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Organic acid fluorescence: applications to speleothem palaeoenvironmental reconstruction

Siobhán F. McGarry[a,b,*], Andy Baker[c]

[a]Department of Earth Sciences, Open University, Walton Hall, Milton Keynes MK7 6AA, UK
[b]Department of Geography, School of Geography and Archaeology, University of Exeter Amory Building, Exeter, EX4 4RJ, UK
[c]Department of Geography, University of Newcastle, Daysh Building, Newcastle-upon-Tyne NE1 7RU, UK

Abstract

Recent advances in fluorescence spectrophotometry permit the non-destructive analysis of speleothems (secondary carbonate cave deposits) with a view to palaeoenvironmental reconstruction. The fluorescence of speleothems is derived from organic acids that have been carried by groundwater from the overlying soil, and coprecipitated with the speleothem calcite. Organic acids are formed as one of the many products of humification and their chemical structures are such that they are particularly suitable for analysis by fluorescence spectrophotometry. Numerous studies have been carried out that have demonstrated the structure and hence the fluorescence properties of organic acids to be influenced by many factors, including extraction method, concentration, source, pH, metal and inorganic ion complexing and, most importantly in the case of speleothems, soil type and climate and vegetation change. On the basis of this, recent investigations carried out on contemporary, historical and Quaternary samples have shown it to be possible to obtain palaeoenvironmental information from the fluorescence properties of organic acids in speleothems.

1. Introduction

A variety of indicators of palaeoenvironmental change have been obtained from speleothems. These include oxygen and carbon isotopes (Hendy, 1971), trace element variations (Goede and Vogel, 1991), the timing of speleothem growth (Gordon et al., 1989; Baker et al., 1993a) and pollen (Bastin, 1978). Such records may be accurately dated to a precision of $\pm 1\%$ from samples of less than 1 g of calcite, due to the recent development of thermal ionization mass spectrometric (TIMS) $^{238}U - ^{234}U - ^{230}Th$ dating (Edwards et al., 1987), and its application to speleothems (Li et al., 1989; Baker et al., 1993b).

Most recently, speleothem fluorescence variations have been investigated as a palaeoenvironmental indicator. The fluorescence derives from organic acids that are trapped within the speleothem calcite, having been introduced from the overlying soil and vegetation by the groundwaters feeding the speleothem. 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; Mobed et al., 1996; Erich and Trusty, 1997) and have shown the fluorescence properties to differ depending on the origin of the organic matter. The influence of other factors including pH (Senesi et al., 1991), metal ion complexing (Shotyk and Sposito, 1988, 1990; Yang et al., 1994), soil type (Körschens et al., 1998) and climate (Martin-Neto et al., 1998; Christ and David, 1996) have also been investigated, and it has been shown that they are all interrelated to produce an organic acid fluorescence signal representative of the conditions under which the acids formed. This paper aims to present a review of some of the more significant research which has been undertaken on organic acids and on the applications of speleothem organic acid fluorescence to palaeoenvironmental reconstruction.

2. Organic acid fluorescence

Organic acids make up about 60–70% of the total organic carbon in soils and 40–60% of the dissolved

* Corresponding author. Tel.:0044-1908-653-012; fax: 0044-1908-653-15.
E-mail address: s.f.mcgarry@open.ac.uk (S. F. McGarry).

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organic carbon in natural waters (Senesi, 1993). They may be defined according to their solubility in water. Fulvic acids (FA) are hydrophilic, being soluble in water at all pHs, while humic acids (HA) are slightly more hydrophobic in nature, being insoluble in aqueous solutions with pH < 2, but are soluble at higher pH values. Humins are insoluble in water at any pH (Senesi, 1993). Solubility is related to molecular weight, on the basis of which they may also be differentiated. FA have a lower molecular weight, ranging from 500 to 2000 D, than their HA counterparts which are 2000–5000 D (Senesi, 1993). A schematic HA structure is shown in Fig. 1.

Organic acids are formed as one of the many products of humification, the transformation of macromorphologically identifiable resources into amorphous humic compounds (Zech et al., 1997). The pattern and rate of decomposition of a resource are determined by the various aspects of its physical and chemical composition, which are termed the resource quality. An important aspect of the quality of a resource is its lignin content, which has been shown to be related to its decay rate (Swift et al., 1979). Lignin is laid down in the cell wall of plants during secondary thickening, adding considerable rigidity to the cell wall. It is composed of cross-linked phenyl-propane monomers, the relative proportions of each monomeric unit in the lignin of a particular plant species depending on its phylogenetic origin (Kögel-Knaber et al., 1991). For this reason, different plant organs, species and groups show characteristically different rates of decomposition (Swift et al., 1979).

The main transformations during litter decomposition and humification are loss of polysaccharides and phenolic moieties, side-chain oxidation, strong alteration of lignin leading to increased proportions of carbon-substituted aromatic rings, and increase in carboxyl group contents and accumulation of refractory alkyl components (Zech et al., 1992, 1997). Most of the lignin-derived compounds are released into the hydrophobic and hydrophilic acid fractions of the humified organic layers. The hydrophobic acids show a high carboxyl C content and the major components of the hydrophobic acids are biooxidatively degraded lignin- and ligninocellulose-derived compounds. They may act as a precursor of humic substances and show similarities to the relatively hydrophobic HA. Hydrophilic acids (FA) are composed of similar components to hydrophobic acids, but they show a higher degree of side-chain oxidation. So the bio-oxidative lignin degradation is more advanced than in hydrophobic acids (Gruggenberger and Zech, 1994a), suggesting that FA are genetically downstream from HA.

It is such structural components, as referred to above, that allows fluorescence to be easily stimulated in organic acids (Senesi, 1990). Fluorescence occurs when an electron is excited by absorbing electromagnetic radiation and the atom emits a photon of light as the electron returns to its original energy level. Excitation is most easily stimulated in molecules containing electrons which are free to move between energy levels (Loudon, 1988). As energy cannot be created by this process, the emitted

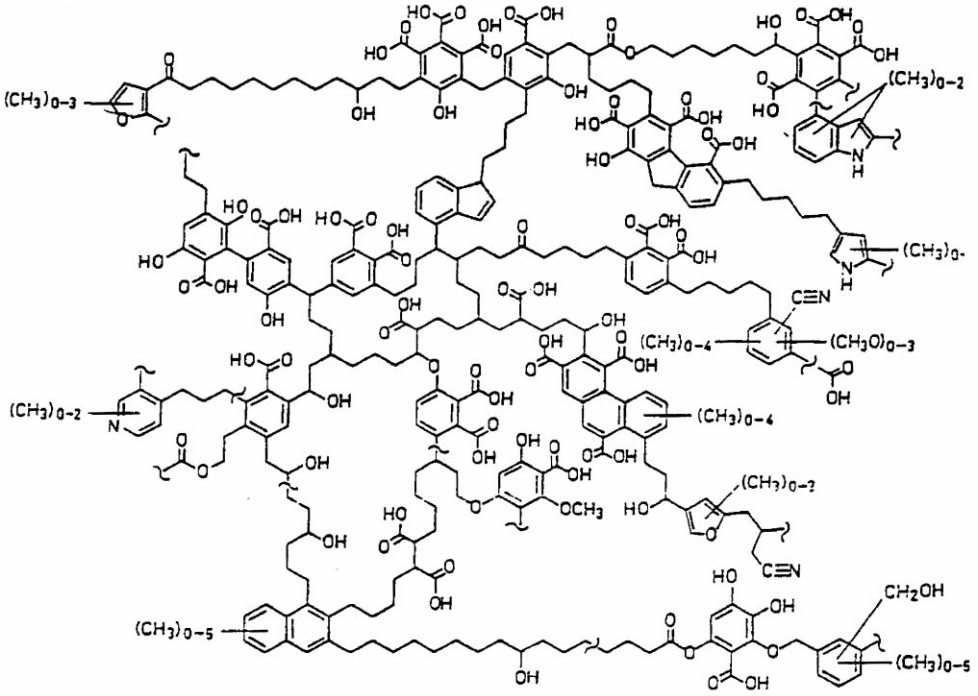


Fig. 1. Schematic humic acid structure (Schulten and Schnitzer, 1993).

fluorescent light is at a longer wavelength (lower energy) than the excitation. The most common form of organic acid fluorescence is emission in the long wave UV and blue wavelengths (350–500 nm) after excitation by UV light (200–400 nm) (Senesi et al., 1991).

The structural feature of a molecule responsible for its fluorescence is called a fluorophore. The most important fluorophore in determining fluorescence characteristics is the number of consecutive conjugated double or triple bonds (i.e. double or triple bonds separated by a single bond). Electrons involved in double or triple bonds are held in π orbitals and can move easily between energy levels, ($\pi \rightarrow \pi^*$), more easily than electrons in other orbitals (s, p, etc.) which are not so easily excited. Therefore, the longer the conjugated system, the higher the wavelengths of excitation and emission as less energy is required and the lower the intensity of the fluorescence of the molecule.

Aromatic rings (containing conjugated double bonds) fluoresce for the same reason. The content of substituent groups such as carboxyls and carbonyls (having double bonds) also increases the wavelengths of excitation and emission. Molecules containing oxygen or nitrogen atoms, having lone electron pairs, are capable of resonance, as are aromatic rings, and this also enhances fluorescence (Loudon, 1988).

HA have higher wavelengths of excitation and emission and lower intensity than FA due to: (a) the presence of higher molecular weight fractions in HA (Senesi et al., 1991), (b) a greater degree of aromaticity and polycondensation in HA, while FA have a more aliphatic character (Barancíková et al., 1997), (c) the higher content of electron withdrawing substituents such as carbonyl and especially carboxyl groups in HA than in FA (Senesi et al., 1991), (d) inter- and/or intramolecular bonding, and (e) reabsorption of the emitted radiation by other absorbing centres of the molecule or neighbouring molecules (Miano et al., 1988).

Therefore, it might be expected that organic matter from soil with a higher proportion of HA to FA would have a higher wavelength of excitation and emission than those with a low HA/FA ratio, with the intensity of this fluorescence per unit mass increasing with the concentration of the fluourescing substances present (Miano and Senesi, 1992).

3. Analytical methods

Initial research in the late 1970s and 1980s adopted single-scan methodologies to study the excitation and emission spectra of organic acids. As the speed and optical resolution of spectrophotometric techniques improved, this was later followed by synchronous-scan excitation spectra, from which slightly more characteristic spectra were obtained. In recent years, further technological advances have allowed the development of excitation and emission matrix spectra.

3.1. Single scan methodologies

3.1.1. Excitation spectra

These are obtained by holding the emission wavelength constant, while scanning over a range of excitation wavelengths. The results are generally better resolved than emission spectra and are characterised by a number of peaks or shoulders that are localised into three wavelength regions, long (480–440 nm), intermediate (400–380 nm) and short (360–300 nm). They produce relative fluorescence intensities, which are typical of each group of humic materials (Senesi et al., 1991).

3.1.2. Emission spectra

Emission spectra are recorded over a range of emission wavelengths, at a constant excitation wavelength. Senesi et al. (1991) showed that emission spectra are generally characterised by a broad band of relative intensity and maximum wavelengths that generally vary in a limited range, within a group of samples of similar origin and nature, but are highly dependent on the group of humic materials considered. In similar studies by Miano et al. (1988) and Ramseyer et al. (1997), the same general trends were found.

3.2. Synchronous-scan excitation spectra.

In this case, the spectra are formed by recording the intensity while synchronously scanning over both the excitation and emission wavelengths. The wavelength difference is maintained constant as the spectrum is recorded (Miano et al., 1988; Senesi et al., 1991; Ramseyer et al., 1997). This method allows better fingerprinting of organic matter than previous single line scans.

3.3. Excitation-emission matrix spectra

In the past 3–4 years three-dimensional excitation–emission-matrix (EEM) spectra have been used, to provide a complete representation of the fluorescence properties of a sample in the form of an excitation–emission matrix, in which fluorescence intensity is presented as a function of excitation wavelength on one axis and emission wavelength on the other (Matthews et al., 1996; Mobed et al., 1996; Baker et al., 1998; Baker and Genty, 1999). Using this method excitation and emission wavelengths can be varied from 200 to 700 nm. Slit-width can be set from 2–15 nm for excitation and emission depending on the intensity and resolution required (wide slits gives greater fluorescence intensity but coarser wavelength resolution).

Two ranges of excitation and emission wavelengths are usually used. Initially, to find the number and location of fluorescence centres, excitation is stimulated from 200–500 nm at 5 nm steps, and for each excitation

wavelength the emitted fluorescence is detected at 0.5 nm steps from 300 to 500 nm. Following this, high-resolution analysis of one particular peak may be undertaken. The excitation wavelength is always less than or equal to the emission wavelength, to minimise the Rayleigh–Tyndall scattering which lies along the $\lambda ex = \lambda em$ diagonal. At the beginning of each period of data collection, the spectrophotometer is calibrated against the 365 nm Raman and Rayleigh–Tyndall (Primary and secondary) peaks, of a sample of double distilled water in a 5 ml UV transparent quartz cuvette, as the wavelength of the Rayleigh scatter and Raman oscillations of water are constant. The intensity of the Raman oscillation can also be used to calibrate the excitation source output between analyses and between machines. Sample collection is only commenced when the signal : noise ratio of the spectrophotometer is $> 500 : 1$.

The fluorescence spectral features are represented as an excitation-emission matrix (EEM), an example of which is shown in Fig. 2. Fluorescence is measured as the maximum intensity at an excitation–emission pair.

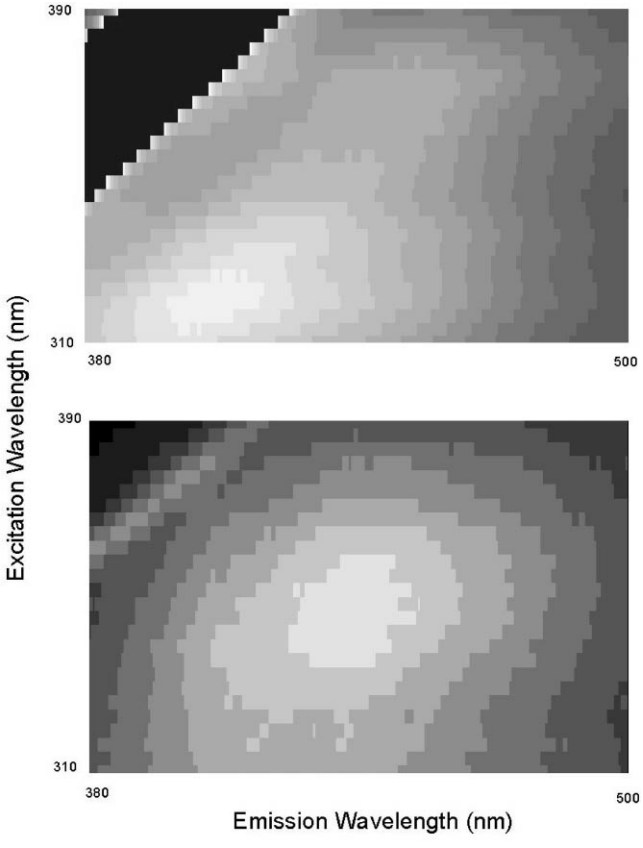


Fig. 2. 3D excitation-emission matrices. Top: stalagmite excitation–emission matrix from Brown's Folly Mine, Wiltshire (BFM-B). (Slit Width = 15 nm) Bottom: FA standard (2 mg/l) (Slit width = 5 nm).

4. Controls on organic acid fluorescence wavelength and intensity

4.1. Extraction method

4.1.1. Water samples

Soil extracts and water samples whose fluorescence is to be studied, must be collected in glass bottles, not plastic, to prevent the leaching of fluorophores from the plastic into the water sample (Coble, 1996). The glassware must be precleaned to remove organic matter, non-fluorescent detergent must be used throughout, and deionised water must again be stored in glass (Baker and Genty, 1999). If water samples need to be filtered to measure only the dissolved organic fraction, glass microfibre filter papers (Baker and Genty, 1999) or nylon filters (Coble, 1996) should be used that have been pre-ashed to destroy any organic residues. On return to the lab samples must be refrigerated and analysed within 48 hours or can be frozen in glass bottles for up to 1 yr prior to analysis (Coble, 1996).

4.1.2. Solid samples

In a study of organic acids in corals, speleothems and marine cements, Ramseyer et al. (1997) found that the most soluble organic matter was released after aqueous and alkaline treatment, while samples extracted under low pH show the most intense fluorescence as the more tightly bound fraction was also released. This showed that different treatments reveal a direct relationship between the complexity of organic molecules and their binding to the crystals. It also indicates that the fluorescence characteristics of organic materials may depend on the method used to extract them from their host medium as different chemical treatments and pHs can affect the conformation of the organic molecules, and for this reason, non-destructive analysis methods are preferable.

Recent advances in spectrophotometry permit the non-destructive analysis of organic acids in solid samples (e.g. speleothems, corals) through the use of fibre optic extensions. Samples need only be cut and polished for analysis in thick section (Isdale, 1984; Milne and Swart, 1994). In the case of speleothems, data are collected at points at 1–3 mm intervals along the axis of growth. The fibre optic extension, which is held at a height of 1–5 mm above the sample surface, has a beam width of 1–2 mm, giving a typical temporal resolution of 10–40 yr depending on the growth rate.

4.2. Concentration

Increased organic acid concentration increases fluorescence intensity with no effect on fluorescence wavelength, at low concentrations, as is the case in groundwater studies where concentrations have been shown to range from 0.5–2.0 m l^{-1} in stalagmite drip waters in Lower

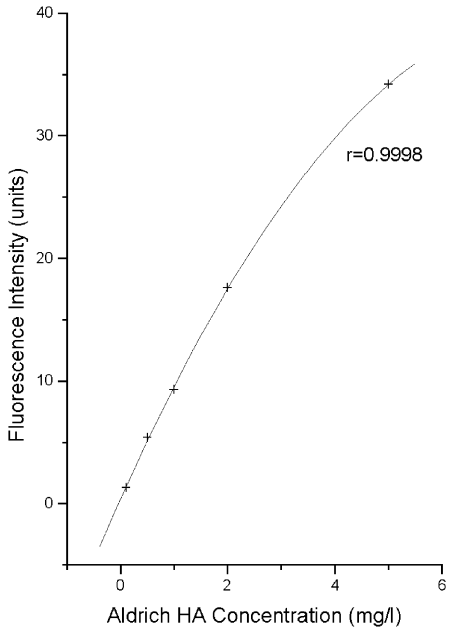


Fig. 3. Effect of increasing organic acid concentration on fluorescence intensity.

Cave, Bristol (Baker et al., 1997). A typical relationship between organic acid concentration and fluorescence intensity is presented in Fig. 3. However, as the concentration gets higher, absorption effects interfere with fluorescence wavelength, and correction is necessary (Mobed et al., 1996). Yang and Zhang (1995) found that at humic substance concentrations of greater than 15–30 mg/l red shift and quenching of the lower wavelength peaks, and distortion of the excitation spectra occur. They also found the emission spectra to be concentration dependent. The emission spectra deviate in the same manner as the corresponding excitation spectra as, at high concentrations (50 mg/l) the emission of a fluorescent unit can be reabsorbed in the form of exciting light by a nearby fluorophore as the result of inter- or intramolecular transfer (Miano and Senesi, 1992). Mobed et al. (1996) and Shotyk and Sposito (1990) also attributed the shift of fluorescence maxima to longer excitation and emission wavelengths, at increasing humic substance concentration, to the attenuation of the shorter wavelength emitted fluorescence, caused by inner filtering. This effect is due to more light being scattered at higher concentrations.

Absorbance correction is therefore necessary to avoid confusion between actual changes in fluorescence spectral features and inner filtering effects (Mobed et al., 1996). However, this is generally a problem when extracts have been artificially concentrated, e.g. soil extracts; organic acid concentration is normally below the range needed for absorbance correction in natural waters and sediments.

4.3. Source of organic matter

Miano et al. (1988) obtained fluorescence excitation and emission spectra and synchronous-scan excitation spectra for a variety of HA and FA from various peats and loams. It was shown to be possible to differentiate between HA and FA from the same source on the basis of the excitation and emission spectra, with HA exhibiting higher wavelengths of excitation and emission in each type of scan due to structural differences, as discussed previously. The excitation spectra of FA were found to be sensitive to the origin of the humic substance, whereas those of HA were relatively insensitive to provenance.

Senesi et al. (1991) investigated 50 samples of HA and FA isolated from various soils and soil-related materials, (including palaeosols, peat, leonardite, composted and earthworm composted organic materials, sewage sludges and materials synthesised by soil fungi), by emission, excitation and synchronous-scan excitation fluorescence techniques. It was found that HA generally exhibited longer excitation and emission wavelengths than FA, the latter having a greater intensity of fluorescence. When the wavelengths of the emission and excitation maxima were plotted against each other, HA and FA fell into five classes, based on their fluorescence behaviour, which is influenced by their origin. The synchronous-scan excitation spectra obtained also allow the organic acids to be grouped according to their origin and nature. In general, the longest wavelengths and lowest intensities were shown by palaeosol, soil peat and leonardite HA. The shorter wavelengths and higher intensities were measured for the main fluorescence peaks of composted HA and soil peat FA. These distinct groupings are thought to be due to structural differences (as discussed earlier).

Mobed et al. (1996) found it to be possible to distinguish between soil and aquatic organic acids and between HA and FA from the same source, by EEM spectroscopy. HA exhibited fluorescence maxima at longer emission and excitation wavelengths than FA, while FA were more intensely fluorescent. The fluorescence spectral maxima of the soil samples were at longer excitation and emission wavelengths than those of aquatic samples. Coble (1996) demonstrated that fluorescence maxima occur at shorter wavelengths for marine samples than for fresh water samples, allowing differentiation between marine and terrestrial dissolved organic matter in water from rivers, coastal, shallow marine and deep marine environments.

4.4. External environmental factors.

4.4.1. pH

Numerous studies have shown that fluorescence intensity increases with increasing pH (Coble, 1996, Senesi et al., 1991; Yang and Zhang, 1995). Such changes in the fluorescence spectra are due to changes in the fluorescence

characteristics of the acidic functional groups (phenols and phenolates) in the organic molecules, and changes in the conformations of the organic molecules which could expose different functional groups to the bulk aqueous solvent (Miano et al., 1988; Coble, 1996).

Increasing pH also affects fluorescence wavelengths, causing a red shift in the longer wavelength peak region due to changes in the fluorescence characteristics of the acidic functional groups (phenols and phenolates) in humic molecules (Mobed et al., 1996). Phenols are known to exhibit two fluorescence maxima, of which the one at longer wavelengths becomes dominant in high pH solutions. Spectral shifts may also reflect the changes in the conformations of the humic molecules at different pHs which could cause changes in the exposure of functional groups to the solvent, as already discussed.

4.4.2. Metal and inorganic ion complexing

Fluorescence intensity is affected by the presence of metallic ions. Iron quenches fluorescence, and aluminium may either quench or increase intensity, depending on the types of bonds it forms with organic compounds (Shotyk and Sposito, 1988, 1990; Yang et al., 1994). Increasing copper concentrations causes fluorescence to decrease (Sposito et al., 1988, Blaser and Sposito, 1987). Erich and Trusty (1997) investigated the effect of liming on fluorescence spectra. They found that liming caused chemical changes including a decreasing the amount of Al and Fe in the soil organic matter extracts, a decrease in the overall size of organic molecules and a decrease in the degree of aromatic condensation. These changes were indicated by shorter wavelengths of excitation and emission after liming of the soil, particularly in the case of HA, which appear to be preferentially flocculated as opposed to the lower molecular weight FA.

4.4.3. Soil type

Soil organic matter stability, and therefore the structure of its organic acids, depends on soil physical properties, and in particular the clay concentration. For example Körschens et al. (1998) demonstrate that soil organic matter (SOM) stability, concentration and extractability were affected by the clay composition. The stability of the organic acids is reflected in their molecular weight and thus their fluorescence properties. Clays are among the most important components of soil which affect the rate of decomposition of SOM, as organic molecules can be adsorbed by clay minerals (Zech et al., 1997). Organic matter can enter the interlayers of clay minerals and also be enclosed in micropores that cannot be entered by microorganisms, so inhibiting the rate of decomposition and SOM turnover. Meredith (1997) showed there to be a greater concentration of old SOM in a clay-rich Kenian vertisol than in a sandy Zambian oxisol. The functional groups of organic matter that mainly interact with the minerals are carboxyl groups. In a study of Andosols, Zech et al. (1994) demonstrated that highly aromatic SOM is more stable than polysaccharide-rich organic matter, hence advanced humification, resulting in high aromaticity may additionally contribute to the high stability of SOM. Therefore, the stability of organic acids is reflected in their molecular weight and structure (controlled by the rate and degree of organic matter breakdown) and so may be indicated by their fluorescence properties.

4.4.4. Climate

Climate (specifically temperature and moisture) influences the rate and degree of humification of organic matter (Swift et al., 1979) and therefore affects organic acid production and the relative proportions of HA and FA present in soil/water, etc. Consequently, it may be expected that the fluorescence properties of these acids may be useful as indicators of the climatic conditions prevailing at the time of their formation providing other factors (soil type, etc.) remain constant.

Christ and David (1996) investigated the effects of temperature and moisture on the production of dissolved organic carbon in a laboratory experiment on a spodosol from Howland, Maine. They found that higher moisture contents increased the dissolved organic carbon production rates, with the proportion of hydrophobic acids increasing more than hydrophilic acids as moisture levels were increased. This may be due to less decomposition/humification taking place and hydrophobic acids not being broken down.

Higher temperatures were shown to also increase the absolute concentrations of all DOC components, but they increased the fraction of hydrophilic acids more than the others do. It was suggested that this might be due to higher temperatures accelerating the activity of microbes in transforming undissolved carbon into hydrophilic acids, more than the activity of other DOC producing microbes. Also, higher temperatures may accelerate the transformation of hydrophobic acids into hydrophilic acids (Christ and David, 1996). Summer DOC maxima, due to a period of drier climate leading to increased humification, have been observed by Gruggenberger and Zech (1994b).

A similar relationship was found by Martin-Neto et al. (1998) who studied the effect of mean annual rainfall on humification along a temperate grassland climosequence. They used the E_4/E_6 ratio (defined as the ratio of optical absorbance at 464 nm to that at 665 nm for humic substances in aqueous solution (Stevenson, 1994)) of humic substances in the soil to characterise the progress of humification. This ratio has been correlated negatively with aromaticity and molecular weight. The more complex (less humified) the soil humus, the lower the E_4/E_6 ratio (Stevenson, 1994). A negative correlation was found between this ratio and mean annual rainfall, (Table 1)

Table 1
Table showing the characteristics of soil types under various conditions from a study by Martin-Neto et al. (1998). The E_4/E_6 ratio is analogous to the fluorescence characteristics of the humic acids investigated, as both have been negatively correlated with increasing aromaticity and molecular weight of soil humus. Rainfall may be negatively correlated with humification levels in soils. Therefore, as rainfall increases (i.e. humification decreasing), the E_4/E_6 ratio (or fluorescence) decreases. (correlation between rainfall and E_4/E_6 ratio (R^2) = 0.7)

Location	Name	Depth	Annual rainfall (mm)	Clay(%)	pH	Organic carbon (%)	C_{HA}/C_{FA}	E_4/E_6 Ratio
38°28′S 64°53′W	Cuchilloco sandy loam	0–33	365	10.7	7.5	0.38	0.43	4.93
38°33′S 63°40′W	Las Gaviotas sand	0–23	379	4.3	6.5	0.95	2.1	3.77
38°33′S 63°20′W	Chapalco sandy loam	0–26	400	11.0	6.5	1.84	3.8	3.91
38°32′S 62°36′W	Neva Roma sandy clay loam	0–11	490	20.0	6.5	2.56	4.6	2.87
38°16′S 60°33′W	Trés Arroyos Loam	0–24	739	21.1	6.5	3.86	2.5	2.82
38°23′S 59°57′W	San Cayetano Sandy Clay loam	0–26	767	20.4	6.5	2.84	2.5	2.87
38°32′S 58°57′W	Necochea Sandy Loam	0–20	916	21.3	6.5	3.17	3.9	2.36

which implies that as rainfall increases, humification decreases on temperate grassland soils.

Baker et al. (1997) and Baker and Genty (1999) found that material flushed into groundwater in the autumn includes a greater proportion of soil breakdown products from the preceding spring and summer, which may have been undergoing decomposition for up to 9 months, whereas the winter flush contained a greater proportion of poorly humified material which accumulated during the winter climate. Similarly, Visser (1984) showed an increase in FA (indicated by the E_4/E_6 ratio) during the period May–September, which may be due to an increase in the degree of humification of material during the summer season.

This relationship between humification and climate is found to hold true for other climatic regimes, as shown by Martin et al. (1998) in a study of humic substances in Himalayan forest soils. It was found that higher annual rainfall at higher altitudes appears to reduce lignin degradation and the formation of high molecular weight condensed aromatic structures. Humic substances of higher, wetter, cooler altitudes were less humified than those extracted from lower, warmer, drier sites where greater oxidation and increased humification was seen. This was due to the higher temperatures stimulating the mineralisation of polysaccharides and lignin and the formation of non-lignin aromatics rich in carboxylic groups and poor in phenolic and methoxyl groups, as observed earlier by Zech et al. (1992).

On the basis of these studies and organic acid fluorescence characteristics, it may be concluded that soil organic matter is in a state of dynamic equilibrium with local climatic and environmental conditions. In general, (a) colder, wetter conditions cause decreased humification and a greater proportion of more complex HA in the soil, such conditions therefore being indicated by fluorescence at higher wavelengths, characteristic of HA, and (b) a warmer, drier climate leads to increased organic matter breakdown and the formation of simpler products further along the decay chain, including FA. Such conditions therefore ultimately lead to fluorescence at shorter wavelengths.

5. Organic acids in speleothems

5.1. Origin

The organic matter present in natural waters has an origin that is partly allochthonous — leached or eroded from the soil of the drainage area — and partly autochtonous — derived from the cellular constituents and exudates of indigenous aquatic organisms (Cheng, 1977). Organic matter is flushed from the soil and plant at times of greater runoff and during storm events (Visser, 1984; Baker et al., 1997; Baker and Genty, 1999). This flush carries organic substances into the saturated zone of groundwater or into streams and rivers by surface runoff

and is a major contributor of organic carbon to groundwater and surface water.

Soil carbon turnover times range from < 20 yr for modern fractions to > 100 yr for old carbon (Milton and Kramer, 1998). Genty et al. (1998) model a 1–10 yr lag between soil carbon production and transport to a stalagmite using bomb ^{14}C as a marker. This lag may be due to a mix of soil carbon of different ages, which vary depending on the presence of grassland soil (slow turnover) or forest soil (faster turnover) and whether the organic matter decomposition is slow (dead roots, leaf veins, twigs) or fast (litter, root exudates) (Dörr and Munnich, 1986). It may also be due to mixing of groundwater of different ages (Genty et al., 1998).

This organic-rich water may eventually pass through the soil and epikarst zone and enter a cave where the organic acids are coprecipitated or adsorbed onto the surfaces of the speleothems which are being formed (White, 1981). Fig. 4 shows the pathway involved and controlling factors, from initial humification through groundwater transport and mixing to the final organic acid signal being preserved in the calcite.

FA, being hydrophilic, are readily soluble and thus may be expected to enter speleothem feedwaters. The more hydrophobic HA may not be as readily transported from the soil zone (Baker et al., 1998). Greater concentrations of soil organic matter are transported onto speleothems by groundwater at times of high winter discharge in temperate climates (Baker et al., 1993a, 1997).

Organic molecules are preferentially adsorbed onto the surface of a growing calcite crystal, rather than being taken up as a fluid inclusion, which would be suggested by a "Raman" Peak in fluorescence spectra (Ramseyer et al., 1997). Carboxyl groups are the most important acidic functional groups in organic acids, and the pKa value is typically in the range 3.5–4.5 (Perdue, 1985). The pH values of most natural waters fall in the range 4–8 (Krauskoph, 1979) meaning that in most aquatic systems the organic acids dissociate and have a negative charge. The calcite surface has a zero point charge of 9 (Parks, 1967) which means that in virtually all aquatic systems the calcite surface has a net positive charge. Therefore, calcite is an effective adsorbent for dissolved humic materials in natural waters. Lauritzen et al. (1986) found strong positive correlations between both HA and FA and inorganic detrital content in speleothems. This may reflect that HA and FA may also be transported in solid phase, adsorbed onto detrital components, like vadose silt.

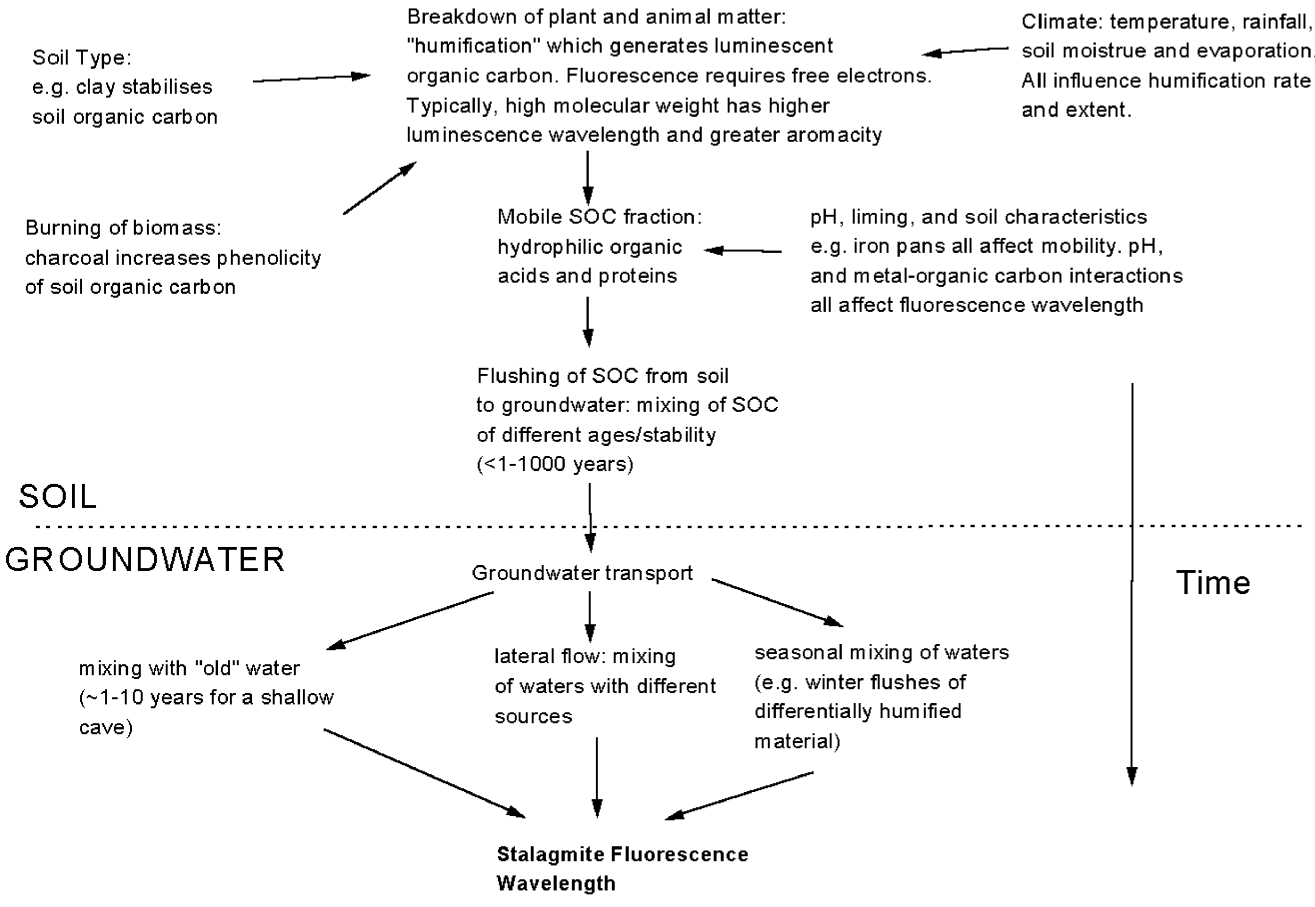


Fig. 4. Pathway of dissolved organic matter in karst system.

5.2. Controls on speleothem organic acid fluorescence

There are numerous factors that control organic acid fluorescence, as discussed in Section 4. However, in the case of speleothems, some effects that could be expected to influence fluorescence may actually be considered to be negligible.

The effect of metal ion interactions with HA and FA may be considered irrelevant due to the organic matter being bound with the calcium that is in solution (Baker et al., 1996a). In most karst groundwaters, for example, calcium and magnesium ions may be present in 40–400 ppm concentrations and therefore dominate any organic acid–metal ion interactions. Other metal ion concentrations that have significant quenching effects (Fe, Pb, Mn) are typically low (< 0.1 ppm) in karst groundwaters and therefore unlikely to have a significant effect on the fluorescence properties of organic matter. Where they do occur in significant quantities, however, they compete with the binding sites used by the calcium and magnesium ions and are generally more reactive, and will thus exert a quenching effect.

Increased porosity of the speleothem calcite has no effect on the wavelength of the emitted light but as it generates more internal reflection of the exciting light at the calcite surface, as porosity increases fluorescence also increases.

HA and FA may be adsorbed onto the walls of the fissures feeding a speleothem as a calcite is a good adsorbant for dissolved organic matter due to its zero point charge, as discussed earlier. Therefore, a decrease in speleothem fluorescence intensity with depth may be expected due to removal of HA and FA out of the groundwater by adsorption. However, organic matter flux variations with depth can be ignored if careful sampling is undertaken from a similar depth in the aquifer.

This leaves soil cover, vegetation change and climate as the factors exerting the strongest influence over speleothem fluorescence. For example, Baker and Genty (1999) have shown that organic matter in speleothems derived from poorly humified peat soils has a higher wavelength of excitation and emission and lower fluorescence intensities than sites overlain by more humified mineral soils such as under deciduous woodland. Therefore, it may be expected that the fluorescence signal preserved in speleothem samples may be a useful record of palaeoenvironmental change.

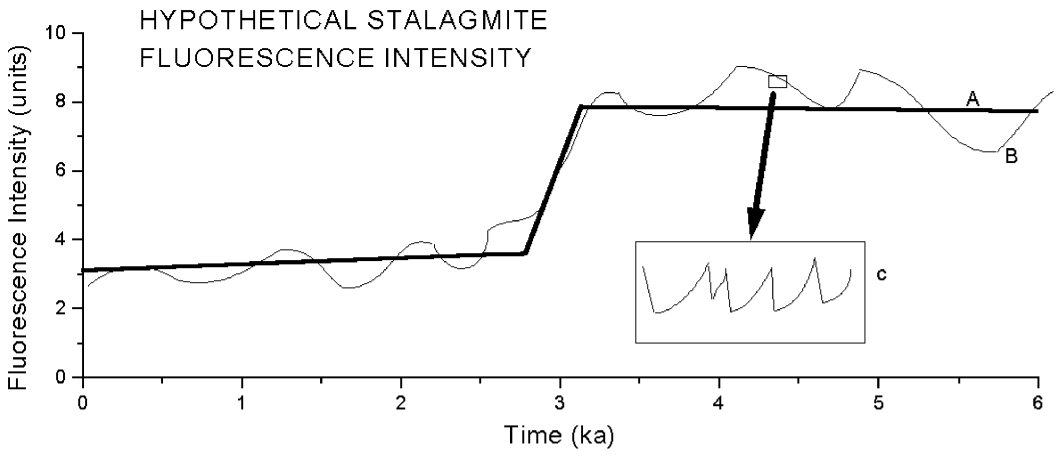
5.3. Analysis

Early studies into the fluorescence of speleothems utilised UV laser technology, with a fixed excitation wavelength (Shopov et al., 1994; Baker et al., 1996a), or UV microscope technology (Baker et al., 1993b). Lasers with a UV source, such as HeCd at 325 nm, were utilised to investigate fluorescence intensity variations. However, fluorescence emission wavelength data demonstrated a broad peak and could discriminate between samples. Laser technology allowed a very high resolution analysis, with a spot size of < 10 μm. However, this also brought inherent problems, as this is the scale of crystal defects on the speleothem calcite surface. Unless a sample comprises a non-porous structure that permits a high-quality polish, fluorescence intensity variations record variations in calcite porosity and not total fluorescent carbon fraction. Working with thin sections or transmitted light helps lessen this problem, but not eliminate it completely.

UV microscope analysis provides a good tool for investigating annual fluorescence intensity variations (Baker et al., 1993b; Tan et al., 1999), although, wavelength data is not obtainable. When combined with image analysis it provides a powerful tool; with a 1 mm field of view, for example, between 10 and 100 laminations may be visible given a typical stalagmite growth rate of 10–1000 μm per year (Dreybrodt, 1988). However, the generation of long annually laminated series requires the tiling of many individual UV images and thus care is need not to incorporate systematic errors when trying to construct a long-time series of fluorescence intensity. Successful results include the publishing of a 1100 yr series annual laminae from a Chinese stalagmite (Tan et al., 1997; Qin et al., 1998) and the correlation of fluorescent laminae structures with rainfall variations for two Derbyshire stalagmites for the last 80 yr (Baker et al., 1999a). Stalagmite fluorescence wavelength variations also exhibit potential as a palaeoclimate proxy, especially with the development of EEM spectrophotometry (discussed earlier in Section 3.3).

5.4. Palaeoenvironmental applications

Fluorescence intensity variations in speleothems have been investigated for the last 5–10 yr (Baker et al., 1993b, 1996a; Shopov et al., 1994) and permit a variety of data on climate to be obtained, on various timescales, as shown in Fig. 5. Over the sub-annual to annual period, research has suggested that high-resolution climate, and in particular palaeoprecipitation proxies, can be obtained (Tan et al., 1997; Baker et al., 1999b). Over longer time periods, it is possible to obtain climatic series but their interpretation is confounded if variations in stalagmite porosity occur. When samples are particularly pure however, intensity variations may yield results. For example, spectral analysis of the intensity data presented in Baker et al. (1996a) for stalagmite SU-80-11, from NW Scotland, using the software SPECTRUM (Schulz and Stattegger, 1997) yields dominant spectral frequencies of 207 and 95 yr, similar to that observed in the overlying peat and in stalagmite fluorescence wavelength variations form other samples from the same site (Baker and Genty, 1999).



Timescale	% Variation in Fluorescence Intensity	Cause
A 10^3 yr	x1.5 to x4	Palaeovegetation Change Calcite Porosity Variations Change in growth rate
B 10^1-10^2 yr	x1.1 to x1.3	Climate or Vegetation Change Calcite Porosity Variations Change in Growth Rate
C subannual to annual	<x1.1	Seasonal hydrological variations Seasonal Porosity Variations

Fig. 5. Hypothetical stalagmite fluorescence intensity showing the various timescales over which fluorescence intensity can change, the percentage variation involved and some possible causes.

Variations in the wavelength of stalagmite fluorescence have also been shown to contain a Late Quaternary signal of environmental change. Table 2 demonstrates the contemporary spatial variability of fluorescence wavelengths from two cave sites in the UK. The first, Brown's Folly Mine, Wiltshire, is overlain by secondary woodland and a brown rendzina soil cover and the stalagmites are all less than 140 yr old and within a 20 m^2 area and at the same depth. Despite differences in drip hydrology, all samples are indistinguishable in fluorescence wavelength properties, demonstrating that variations in hydrology or fissure size are not limiting the organic acid properties. The second site, Stump Cross Caverns, Yorkshire, is overlain by till covered by gley soils and patchy thin peat. Samples taken from the top of actively forming stalagmites at the site demonstrate a greater spatial variability, representing variations in the overlying soil cover. Samples from Raistick's Cavern have high wavelengths and are overlain by thin peats that have formed over relatively impermeable till. Because till cover is patchy, at other locations the soil is well drained and brown earth soils have formed, stalagmites in the cave underneath this soil cover have a low fluorescence wavelength. Because of the variability of soil cover, Stump Cross Caverns have a higher and more variable mean fluorescence emission wavelength (412.8 $\pm$ 7.4 nm) than that of the samples from Brown's Folly Mine (individual sample means of 406.5–411.9 nm).

When comparing spatial variability of fluorescence wavelength variations to temporal variability (such as that of Vil-Stm1 reported in Baker et al. (1998) and the stalagmite series presented in Table 2) results suggest that spatial variations at a site may be of similar proportions to temporal variations, at least over the decadal to centennial time periods. Therefore, the analysis of individual stalagmite time-series data must primarily consider the relative trends in fluorescence wavelength, rather than absolute values. However, it must also be remembered that time-series data is time averaged due to the present limitations in fibre-optic technology, with a fibre-optic spot size of 1–2 mm fluorescing a time average of 1–200 yr of stalagmite growth. In order to assess the actual range of fluorescence variations that occur on an annual basis, a stalagmite from Poole's Cavern has been analysed. Some stalagmites from this cave are formed by the reaction of calcium hydroxide with carbon dioxide and thus have a faster growth rate than typical speleothems (1–8 mm yr^{-1}). This site is overlain by secondary

Table 2
Fluorescence intensity and wavelength variations from speleothems from Stump Cross Caverns, Yorkshire and Brown's Folly Mine, Wiltshire. (a) Paired stalagmite and dripwater fluorescence dripwater discharge for the period 1997–1998. (b) Stalagmite fluorescence wavelength data for contemporary samples at Stump Cross Caverns. 150 m separates the groups of samples; ID are the same as that reported in Baker et al. (1997)

Station	Dripwater discharge drips/s	Stalagmite emission wavelength (nm)	Dripwater emission wavelength 1996–97 (nm)
(a) *Brown's Folly Mine, Wiltshire*			
B	0.245 ± 0.638	408.0 ± 1.5	406.7 ± 4.6
DO	0.016 ± 0.004	407.0 ± 1.4	407.5 ± 5.0
J	0.074 ± 0.121	407.2 ± 1.5	409.9 ± 5.8
NL	0.012 ± 0.003	411.9 ± 3.9	410.3 ± 4.3
F1	0.036 ± 0.008	409.0 ± 1.8	406.8 ± 5.8
F2	0.015 ± 0.003	406.5 ± 1.0	405.0 ± 6.4
F4	0.009 ± 0.001	406.5 ± 1.6	410 ± 3.8

(b) *Stump Cross Caverns, Yorkshire*		
SC10, SC11	Jewel Box	410.0, 411.0
SC6, SC7	Policeman's Truncheon	407.0, 414.0
SC8	Sentinel	409.0
SC32, SC33	Raistrick's Cavern	414.0, 420.0, 406.0, 407.0, 433.0
SC34, SC35		
SC36		
SC30, SC31	Fourth Level	410.0, 413.0

woodland planted in 1800 AD, which remained undisturbed until the mid 1970s when it became a nature reserve and light woodland management practises were introduced. Fig. 6 presents data for the period of stalagmite deposition for sample PC-97-1 (AD 1927–1996); the inset shows the relationship between fluorescence wavelength and intensity over an annual cycle. The graph shows that the fluorescence wavelength decreases in the high fluorescence intensity autumn flushes of soil organic acids; suggesting that this flush comprises a greater proportion of low molecular weight organic material that has been produced in the previous summer. This results agrees with observations that CO_2 produced in summer is more likely to be from the "young" (< 1 yr) carbon component, whereas the CO_2 produced in winter is more likely to be "old" (Dörr and Munnich, 1986). A low molecular weight organic acid flush has also been observed in river waters (Visser, 1984). Over the whole time series, one can observe that seasonal fluorescence wavelength variations can be considerable (from 5 to 15 nm), suggesting that fibre-optic spot size is limiting the palaeoenvironmental signal obtainable from normal growth rate speleothems. In addition, the time-series suggests that the high fluorescence intensity (autumn flush) and low fluorescence intensity (base-flow) components have different responses over time. Although the high-intensity (autumn and winter) fluorescence has maintained low wavelength values of between 410 and 417 nm throughout the growth period, the low-intensity (summer) fluorescence has demonstrated an increase from a mean of ca 417–427 nm. This has occurred around a transition of AD 1975–1985. Given the high proportion of this fluorescent material that may have derived from an "old" carbon source, this transition might be a lagged one, and reflect a slow response of the soil carbon to climate and environmental changes (Genty et al., 1998). A climatic cause of this fluorescence increase is hard to invoke, however, as no significant climate changes have occurred at the site. However, environmental changes are more likely, as the overlying woodland has lost 30% of its trees to Dutch Elm Disease over the 1970s and 1980s, and some trees to storms in the late 1980s and 1990, directly over the stalagmite a mature elm and beech tree were lost to disease and storm damage, respectively, and might account for the change in fluorescence properties as they would have removed substantial quantities of soil water.

Fluorescence wavelength studies undertaken at a high temporal resolution therefore suggest that long term (decadal to centennial) variations in fluorescence wavelength may reflect either a change in either or both of the old and young carbon fractions. If just the young fraction responds to environmental change then there should be no lag time, but if there is a response by the older, more stable organic carbon, then the signal may be buffered and lagged. Over time periods of interest in the Quaternary, stalagmite fluorescence variations may provide useful information about soil and environmental conditions and by inference the palaeoclimate. Fig. 7 presents the already published flowstone fluorescence data for the last 140 ka from Lancaster Hole, Cumbria (Baker et al., 1995), with that of Stump Cross flowstone SC-90-4/5 from Stump Cross Caverns, Yorkshire. TIMS U–Th dates on both sequences suggest that there are periods of overlap between them (Baker et al., 1996b); fluorescence wavelength data demonstrates considerable differences. Mean fluorescence wavelength over the 140 ka time period at Stump Cross is lower than that at Lancaster Hole (413 ± 10 vs. 436 ± 19 nm, $n = 40$ and 85, respectively), despite being only 45 km apart and at a similar altitude. Where flowstone deposition may have occurred at the same time at both sites (for example at about 58 ka), the fluorescence within the Stump Cross flowstone is lower than in the Lancaster Hole sample. This difference again reflects local soil conditions, for example, today a thicker peat cover overlying relatively impermeable till occurs at Lancaster Hole. Time series trends are also considerably different, with the Stump Cross record exhibiting less variability than that at Lancaster Hole. The reason for this is unclear, but may reflect a more stable soil carbon pool above Stump Cross Caverns. For example, woodland soils have been

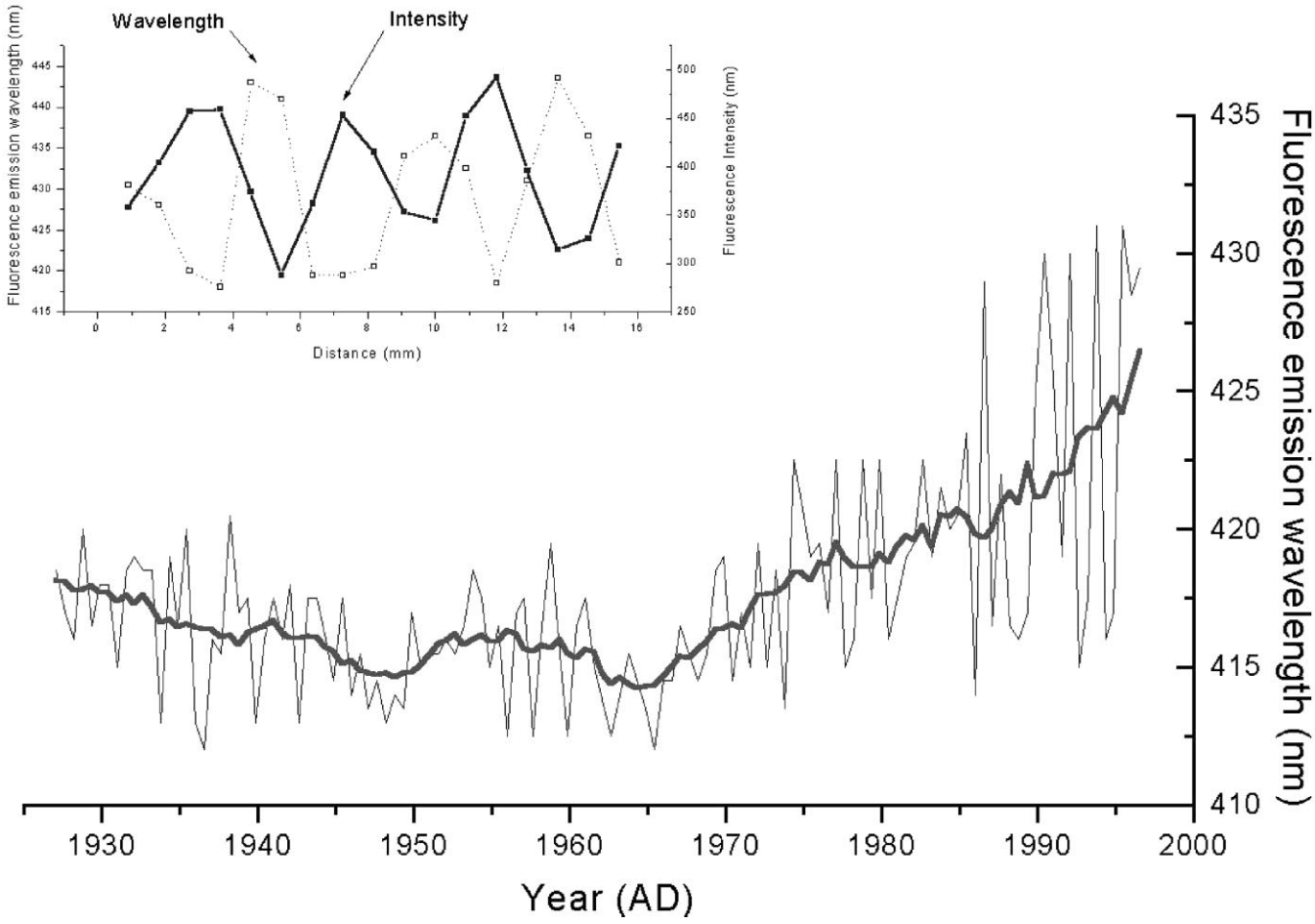


Fig. 6. Fluorescence record for a Poole's Cavern (Derbyshire) stalagmite from 1927 to 1996, with inset showing relationship between fluorescence wavelength and intensity over an annual cycle.

demonstrated to have a larger old carbon pool which is more stable than grassland soils (Genty et al., 1998); groundwaters derived from such a soil will therefore be relatively buffered. Increased clay content at Stump Cross due to local differences in glacially derived clay deposition could also explain the differences in fluorescence variability due to its stabilising effect on soil carbon. Inter site variations in variability of fluorescence signals again suggests that relative trends rather than absolute values may be more useful in interpreting fluorescence data, and that multiple samples or multi-proxy data are needed to obtain the best palaeoenvironmental interpretation of speleothem fluorescence.

6. Conclusions

Extensive research on organic acids has shown their formation, structure and fluorescence characteristics to be controlled by numerous complexly interrelated factors. Possibly the most important of these, in terms of environmental reconstruction, are soil, vegetation and climate change as they influence organic acid production (through humification). Therefore, it may be expected that organic acids would preserve a record of palaeoenvironmental conditions which may be investigated through their fluorescence properties.

Recent research has demonstrated that useful palaeoenvironmental information can be obtained from speleothem fluorescence properties. On the annual scale, fluorescence intensity variations have been demonstrated to correlate with climatic changes (Tan et al., 1997) and wavelength variations with variations in soil carbon dynamics. Over longer (decadal to centenial) timescales, fluorescence wavelength variations may reflect longer-term soil responses to climatic change; over millennial and longer timescales, where changes in soil and vegetation type are possible due to glacier advance and retreat, the speleothem fluorescence record may reflect these changes as well as a superimposed climate signal. Like all palaeoclimate proxies contained within speleothems, the fluorescence record is one which is a complex environmental and climate proxy, and will come to full fruition when combined with other proxies such as trace element, fluid inclusion and ^{18}O, pollen and fungal analyses. These are the focus of current research.

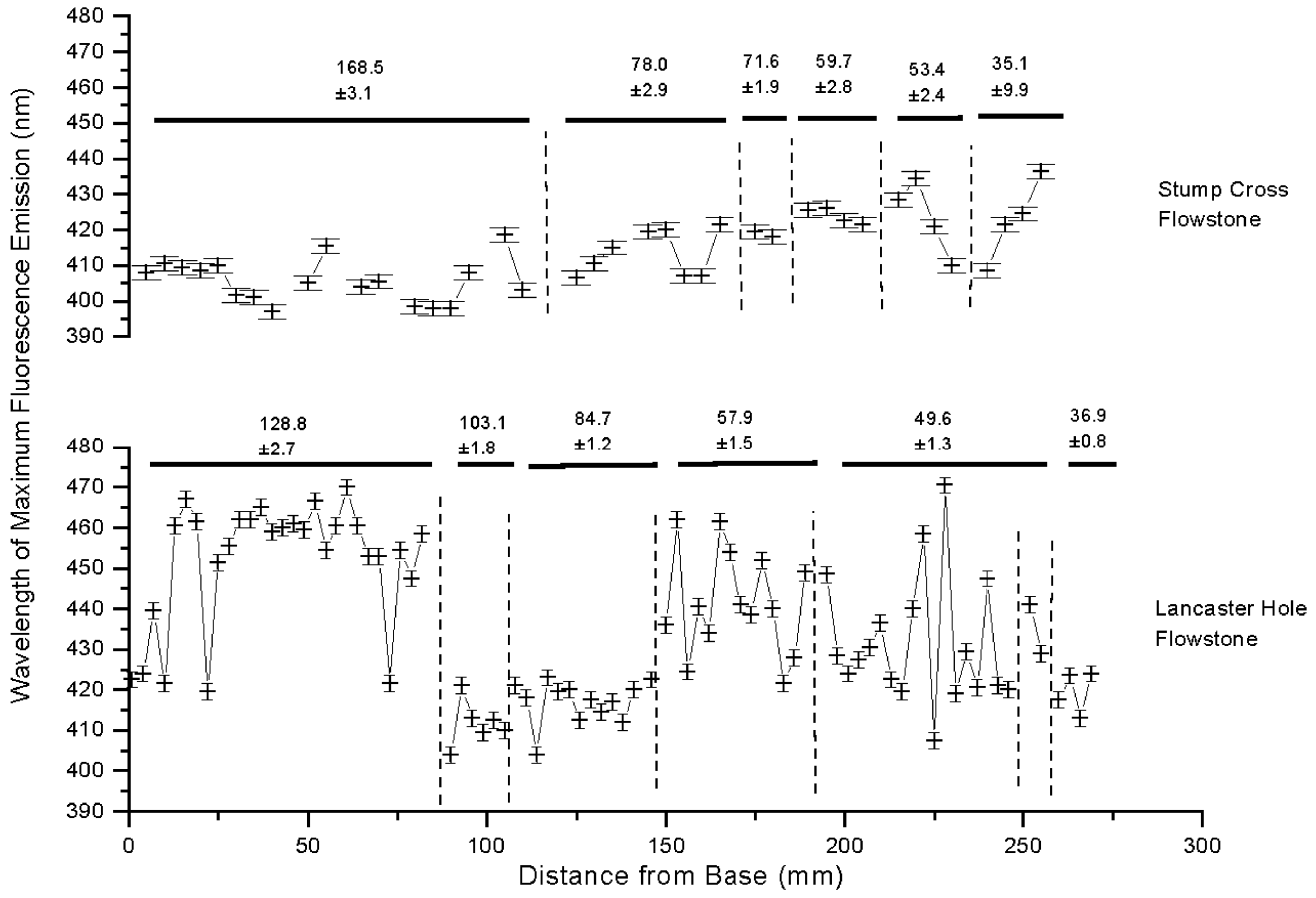


Fig. 7. Quaternary record of fluorescence from Lancaster Hole, Cumbria and Stump Cross Caverns, Yorkshire. (Dates from Baker et al., 1995, 1996b, Lancaster Hole Fluorescence record from Baker et al., 1998.)

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