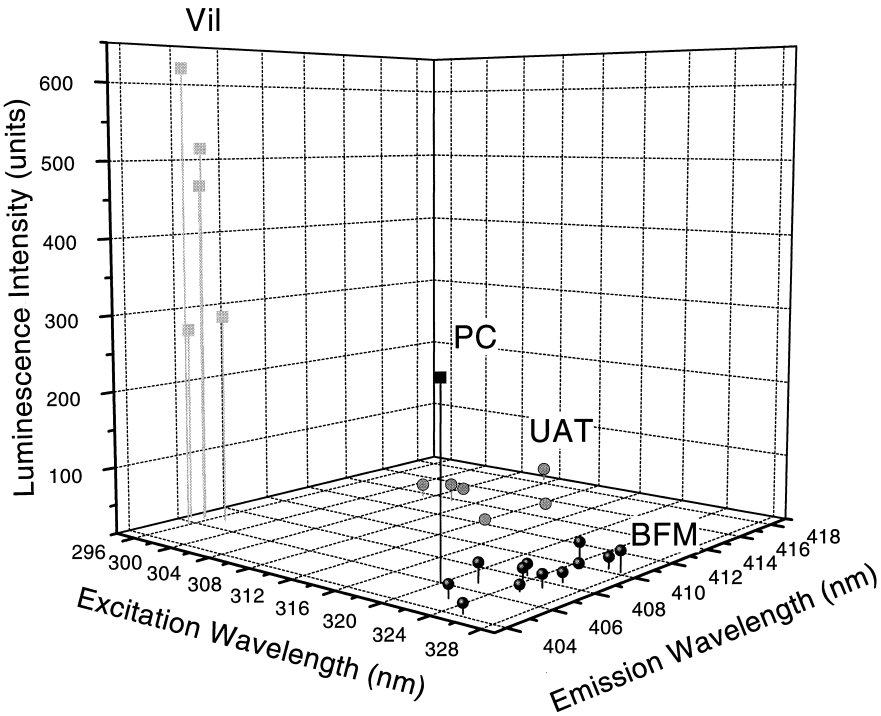

Fig. 4. 3D plot of mean fluorescence excitation and emission wavelengths and fluorescence intensity for VIL (light squares), PC (dark squares), UAT (circles) and BFM (spheres).

wavelength or intensity of groundwater fluorescence of the 290–340:395–430 nm wavelength pair, partly a reflection of the lack of statistical difference in fluorescence properties of all the stations at the site. In particular, station pairs DO/DC and NL/NR, which are within 1 m of each other, demonstrate remarkably similar means of fluorescence wavelength and intensity. Elsewhere at the site, five sites, which have completely different hydrological characteristics within a $4\,m^2$ area exhibit no differences in fluorescence properties; F1 is a constant discharge, storage flow source (mean discharge = 0.036 drips/

Table 2
Groundwater discharge and fluorescence characteristics at BFM

Station	Discharge drips/s	Excitation wavelength (nm)	Emission wavelength (nm)	Intensity (units)
B	0.259 ± 0.638	321.9 ± 7.9	406.7 ± 4.6	36.2 ± 30.2
DC	0.007 ± 0.003	324.3 ± 10.5	407.4 ± 3.9	31.7 ± 29.9
DO	0.016 ± 0.004	325.7 ± 7.2	407.5 ± 5.0	28.0 ± 15.9
J	0.074 ± 0.121	324.7 ± 7.2	409.9 ± 5.8	16.1 ± 8.8
NL	0.012 ± 0.003	326.5 ± 7.8	410.3 ± 4.3	26.3 ± 10.4
NR	0.005 ± 0.002	327.6 ± 5.2	410.2 ± 3.9	38.6 ± 36.0
F1	0.036 ± 0.008	325.1 ± 7.8	406.8 ± 5.8	20.2 ± 8.8
F2	0.015 ± 0.003	322.6 ± 5.5	405.0 ± 6.4	28.7 ± 15.9
F3	0.003 ± 0.0003	323.3 ± 7.8	408.2 ± 5.1	26.2 ± 13.4
F4	0.009 ± 0.001	323.8 ± 6.0	410.5 ± 3.8	34.7 ± 29.2
F5	0.024 ± 0.017	326.0 ± 3.9	408.3 ± 4.5	24.1 ± 17.0

s, CV = 20%), F5 responds rapidly to surface climate variations with a direct feed from the gull fissure (mean discharge = 0.024 drips/s, CV = 85%) and F2, F3 and F4 seasonally variable karst dripwaters (mean discharge = 0.003–0.3 drips/s, CV = 10–20%). The homogeneity of fluorescence properties at BFM, as well as VIL and UAM, probably reflects that of the overlying soil and vegetation at the sites, with the PC site being different for the reasons explained earlier (see previous section (Section 4.2)).

4.4. Time series trends of groundwater fluorescence at BFM

Fig. 5 presents fluorescence emission wavelength variations, together with drip rates and fluorescence intensity data, for water samples from seven of the sites that were sampled at 10–20 days intervals over the period 1997–1998. These sample sites were chosen for their low discharge (< 0.02 drips/s) and coefficient of variation of discharge ($< 20\%$) so that individual fluorescence events could be resolved by the water-sampling interval.

Fluorescence intensity can be observed to peak in autumn and winter, results matching previous studies (Baker et al., 1997). Fluorescence intensity maxima occur after the autumn recharging of the groundwater, which is reflected in the increase in discharge at all sites after Julian Day 240 (JD; JD 1 = 1 January 1996), and are caused by increases in hydrologically effective precipitation in autumn winter. Fluorescence emission wavelength exhibits remarkably little variation throughout the year, except a decrease in emission wavelength that correlates with an increase in fluorescence intensity in autumn (JD 275–300; October 1997). However, this relationship reverses for the second flush of organic matter from the soil, which reaches the cave sources in JD 360–400 (January 1998). Instead, this water is characterised by a decrease in fluorescence excitation wavelength for six of the seven sites (Fig. 6) to a level equivalent to that observed all year at VIL and at some sites at PC. Several explanations for these wavelength variations include:

1. The arrival of dissolved organic matter that has undergone different extents of organic matter breakdown. Despite both long and short residence time components of OM in the soil, if significant variations in the humification rate/residence time of the short residence time component occur, then this may be reflected in the fluorescence variations. For example, material flushed into the groundwater in autumn is likely to include a greater proportion of soil breakdown products from the preceding spring and summer, and which may have been undergoing decomposition for up to 9 months, whereas the winter flush may contain a greater proportion of poorly decomposed material formed in late summer/autumn, with a shorter time for breakdown and during a period of colder and wetter climate.
2. Differences in metal-organic acid interactions that may alter the fluorescence excitation–emission properties. Both the autumn and winter flushes are characterised by higher concentrations of Ca ions (2.5–3.0 mmol l^{-1}) in comparison to the annual mean values (1.4–2.7 mmol l^{-1}; Table 1). However, given the $\backsim 100\times$ higher concentration of Ca ions compared to dissolved organic matter in solution, it might be assumed that such seasonal trends in Ca may be of negligible importance. However, a higher concentration of other metal ions in the first autumn flush, which may have a greater proportion of waters which have experienced a long residence time in the soil zone (e.g. Al), could explain the fluorescence wavelength variations if they act as fluorescence quenchers.
3. Inclusion of a fluorescence source derived from organic matter contained in the limestone bedrock and dissolved during groundwater flow. Coble (1996) demonstrates the presence of a marine fluorescence source in shallow marine waters as a decreased excitation wavelength of $\backsim 30$ nm and emission wavelength of 5–20 nm. If this source is present in the Jurassic limestone bedrock, and is dissolved into solution together with the soil derived fluorescent material, then it may affect the overall fluorescence signal. However, the low concentrations of this bedrock source in comparison with the relatively high organic matter soil zone makes this an unlikely factor, together with the fact that only $15 \pm 5\%$ of carbon precipitated in cave speleothems has a bedrock source, the rest deriving from the soil zone (Genty and Massault, 1997).

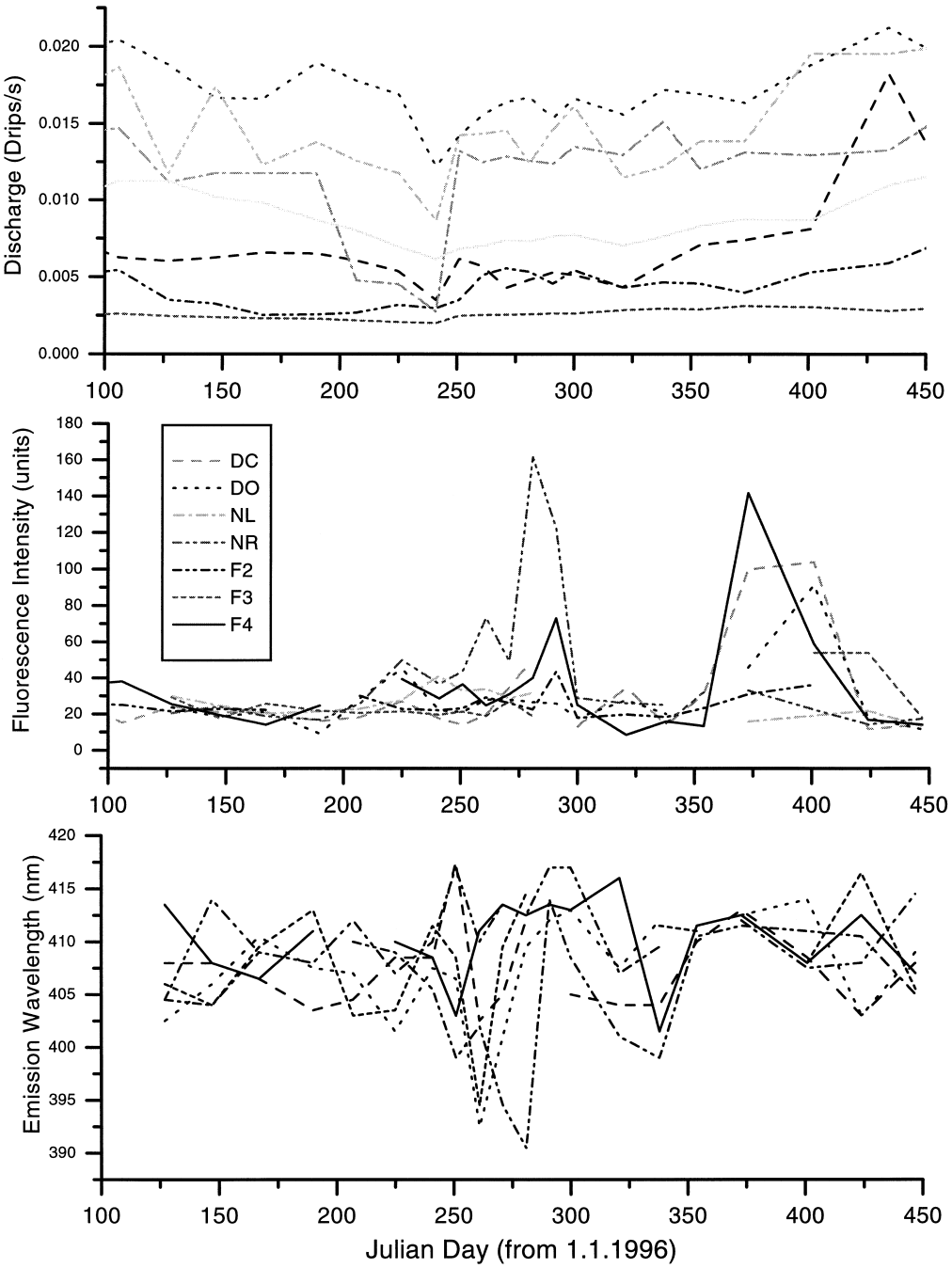

Fig. 5. Top: time series of fluorescence intensity variations for seven drip waters at the BFM site. Mid: fluorescence emission wavelength variations for the same samples. Base: drip discharge of the seven groundwater sources.

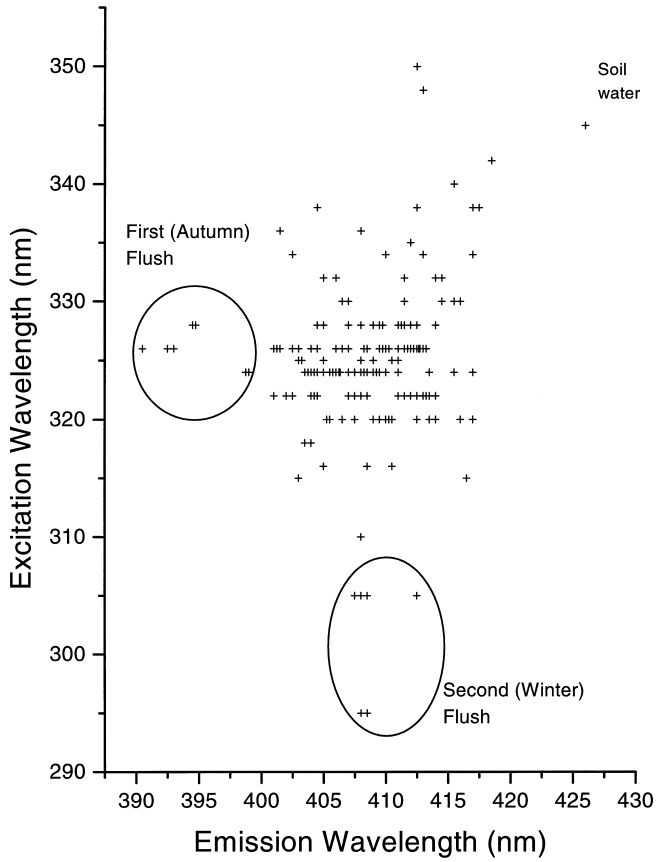

Fig. 6. Scatterplot of excitation and emission wavelength variations of the 290–340:395–430 nm fluorescence pair for seven groundwaters in BFM, together with the soil water sample. Outlying samples with shorter excitation or emission wavelengths occur solely during the autumn and winter fluorescence intensity peaks.

Three factors have been proposed to explain the time series fluorescence wavelength trends, however, only the first adequately explains the difference between the first and second flushes of groundwater by differential degrees of decomposition of the organic material. Time series variations in the fluorescence wavelength have not been previously analysed, and further research under controlled conditions is necessary to elucidate the controlling factors. In particular, the presence of seasonal variations in the fluorescence wavelength which are of the same order of magnitude as inter-site variations suggests that temporal variations of fluorescence wavelengths over periods longer than intra-annual may be significant.

5. Conclusions

The fluorescence wavelength and intensity variations of groundwaters from the four sites have demonstrated a good agreement with existing literature, particularly with that from soil extracts. All groundwaters appear to omit the long wavelength, HA fluorescence, possibly due to its relatively hydrophobic nature and by its flocculation by Ca ions in the soil zone. In addition results from this study suggest the presence of one, or possible two fluorophores at an excitation wavelength of $\backsim$ 250 nm, which may be ascribed to a humic-like source or a protein source, or a mixture of the two. Detailed time series and spatial investigations at BFM demonstrate that spatial

variability of fluorescence wavelength and intensity variations was less than temporal variations observed at each site. Time series data from BFM suggest a possible trend to a lower fluorescence wavelength, but only at the first intensity peak of the hydrological year, with the second groundwater flush in winter exhibiting a decrease in excitation wavelength and fluorescence properties which resemble those observed all year at VIL and PC. The causes for these changes are unclear and require further studies; the autumn flush may contain more humified organic material as it contains a greater proportion of decomposed organic material which has been in the soil zone for several months, whereas the winter flush may have fresher organic remains.

Subtle differences in the wavelength of the fulvic acid fluorescence peak have been observed in the groundwater at the four study sites. This differentiation is improved when fluorescence intensity is included as a characteristic. Explanation of site differences in terms of climatic variations, pH or metal ion quenching, or differences in vegetation and soil types is difficult due to the inter-relationships between the different factors, although the latter two factors appear to be the most important. UAT has the highest fluorescence emission wavelengths which may reflect the overlying humic acid rich peat bog, a result that agrees with fluorescence in solid stalagmites overlain by peat deposits (Baker et al., 1998a), and the highest fluorescence intensities occur at VIL and PC which have a well developed soil cover and mature deciduous woodland vegetation.

Results presented here suggest many avenues for future research. In particular, controlled experiments on the decomposition of organic material in karst soils and groundwater, and the effect on their fluorescence properties are required, in order to assess the relative importance of Ca-quenching, pH effects, soil and vegetation variations and climate. In addition to this process study, several potential applications of karst groundwater fluorescence studies can be envisaged:

1. The sourcing of groundwaters in karst terrains, especially at springs and other recharge sources where the groundwater may derive via different and potentially unknown flow routings and from source regions with different soil and vegetation covers.
2. Assessing the effects of pollution/land use change. Results here demonstrate a remarkable within site homogeneity of fluorescence intensity and wavelength variations, which may be utilised as a benchmark for future changes in e.g. farming, heavy metal pollution, sewage pollution.
3. Palaeoenvironmental reconstruction. Organic matter contained within groundwater may become preserved in speleothem calcite. Empirical evidence from stalagmites has demonstrated the presence of fluorescence wavelength cyclicity of the 290–340:395–430 nm wavelength pair over 100–1000 y periodicities (Baker et al., 1998a). Results presented here from the time-series analyses from BFM demonstrate the occurrence of seasonal variations, which may be correlated with soil decomposition or climate. Longer time series from waters are needed over inter-annual cycles, in order to calibrate the speleothem record for longer time periods.

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