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INVESTIGATIONS INTO THE FLUORESCENCE OF CALCITIC SPELEOTHEMS

By

PHILIP EDWARD VAN BEYNEN, M.A.

A Thesis

Submitted to the School of Graduate Studies

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Abstract

Variations in fluorescence in calcite speleothems may record environmental changes above a cave. It is established that many display annual banding of colour or fluorescence or both, but it is not known what produces the bands or what is the environmental significance of the variability in their fluorescence or thicknesses.

Analysis of speleothem feed waters at Marengo Cave, Indiana, found that peak fluorescence values are highest in the spring when soil organics are flushed into the cave or during the early Fall drought. These seasonal characteristics permit annual sequences of fluorescence intensity to be recognized in many speleothems. A comparison of seasonal variability in quantity and types of organic substances in soil and cave waters found that the former display little seasonality in organic concentrations compared to cave waters' peak yields in the spring and fall. Fluorescence studies revealed shorter peak excitation and emission wavelengths in the cave waters, due to differences in concentration and significant changes in the proportional organic assemblages. Precipitation affects the fluorescence in both waters, with the dry fall producing the highest fulvic acid and particulate organic matter yields. Molecular size fractionation determined that larger hydrophobic compounds are preferentially removed from the percolation water and the smaller hydrophilic compounds become the dominant fluorophore in cave drips.

Fluorescence of twelve sample speleothems (and extracts) from a wide range of geographical environments gave similar spectra, with broad emission maxima centred around 410-430 nm and two excitation maxima at approximately 255 nm and 330 nm. Trace elements are not responsible for these spectra. Organic acids, particularly fulvic acid, were the dominant fluorophore in the calcite, confirming the Marengo Cave results that show low molecular weight (<1 kD) substances were the dominant organic fraction.

Concentration of organics does not relate directly to colour hue or density in the speleothems. Annual bands were found in speleothem fluorescence/ phosphorescence signals, with strong similarities between speleothems from the same cave suggesting that the signal is generated by the overlying environment. Correlations between fluorescence/phosphorescence and standard climatic variables are very tentative at present.

Acknowledgements

This thesis was only made possible by the assistance of many people. I am deeply indebted to Derek Ford for his constructive criticism, encouragement and support throughout this process. Much is owed to Henry Schwarcz for his extreme patience while teaching me how to write and think like a scientist. I would also like to thank Wayne Rouse for his comments and help during the process of this thesis. The co-authors of the papers, Rick Bourbonierre and George Timmins, helped greatly in the analysis of this dissertation. The Marengo Cave staff were most helpful in collecting water samples over the two years of data collection, without their help this work would not have been possible. For having the opportunity of working at Marengo Cave I have to thank Gordon Smith and Gary Robertson, the owners of the cave.

I wish to thank Trish Beddows, Scott Smith and Steve Worthington for their insightful discussions. Jim Kramer was usually helpful in allowing access to his equipment, and his technician Pam Collins was wonderful in keeping the DOC analyzer in working order and demonstrating the use of his other equipment. This project would not have been possible without the funding from NSERC. Finally, I would like to thank the office staff, Darlene Watson and Jude Levett, for their help and patience during my PhD.

On a more personal note, I would like to thank the geography grads who hung out in the purple room for providing pleasant conversation during coffee and lunch. It certainly made life as a struggling PhD student all the more enjoyable. To my parents I owe a huge debt for their support and encouragement which has allowed me to get this far in my academic career. Finally, to Lynn MacLeod I owe so much, for her patience, love and understanding during this last year. Without your support, completing this thesis would have been a lot less enjoyable.

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Preface

Contributions to co-authored chapters of this thesis

Chapter 2: Humic substances in cave waters, Marengo Cave, Indiana: characterization and seasonal fluctuations. P.E. van Beynen, D.C. Ford and H.P. Schwarcz. Submitted to Geology. Nine months of data collection and DOC analysis was undertaken by Veronica Toth, and I added seven additional months of data. Marengo Cave staff collected the weekly water samples. Only the raw values were used from Ms. Toth's work. Dr Ford and Dr Schwarcz gave advice and editorial comments on the paper. I conducted all data tabulation, subsequent DOC, fluorescence, and size fractionation analysis and wrote the paper.

Chapter 3: Seasonal variability in organic substances in surface and cave waters at Marengo Cave, Indiana. P.E. van Beynen, D.C. Ford and H.P. Schwarcz. To be submitted to Cave and Karst Science. I designed the experiment, conducted the data analysis, tabulation and wrote the paper. Dr Ford and Dr Schwarcz gave advice and editorial comments on the paper.

Chapter 4: Causes of Colour and Fluorescence in Speleothems. P.E. van Beynen, D.C. Ford, R.A. Bourbonniere and H.P.Schwarcz. To be submitted to Chemical Geology. I designed the experiment, undertook the fluorescence, absorbance and size fractionation analysis, and wrote the paper. Dr Bourbonniere carried out the DOC analysis and the fulvic acid fractionations. Dr Ford and Dr Schwarcz gave advice and editorial comments on the paper.

Chapter 5: The Presence and Environmental Significance of Fluorescence in Annual Laminae of Modern Speleothems. P.E. van Beynen, G. Timmins, D.C. Ford and H.P. Schwarcz. To be submitted to Cave and Karst Science. I designed the experiment, collected the data, conducted the analysis and wrote the paper. George Timmins provide technical advice on the use of the laser Raman spectrophometer. Dr Ford and Dr Schwarcz gave advice and editorial comments on the paper.

Chapter 1: Overview of Previous Work on Speleothems and Structure of Thesis

"Speleothem" is a general term derived from the Greek words for "cave" (speleo) and "made in" (them). In common usage for the past 25 years, it is taken to mean calcite and aragonite precipitates such as stalagmites, stalactites and flowstone, usually in limestone caves. Most speleothems of these types form in the vadose groundwater zone (i.e. above the watertable) from subsoil seepage that has been enriched in soil CO_2 and consequently become saturated with $CaCO_3$ from dissolution of limestone at the base of the soil. During passage through the soil, the water may also entrain humic substances produced by organic decay processes there. In an airfilled cave CO2 partial pressure is usually lower than in the soil, permitting CO_2 degassing from the seepage that causes supersaturation with $CaCO_3$ and consequent precipitation of calcite or aragonite. A proportion of any humic substances, trace elements in solution, etc, carried by the water are trapped in the precipitating mineral and become part of the speleothem.

Cut normal to the growth axis, most calcite and aragonite speleothems display growth layering at various scales. Under magnification even tiny couplets of light and dark calcite may be apparent. Speleothems are readily analyzed for the carbon and oxygen isotope ratios and Hendy and Wilson (1968) showed that if the speleothem was deposited in thermodynamic equilibrium, then inferences could be made about past climates. Schwarcz et al (1976) determined that analysis of the D/H ratio in fluid inclusions in speleothems can provide accurate estimates of paleotemperature change. This relies on the relationship which defines the meteoric water line:

$$\delta D_{inclusion} = 8\delta^{18}O_w + 10$$

Using fluid inclusions in speleothems, Harmon et al. (1978) suggested a 8°C difference

between glacial and interglacial periods for North America.

^{14}C and U-series dating (Broecker et al. 1960, Gascoyne 1992, Li et al 1989) are used to produced accurate timelines for the precipitation of the calcite and, hence, any paleoclimate estimates. The ^{14}C dating technique uses decaying atmospheric ^{14}C incorporated within the speleothem to estimate ages: however, it is adversely affected by the incorporation of old carbon from the dissolved bedrock above the cave and limited to the last 40,000 years or so. U-series methods can date much older speleothems (to ≈600,000 years B.P.) using the $^{230}Th/^{234}U$ ratio and, in exceptional cases, to the oldest limestones using U/Pb ratios. Studies by Hennig et al. (1983) showed that the distribution of the growth phases of speleothems in temperate Europe and North America matched precisely with the glacial/interglacial cycles. Using U-series to date oxygen isotopic changes in vein calcite from Devils Hole, Nevada, Winograd et al. (1992) have produced a 500,000 year record of climate cycles during the Quaternary which calls into question the timing of the last interglacial and challenges the standard "Milankovitch model" for the causes of the Earth's ice ages.

Broecker et al. (1960) used ^{14}C dating to investigate the resolution of the fine laminae that they observed in some sectioned speleothems and showed that they were probably annual bands. Colour variations in the calcite are what appeared to delineate these laminae. Gascoyne (1977) suggested that such colour may be due to the presence of organic compounds, rather than to trace elements as had been supposed by earlier workers.

Fluorescence in speleothems has been reported since the early use of powerful flash powders to photograph them in completely dark caves. Gilson and MacCarthney (1956) were the first to suggest that it may be caused by organic compounds coprecipitated in the calcite. White (1984) reported the same conclusion, stating it was probably fulvic acid

which generated the fluorescence. Lauritzen et al. (1986) established that organic compounds can be co-precipitated in calcite and found that fulvic acid was the principal compound (and, presumptively, fluorophore) in extracts from sample speleothems. Shopov et al. (1987) returned to the study of the annual banding phenomenon using laser induced luminescence microscopy and confirmed that many of these microscopic structures were probably annual. Baker et al. (1993) also found annual banding in portions of certain speleothems using fluorescence measurements.

The use of fluorescence measurements to determine the presence of annual bands has become more prominent in recent years. Shopov et al. (1994) showed strong correlations between sunspot activity and atmospheric ^{14}C with fluorescence-delineated annual laminae widths. Genty and Quinif (1996) examined one speleothem from a tunnel in Belgium and found that its laminae were annual and that their widths correlated with water excess above the tunnel. They also discovered that dark calcite was deposited during one year with particularly high precipitation. Genty et al. (1997) used luminescence intensities to define annual band widths in one Belgian speleothem but found no significant correlations between band width and annual precipitation, water surplus or temperature except for the year, 1975, when band width matched a water surplus. They suggested that variations in Ca ion loadings or complex links between precipitation and cave drip rates may better explain the laminae variations than simple statistics derived from temperature or precipitation alone.

Both theoretical and field research has been undertaken on these annual laminae. Dreybrodt and Buhmann (1987) show that temperature and water film thickness are what govern the depositional thickness of a laminus. Field research by Baker et al (1998), although confirming Dreybrodt and Buhmann's general models, found that in nature the actual mechanisms are more complex. Ca ion concentrations in drip water appears to be the

overall governing factor, which in turn is influenced by soil temperature, soil moisture, seasonality of surface climate, soil depth, vegetation type, bedrock porosity, permeability and purity, and the evolutionary path of the groundwater between open and closed system end-member conditions as it moves from the soil to the speleothem deposition site.

Research on the characteristics of organic compounds in speleothems, and in particular their role in fluorescence and annual banding, can be said to be an early stage. The purpose of this thesis is to explore some of the principal questions that remain unanswered. First, there has not been any detailed study of the organic content of drip waters depositing calcite to determine whether it is variations in them throughout a year that lead to the production of an annual band structure. Second, there has not been a study of relationships between conditions in the soil and in the drip waters beneath it. Third, it has not been conclusively proven that trace elements are not the dominant source of fluorescence; if organics are dominant, the sources within them remain to be determined in detail and their relation to the colour hues and densities of the calcite. Finally, much more work is required to link weather, climate and climate change to any organic fluorescence in speleothems.

When it was begun, the thesis was intended to contain four substantive papers plus an introduction and conclusion to synthesize the work. Due to delay in obtaining satisfactorily complete results, the final three papers have not as yet been submitted to journals. In some areas more work is still needed, especially for the fourth paper. In the thesis sequence, chapters 2-5, follow that of the four papers originally designed.

Chapter 2 investigates seasonality in cave drip water fluorescence. It is hypothesized that conditions at a certain part of the year may produce higher yields of organic compounds reaching the cave in the drip waters thereby producing darker calcite and consequently affecting fluorescence, with the rest of the year yielding lower

concentrations and consequently lighter calcite. Field studies were conducted at Marengo Cave, Indiana. The temporal and spatial variations in drips from speleothems in this rather simple, homogeneous natural site are the focus of this chapter. One aim is to determine if different parts of the cave show the same results throughout the year. This has important implications for paleoenvironmental research because if the same signal does appear in different parts of the cave, then that must be produced by influences outside the cave and therefore a regional interpretation may be made if one progresses to the longer time frames recorded in the speleothems. To a lesser extent, determination of the dominant fluorophore present in the drip waters is undertaken to determine whether these are fulvic acids as claimed by Lauritzen et al (1986).

Although chapter 2 looks at the seasonality in organics in the drip waters entering a cave, it does not ascertain if there is a connection between them and any seasonal changes in organic substance assemblages and concentrations in the soil waters that feed the cave. Chapter 3 is the first attempt to ascertain if what is happening at the surface is reflected in the cave, and if so, how this may affect the fluorescence of the cave waters and consequently the speleothems. Both changes in the quantity and type of organics are investigated.

The nature and causes of fluorescence in calcite speleothems is the central topic of chapter 4. This is the most detailed attack thus far on the nature and causes of fluorescence in speleothems, following up the work of Lauritzen et al. (1986) in which they found fulvic acid to be the dominant fluorophore in speleothems, and that of White and Brennan (1989) who characterized the fluorescence of many solid speleothems. However, the proportional contributions of humic acids, particulate organic carbon and of trace elements to speleothem fluorescence is unknown. How the concentrations of organic compounds influence the intensity of fluorescence has yet to be ascertained. If both organic acids and trace elements are abundant, the dominant fluorophore can be determined by measuring

which trace elements and organic substances fluoresce at the same wavelength as the calcite. Where present, an annual band is usually a couplet of lighter and darker calcite. Unfortunately, due to the microscopic thickness of reported bands (e.g. varying between 0.05 - 1.56 mm in the work of Baker et al, 1998), it is not possible to carry out the chemical analysis suggested above to determine the differences between light and dark calcite. Larger but still uniform calcites are required to study light-dark colour variation in detail. Twelve speleothems were chosen from the collection at McMaster University which display the widest range of colour hue and density (from colourless-translucent to very dark brown and opaque) and from a wide range of environments (alpine to hot desert margin via temperate and tropical grasslands and forests). These were systematically analyzed for trace elements, POC, HA and FA and the latter were further fractionated into the hydrophilic and hydrophobic fractions. Finally, in keeping with the bulk nature of this work, these chemical differences in the sample speleothems are related to the environment from which they came. It was hoped that some conclusions can be reached about how the environment influences the fluorescence through the organic assemblages or trace elements in the speleothem.

Although Chapters 2 and 3 investigate how climate or the environment affect modern cave drip waters and how this could potentially produce annual bands, Chapter 5 investigates actual sequences of annual bands in speleothems. It is thought that seasonality is important in production of annual laminae, therefore two sites with strong seasonality were selected, Minneapolis, Minnesota, and Aggtelek, Hungary. Pairs of adjoining samples from these locations were selected whose maximum ages can be determined rather precisely from historical data. The fluorescence of these speleothems is used to define laminae that are expected to be annual and to test the count of years from those laminae against the historically determined timelimits. Secondly, the characteristics of the laminae are then compared to standard measured climate statistics from nearby meteorological stations.

With the information from this research, it is hoped to attain a better understanding of the production of annual laminae in speleothems and how or even if their fluorescence is controlled by climate. With this understanding, future interpretation of the fluorescence of Holocene and Quaternary speleothems can be forwarded pertaining to climate change. With the high resolution banding present in the speleothem, accurately timed changes in climate can be given for regions with karst. This may allow the testing of GCM predictions of past climates, and for their fine tuning.

Chapter 2: Humic Substances in Cave Waters, Marengo Cave, Indiana: characterization and seasonal fluctuations

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Submitted to Geology

Abstract

Analysis of speleothem feed waters at Marengo Cave, Indiana, revealed that fluorescence generated from these waters is caused predominantly by low molecular weight (<1,000 MWCO) organic substances similar to fulvic acid. Fluorescence spectra from both unfiltered and ultrafiltered cave water show peak emissions from 370nm to 430nm, with the longer wavelengths being similar to those of the Suwannee River standard fulvic acid. Peak fluorescence values coincide with increasing temperatures above ground,being highest in the Spring when thaw of the soil releases organics to be flushed into the cave. This seasonal characteristic permits an annual sequence of fluorescence intensity to be recognized in many speleothems. Inter-site variability in seepage water flow rates within the cave, as detected by dye tracing and by lag times in the relationships between feedwater fluorescence and external temperature, reveals the nature of flow paths from the soil to the speleothem in many instances.

2.1 Introduction

It is established that much of the colour in calcite speleothems is due to the presence of organic substances (Gascoyne 1977, Lauritzen et al. 1986, White 1984). Organic acids are efficient complexing agents (Scudeler Baccelle and Nardi, 1991); they may bind with the $CaCO_3$ in their capacity as nucleating agents and be coprecipitated as integral parts of speleothems. As many organics are also strong fluorophores (Schnitzer 1978, Larson and

Rockwell 1980, Miano 1988, Bloom and Leenheer 1989, Senesi 1990),they may be responsible for much of the UV-stimulated fluorescence that is generated in speleothems (White 1984, 1986, Shopov 1987, White and Brennan 1989, Shopov et al. 1994). Speleothems often contain laminae parallel to their growth axis, some of which have been shown to be annual (Shopov 1987, Baker et al., 1993). These tend to comprise a dark dense layer alternating with a lighter, more porous layer of calcite, resembling early- and latewood in tree rings. Such variability in fluorescence may be attributable to seasonal fluctuations in drip water organic concentrations. While investigating this phenomenon at a Belgian site, Genty and Quinif (1996) found that the winter period coincided with the dark dense calcite.

The fundamental work to identify the principal organics as precisely as possible in speleothems and their feedwaters remains to be undertaken. The question of the commonality of seasonal signals in different speleothems in a given cave also remains open. In many cases there are great ranges in the seasonal drip rates of different feedwaters. Some display periods of rapid flow or of cessation while others nearby remain constant. Fluctuations in flow rates may lead to varying dilution of organics in the drip water and consequently in the speleothem crystals. Also, the dissolved $CaCO_3$ load may vary seasonally, which leads to seasonal change of growth rates in speleothems (Gams 1965, Pitty 1968).

The objectives of our study were to:

a) Characterize the fluorescing compounds in the feedwater.

b) Relate the fluorescence to other fundamental features of the feedwaters such as dissolved inorganic and organic carbon, solute loads and drip rates.

c) Ascertain if there were any seasonal and shorter term weather effects on these properties.

2.2 Marengo Cave

Marengo Cave, southern Indiana (latitude 38'22"N, longitude 86'20"W) was selected as it ideally suits the above objectives. Typical of limestone caves of the midwest, Marengo Cave is a 500m segment of an abandoned river passage that has been truncated at both ends by natural breakdown. It is approximately 15 metres above the modern Blue River (Figure 2.1) and contained within the Ste. Genevieve and Paoli formations of the Blue River Group of the Middle Mississippian (Powell, 1992). These are thick to massively bedded, near-horizontal limestones capped by a sandstone, the West Baden Formation.

Thickness of bedrocks above the cave varies between 10 and 30m, with the shallower depths being at either end of it. Modern speleothem distribution is related to this; the lesser the overburden, the greater the density of decoration. Drip rates of speleothem feedwaters are also spatially variable, ranging from some that are extremely responsive to precipitation and frequently dry up (e.g. Site A - The Crock) to apparently constant sites (e.g. Site C - Tomtom). Sources and rates of feedwater flow towards the two principal areas of deposition at the ends of the cave were investigated by dye tracing (Figure 2.1). Flow paths to modern speleothems were determined at the Crock and the Natural Entrance, where dyes were recovered 4.5 hours and 45 minutes respectively after time of injection. The mean measured groundwater flow rate from the sink point to the Crock was 15m / hour. Two traces at the southern end of the cave (where dye was injected into sinkholes) failed; it is possible the groundwaters tested there were draining underneath the explored cave into Blue River springs.

Overlying the bedrocks are thin soils consisting of residues from limestone and clastics, plus alluvial clays, sands, silts and gravels ranging in age from Tertiary to recent (Powell, 1992). Above this stands a deciduous forest that, although cleared once in the last century, has recovered and displays little human interference today. Continuous climate

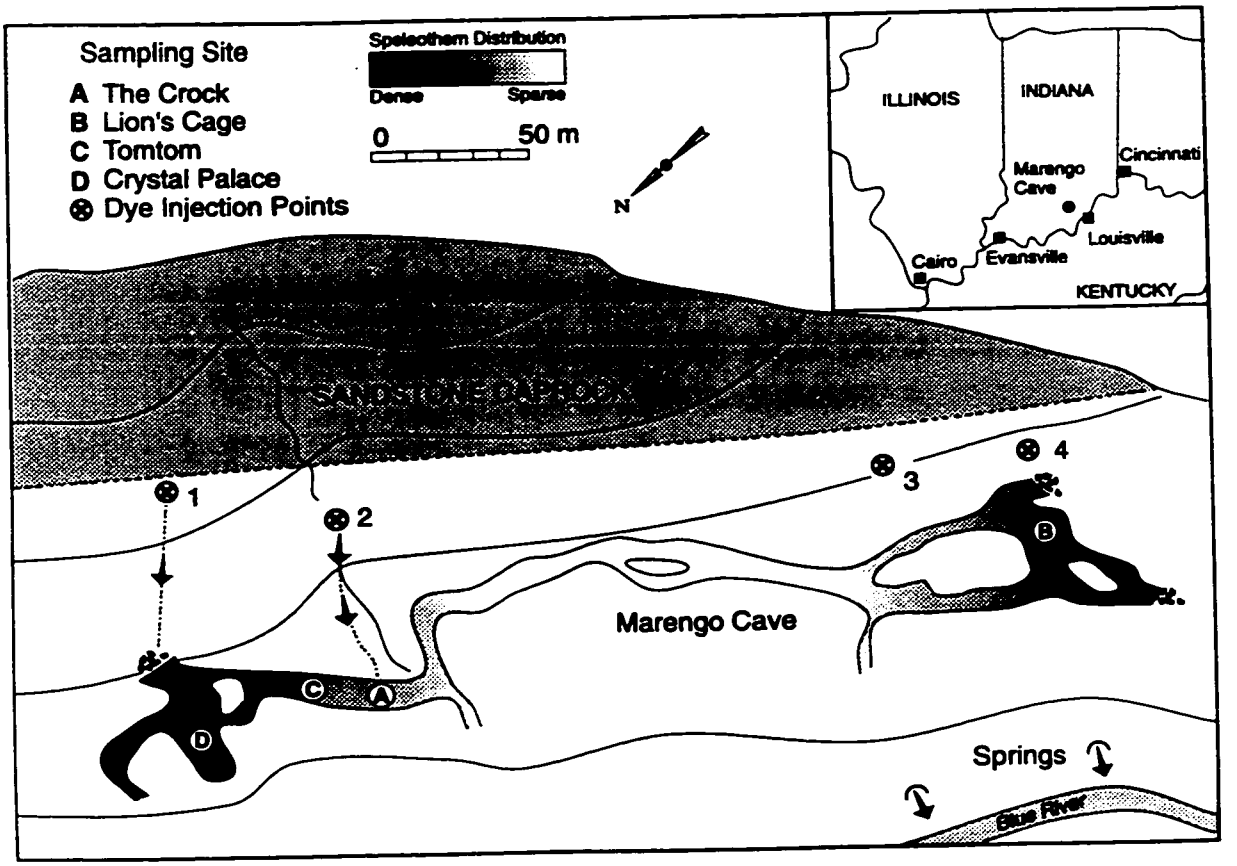

Figure 2.1. Marengo Cave, Southern Indiana, showing the locations of the four principal speleothem drip water sampling sites and the dye traces.

data were obtained from a rain gauge at the site and from the meterological station at Louisville Airport, 50km to the east. 30-year mean annual temperature equals 13.4°C and mean annual precipitation is 1110 mm at Louisville. Potential evapotranspiration for this region is approximately 900mm while actual evapotranspiration is probably 800 mm.

2.3 Field Sampling Design; Analytical Procedures

Drip waters were collected at Marengo Cave between March 1995 and May 1996 in order to obtain one full year's record of the vadose seepage. The program began with repeated sampling at 12 different sites throughout the cave to establish the range of drip rates and water quality variables. Sampling was then reduced to four principal sites encompassing that range (Figure 2.1). As named by the tour guides, they were: (A) the Crock (highly variable flow and responsive to precipitation), (B) Lion's Cage (variable), (C) Tomtom (little variation) and (D) Crystal Palace (nearly constant in all variables). There is net calcite deposition at each site today. For six weeks in May-June 1995 there were daily measurements of water temperature, conductivity, pH, alkalinity and fluorescence within the cave and of calcium and total hardness in a nearby laboratory. Thereafter, weekly samples were accumulated in acid-washed glass bottles. These were retained within the cave until periodic transfer via cold chest to a refrigerator at McMaster University. Maintenance of low temperature and continuous darkness avoided photolysis and other organic reactions before analysis.

Total fluorescence was measured in the cave with a Turner Systems Model 10 fluorometer using 15 ml cuvettes. Routine repeat checking of samples determined that variation of the fluorescence was much less than 10%. Sample fluorescence values were standardized against Amino G Dye in every run. The excitation device is a UV lamp which produces an exciting light in the range of 310-390nm. The excitation filter (10.69) excludes all light except between the range of 300-400nm. The emission filter (blue-98) only passes light between the wavelengths of 400 and 520 nm (Smart and Laidlaw, 1977). This is in

the range required for the excitation and emission of fulvic acids (FA; Schnitzer, 1978).

Dissolved organic carbon (DOC) was determined with a Dohrmann DC-180 Carbon Analyzer after inorganic carbon was removed by acidification to pH 2-3 and bubbling with argon gas for 5-10 minutes. Samples were run three times to ensure reproducibility, and results were only accepted if replication fell within 5%. Fluorescence spectra were measured with a Perkin-Elmer LS-5 Fluorescence Spectrophometer with 5nm slit-widths and a scan speed of 60nm/min for the monochromator. Blank runs using MilliQ water (DOC < 0.5 ppm) and an empty cuvette were analyzed in each run and were within machine background noise of 0.1-0.3 fluorescence units. Emission spectra were standardized by the method of Goldberg and Weiner (1991), utilizing Rhodamine WT. Aldridge Suwannee River Fulvic Acid was used as a reference solution for the spectroscopic analysis, at a 10 ppm concentration, similar to DOC values of the cave water.

Organic fractions were separated by ultrafiltration flowfield fractionation (Beckett et al. 1987) on Millipore Pellicon cassette membranes at 0.45 micron, 10,000 and 1,000 MWCO. The 0.45 micron membrane separated particulate organic carbon (POC) from the solution. The other two provided three fractions, 0.45 micron-10,000 MWCO, 10,000-1,000 MWCO and less than 1,000 MWCO; to quantify these fractions, DOC values were obtained by the method noted above (Buffle et al. 1978, Hayase and Tsubota, 1985).

2.4 Results and Discussion

2.4.1 Characterization of the Organic Compounds.

As a first step, it is important to demonstrate that fluorescence in drip waters is caused by organic substances. Figure 2.2a shows the relationship between fluorescence intensity and DOC at Marengo Cave. There is positive correlation between these two variables at all 12 sites and for much of the climatic year; however the correlation is not as convincing for the summer values. Of the four sites sampled for the full 15 months, the

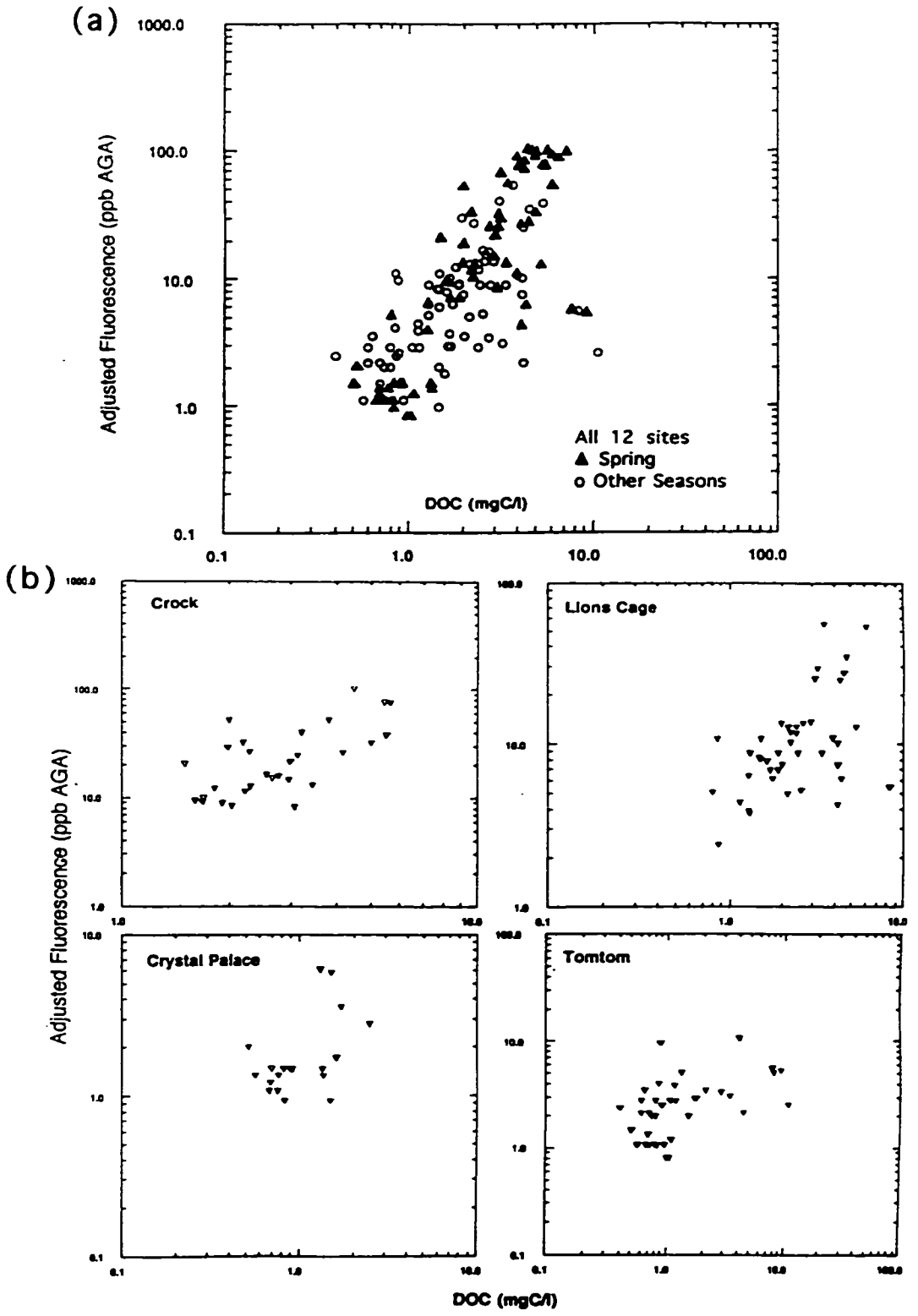

Figure 2.2. The relationship between fluorescence and dissolved organic carbon (DOC) for (a) all 12 sampling sites, and (b) the four sites sampled throughout the 15 months. Adjusted fluorescence is sample fluorescence - blank fluorescence / standard fluorescence (Amino G acid).

Crock and Lion's Cage show positive correlation between fluorescence and DOC (Figure 2.2b), but Tomtom and Crystal Palace (Figure 2.2) do not.

Measurements at all 12 sites during May-June 1995 determined that there were no significant correlations between fluorescence and the standard bicarbonate groundwater hardness variables, except for a weak negative relation (r=-0.48) between fluorescence and conductivity. At Marengo Cave it appears there is no simple association between concentrations of organics in the feedwaters and the rates at which calcite would be precipitated from these waters.

Miano et al. (1988) and Senesi et al. (1991) have provided fluorescence spectra for fulvic acid that closely resemble those of the cave water. The particular example of fulvic acid used by Miano et al. (1991) was that of Aldridge Suwannee River. In Figure 2.3a, it is compared to sample spectra derived for the principal sites. The Crock and Lion's Cage, which display the largest and most responsive flows, are both a close match to Suwannee River FA. The Crock yielded consistent spectra regardless of its volume of flow and the weather conditions. In certain samples at Lion's Cage the peak emission wavelength decreased, however, possibly in response to weather changes. It was more prominently decreased at Tomtom. Peak emission wavelength also shifted for a given sample when the excitation wavelength was changed, suggesting that the fluorescent signal was being produced by a mixture of different organic compounds. Changes in their relative concentration could also produce such shifts in the spectra in samples collected at different times. In addition, simple organic compounds tend to fluoresce at lower wavelengths (peak emissions between 380-410nm) than more complex ones (Larson and Rockwell 1978, Schnitzer 1978). At Tomtom, and on occasion at Lion's Cage, it appears that the simpler compounds are more abundant than the complex ones (Figure 2.3b), a finding that is given added weight by the ultrafiltration results outlined below. At Crystal Palace the concentrations of organics were always very low, making it difficult to obtain significant

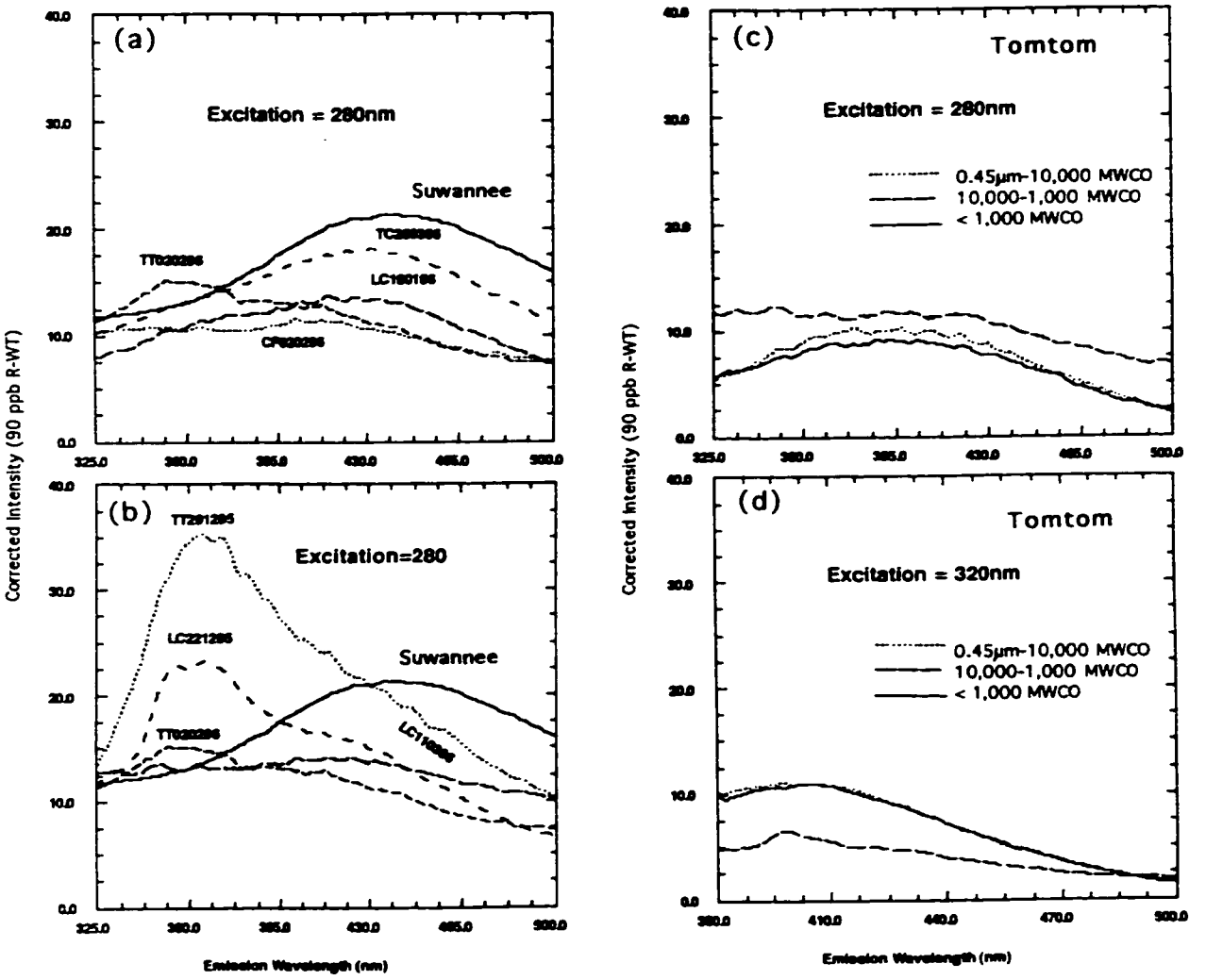

Figure 2.3. Fluorescence emission spectra of the four sample cave waters: (a) displaying the similarity between Suwannee River fulvic acid and most samples, no ultrafiltration; (b) examples of the greatest deviations from the Suwannee River standard; no ultrafiltration; (c) spectra of the various fractions produced by ultrafiltration of a three month accumulated sample of the Tomtom feedwaters (the reliable flow) at an excitation of 280nm and (d) excitation at 320nm.

fluorescent signals.

Ultrafiltration (Buffle et al. 1978, Hayase and Tsubota 1985) was undertaken to substantiate the finding that FA was the principal source of fluorescence. Tomtom was selected for this experiment because its consistent flow at a sufficiently high drip rate allowed 4 litres of water to be collected every week. Using a 0.45 micron membrane, particulate organic matter was first removed from a homogenised four litre sample of three months' accumulation of the water: note that spectra of previous Tomtom samples (Fig 2.3a, 2.3b) are similar in both intensity and peak emission wavelength to the spectrum of this homogenized, ultrafiltered sample, indicating that little or none of the fluorescence in the former can be derived from POC. The filtrate was then fractionated into the components, 0.45 micron-10,000 MWCO, 10,000-1,000 MWCO and <1,000 MWCO. DOC values for these three filtrates were 1.69 mgC/l, 3.21 mgC/l, and 5.31 mgC/l respectively. These values show that the majority of the organics are low molecular weight compounds, such as FA. Spectroscopic analysis of these fractions (Figs 2.3c and 2.3d) revealed slightly simpler compounds, but as the collection site was Tomtom, (the chief location to provide these shorter peak emission wavelengths, cf. Fig 2.3b), this result may not be indicative of the cave as a whole. There were no spectra representative of larger organic molecules such as HA. Consequently, the ultrafiltration results are entirely consistent with the spectroscopy as both types of analysis revealed the spectra and size fractions indicative of FA or even smaller organic compounds and the absence of HA.

2.4.2 Correlations with seasonal weather and climate events

Short term weather might influence fluorescence and DOC of the feed water through the dilution of organics by increased precipitation or, alternatively, increase organic concentrations when higher temperatures stimulate the release of organics from the soil, eg. during the Spring thaw which is a strong climatic feature of this region.

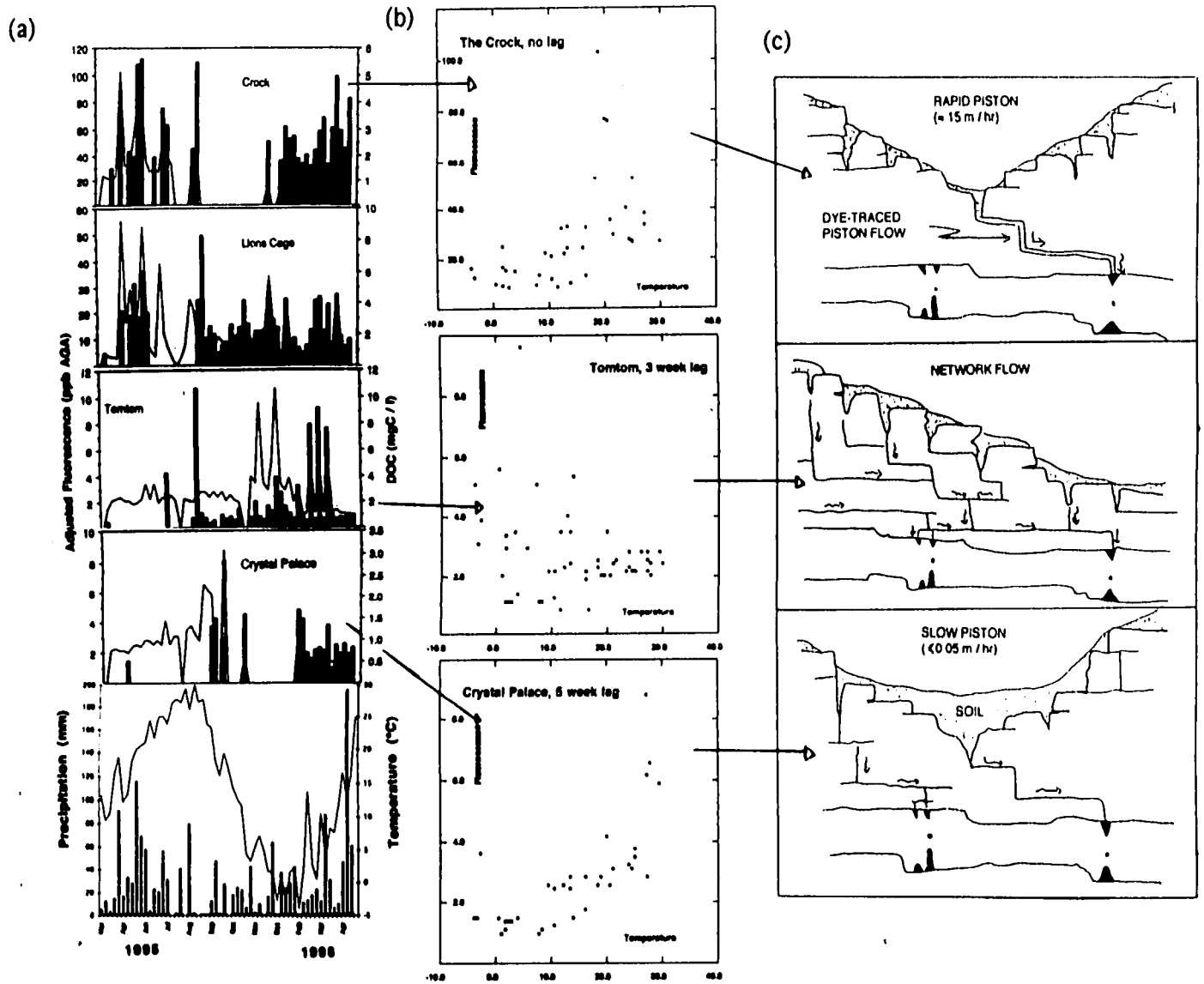

Figure 2.4. Associations of surface temperatures and precipitation with fluorescence and DOC in the cave waters: (a) Time series of the data sets. Temperature is weekly running means, Louisville Airport, precipitation is weekly totals at the cave. Periods of no fluorescence at the Crock and Crystal Palace indicate cessation of flow. Before 01/10/95 DOC was only measured on a representative selection (means and extremes) of the fluorescence results. DOC is the bars, Fluorescence is the line. (b) Sample plots of temperature vs. fluorescence, with differing timelags. (c) Conceptual models of the feedwater flowpaths to the four sites in the cave.

In Figure 2.4a, mean weekly temperature and total daily precipitation at Louisville are compared to the weekly fluorescence and DOC values for each of the four Marengo Cave sites. At the Crock and Crystal Palace, fluorescence and DOC display a weak positive relation with the contemporary temperature (Table 2.1). The Crock is the fast responding site while Crystal Palace is the least reactive, yet climatic variability affects both locations. Lion's Cage and Tomtom produced no truly significant relations, as is seen in Table 2.1, except for a relation between fluorescence and precipitation at Lion's Cage, that had a low correlation coefficient.

Recognising the possibility that many sites will not react immediately to weather changes at the surface, time lags were investigated in stepwise correlations with lags from 1 to 16 weeks (Table 2.2). At Crystal Palace, correlation between fluorescence and temperature improved progressively to a peak at five weeks (Fig 2.4b) and there was a lesser effect in DOC. With its comparatively uniform drip rate and lack of immediate response to rainfall or sudden warmings, Crystal Palace was expected to show a significant time lag; the improvement in the fluorescence correlation coefficient from 0.36 to 0.72 between zero and five weeks is strong. After five weeks, the correlation values steadily decreased. Surprisingly, the most responsive site, the Crock, also shows improved correlation between fluorescence and temperature at lags of four and five weeks; however the overall improvement was marginal.

Both the Crock and Crystal Palace dried up at intervals during this investigation, whereas the other two sampling sites did not. The start and the cessation in flow at Crystal Palace each lag the start and cessation at the Crock by five weeks (Fig 2.4a); the Crock transmits effects of weather events very quickly, and Crystal Palace responds to them with the five week lag. Lion's Cage and Tomtom are intermediate in driprate, do not dry up and on most occasions display no significant correlations between fluorescence or DOC and the climatic variables.

Table 2.1. Pearson correlation coefficients (r) between drip water variables, weekly temperature and precipitation - no time lags.

	The Crock		Lion's Cage		Tomtom		Crystal Palace	
	Fluo	DOC	Fluo	DOC	Fluo	DOC	Fluo	DOC
Temp	0.55 †	0.49	0.11	0.23	-0.34 †	0.01	0.36	0.20
Precip	0.31 †	0.28	0.18 ‡	-0.04	-0.27	-0.11	-0.50	-0.45

Significant correlations: † (P = 0.01), ‡ (P = 0.001)

Table 2.2. Notable improvements in correlations when time lags are applied.

Lag	The Crock	Lion's Cage	Tomtom	Crystal Palace
1 week	T*FL (0.53)	P*FL (0.38)	P*DOC (0.40)	T*FL (0.55) ‡
2 weeks	T*FL (0.43)	-	-	T*FL (0.64) ‡ P*FL (0.44) T*DOC (0.53)
3 weeks	T*FL (0.43)	-	-	T*FL (0.62) ‡ T*DOC (0.52)
4 weeks	T*FL (0.61) T*DOC (0.43)	-	-	T*FL (0.71) ‡ T*DOC (0.54)
5 weeks	T*FL (0.65) T*DOC (0.52) †	-	-	T*FL (0.72) ‡ T*DOC (0.64)

Significant correlations: † (P = 0.01), ‡ (P = 0.001)

For the Crock, it is noted that there is an immediate response of fluorescence to increases in temperature (Figure 2.4a). The probable explanation lies in the thawing of the soil in spring (Thurman, 1985) allowing the flush of organics into the cave. Dye tracing demonstrated rapid flow to the Crock, which suggests a large fracture or bedding plane flowpath within the limestone; this is illustrated as a rapid piston model in Figure 2.4c.

At Crystal Palace the organic concentrations are significantly lower than at the other principal sites (Figure 2.2d), yet their small variations and the starting and stopping of feedwater flow both display the distinct five week lag. It is believed that the lower concentration must be an effect of physical filtration of the soil water in a comparatively deep clay at the source, after which the water follows a simple single path through the limestone to the drip site. The path is probably more constricted than that of the Crock and thus the system may be termed a "slow piston" (Figure 2.4c).

There are no significant temperature correlations, positive or negative, at the intermediate flow response sites, Lion's Cage and Tomtom (Figure 2.4c). This implies that their systems are not simple pistons - there are many tributaries and/or distributaries in the feedwater path, thereby erasing the temperature-fluorescence correlation through homogenization of percolation water (Figure 2.4c). Significantly, these two intermediate sites did not dry up. At Lion's Cage there are many drips close together, suggesting that there is a distributary system a short distance above in the cave roof. In contrast Tomtom is a quite isolated, steadily dripping individual with the distinctive DOC and fluorescence signatures that have been noted above.

Figure 2.4a shows only a weak wet season / dry season contrast as opposed to the strong seasonal temperature regime. Therefore, simple correlations over the year might not reveal associations between precipitation and fluorescence, and DOC loadings. Network type systems and slow pistons will also tend to smear out direct associations with particular

events. However, the strongest fluorescence and DOC loadings at the Crock and Lion's Cage are associated with a consistently rainy period between 20th of April and the 30th of May, 1995. In particular, two large storms at Marengo Cave, on April 21st (65 mm) and May 25th (69 mm), 1995, correlate with high fluorescence and DOC values at both sites. There is therefore event - driven flushing of DOC, as witnessed by colour change of feed water at the Crock.

There was some significant seasonal weighting in the drip waters entering the cave (Figure 2.4a). During the observing period, the Crock and Lion's Cage both display augmented DOC and fluorescence with snap winter thaws and spring rains; at Tomtom and Crystal Palace the findings are not conclusive. Periods of low organic concentration are expected to yield clearer calcite layers, with the darker bands being generated by high concentrations. Seasonal structure in the calcite may also be caused by the cessation of flow that occurs during summer dry spells, as seen at the Crock and Crystal Palace.

2.5 Conclusions

The objectives of this study were to investigate the sources of fluorescence in Marengo Cave speleothem feedwaters and to distinguish the influence of seasonal or shorter term weather effects on organics in them. The spectroscopic results and ultrafiltration experiments demonstrate that fulvic acid is a principal contributor to the fluorescence but that simpler and smaller organics are also present, as seen at Tomtom and, on occasion, Lion's Cage. Amongst the standard limestone groundwater variables only conductivity showed any relation to fluorescence.

The cave experiences; 1) precipitation all year, which forces some event - driven responses; 2) freezing of the upper soil, thereby locking up organic acids to be flushed out by snap thaws or Spring rains; 3) a strong seasonal temperature regime. At those sites which yielded significant statistical correlations, temperature influenced feedwater

fluorescence, with an immediate response at the Crock and a five week lag at Crystal Palace. Both sites are believed to be simple piston flow systems. Network flow conditions are proposed for the intermediate sites at Lion's Cage and Tomtom, smearing temperature-fluorescence relationships. Seasonal effects can be produced by two modes: a) high organic concentrations in Spring feedwaters, and b) cessation of dripwater flow, which may produce sharp delineations in the precipitated calcite. At sites that do not display seasonality, variations in organic concentrations may be event - driven. The occurrence of seasonality in organic concentrations in feedwaters accounts for annual luminescence banding reported in some speleothems.

For paleoenvironmental reconstructions, variability in the temporal behaviour of organic concentrations in feedwater at different sites reported here indicates that only limited inferences may be made about past climates with just one speleothem from a cave or region. The scientific requirements are for (i) the establishment of characteristic feedwater behaviour before sampling, and (ii) the analysis of several samples. The latter conflicts with the conservation ethic practiced by responsible speleologists, calling for great care in the design of field programs.

Chapter 3: Seasonal variability in organic substances in surface and cave waters at Marengo Cave, Indiana.

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Abstract

Water samples from forest soils and a shallow cave underlying them were collected for the hydrological year 1996-1997. The soil waters did not display much seasonal variability in concentrations of organic substances in them but the cave waters yielded distinct highest levels in the spring and fall seasons. Fluorescence studies of the organic compounds isolated from these waters revealed shorter peak excitation and emission wavelengths for the cave waters than for the soil waters, a result of both differences in concentration and probably also of significant change in the proportional organic assemblages in the waters. Precipitation appears to affect the fluorescence in both waters, with the dry fall producing the highest yields. Molecular size fractionation revealed how the larger hydrophobic compounds are preferentially removed from the water before it reaches the cave with the consequence that the smaller hydrophilic compounds become the dominant fluorophore there.

3.1 Introduction

The discovery of annual bands in calcite speleothems (Broecker et al., 1960, Baker et al, 1993, Shopov et al, 1987, 1994) , and their ready detection by fluorescence has created much interest in the potential paleoclimatic information stored in them. Genty and Quinif (1996) attempted to correlate the variability in fluorescence in a young, growing sample in a Belgian canal tunnel with climatic conditions above it, but with inconclusive results. The cause of the fluorescence has been attributed to organic substances

incorporated within the calcite (Gilson and McCarthney 1954, Gascoyne 1977, Lauritzen et al. 1986, White and Brennan, 1989). Organic substances can be transported by percolating waters from the soil and upon reaching the cave may be incorporated into the calcite matrix of any precipitate. Calcite is an efficient adsorber of organic matter, especially of lipoid material and amino acid substances (Suess, 1970, Carter, 1978). Mitterer (1968) showed that organic matter may even induce $CaCO_3$ precipitation, by concentrating calcium ions. In earlier work (van Beynen et al. 1998), we have demonstrated that FA and specifically the hydrophilic acids and neutrals were the predominant fluorophores in some sample speleothems. Some important aspects of this research that merit further study are the comparative magnitudes of fluxes of organic substances in the soil waters where they are generated and in the cave waters underneath, seasonal variation in those fluxes, and whether fluxes underground can be correlated with external weather and climate variables.

The term *organic substances* as used here encompasses fulvic acids (FA), humic acids (HA) and humin. These three components are operationally defined by their solubility in acid-base (Hayes et al., 1989). The residence times in soils may range from decades to hundreds of years for FA and up to thousands of years for HA (Schlesinger, 1977). Molecular weights of FA tend to be smaller than HA and humin (Suffet and MacCarthy, 1987). All of these substances are loosely defined complexes of major functional groups including carboxylic acids, phenols, hydroxyls and alcohols (Visser, 1983). Nuclear magnetic resonance has revealed ketone/aldehyde, carboxyl, aliphatic and aromatic hydroxyl groupings (Wilson et al. 1987).

The organic chemical differences in water samples can be determined by molecular weight analysis and fluorescence spectroscopy. The typical molecular weight sizes are 0.8-1 kilodaltons (kD) for FA and 2-300 kD for HA (Suffet and MacCarthy, 1989); analysis is by gel permeation chromatography or ultrafiltration (Wershaw and Aitken, 1985). Much

work been done on the fluorescence of these compounds. Suwannee River FA (Miano, 1988) is often used as a fluorescent standard for FA; the characteristic maximum excitation peak is at 320 nm, maximum emission peak at 425 nm, and average molecular weight is 2.3 kD (Chin et al., 1994). Humic acid has longer excitation and emission wavelengths, with respective peak intensities at 480 nm and 540 nm (Hayase and Tsubota, 1985). Aldrich HA, the commonly used standard for humic acid has a larger average molecular weight than Suwannee River FA, approaching 4.1 kD (Chin et al., 1994).

3.2 Objectives of Study

Some speleothems possess annual laminae which, it has been hypothesized, are generated by annual (seasonal) variation in the concentration of organic substances in cave drip water.

To test this hypothesis the following investigations were undertaken:

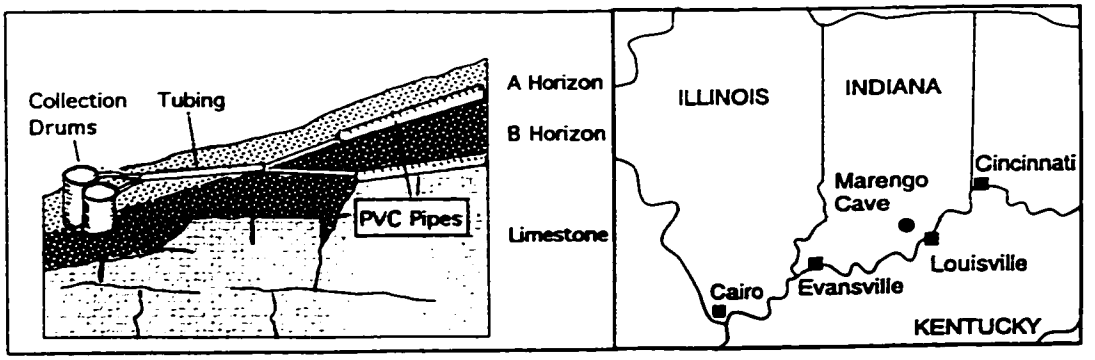
1) Ascertain whether there is seasonal variability in the content and nature of dissolved organic substances in sample cave drip waters precipitating calcite and in the groundwater recharge sites in the soils overlying them.

2) If variability is present, to determine whether it is produced by seasonal changes in the climate above the cave

3) Determine whether there are differences between the amounts and proportions of organic substances found in surface and cave waters

3.3 Study Area

Marengo Cave, southern Indiana (latitude 38°22"N, longitude 86°20"W) has a strongly defined seasonal climate, is easily accessible and is a show cave with a professional staff willing to undertake periodic water sampling. The cave is a 500m segment of an abandoned river passage that has been truncated at both ends by natural breakdown. It lies under a hillside and is approximately 15 metres above the channel of the Blue River (Figure 3.1). The cave is developed in Ste. Genevieve and Paoli formations of



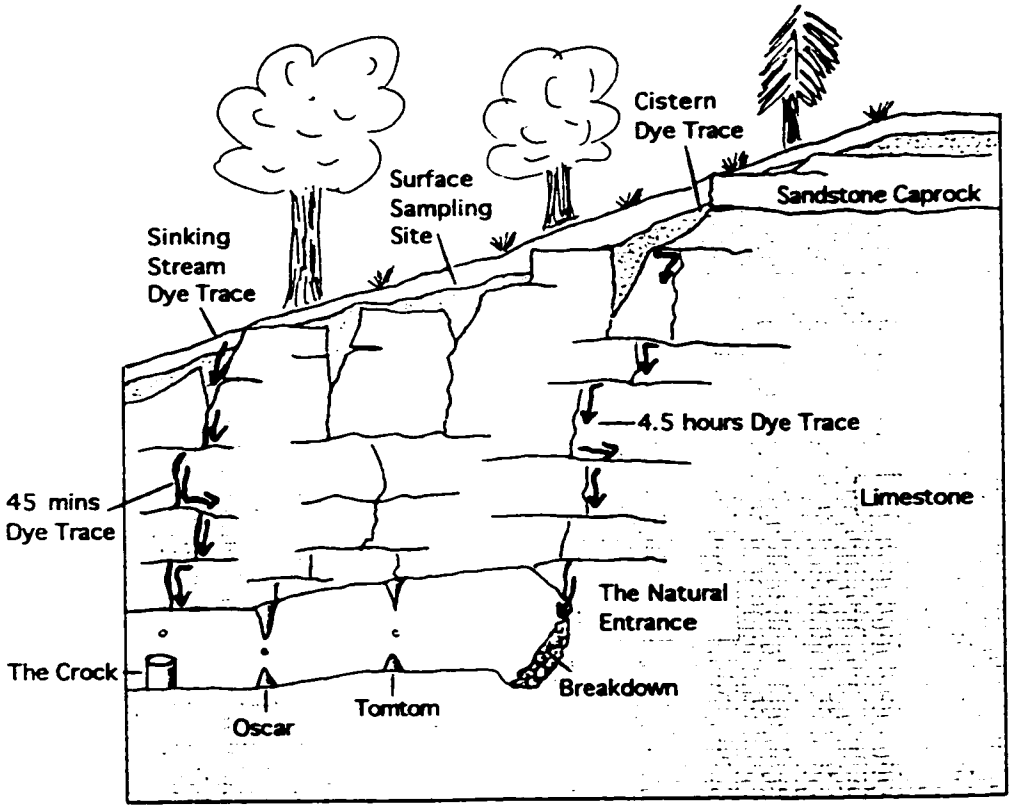


Figure 3.1. Cross-sectional view of sampling sites at Marengo Cave. Sampling design of collection of surface water included.

the Blue River Group, of Middle Mississippian age (Powell, 1992). These are thick to massively bedded, near-horizontal limestones; they are capped by a sandstone, the West Baden Formation.

Thickness of bedrock cover above the cave ranges from 10 m at either end to 30m above the centre. The speleothem distribution is closely related to this; the lesser the overburden, the greater the density of decoration. The drip rates of feedwaters to the speleothems are also spatially variable; the two sites selected for this study, Oscar and Tomtom, appear to be perennial, yet responsive to precipitation. Sources and rates of feedwater flow towards the principal areas of deposition at the two ends of the cave were investigated by dye tracing (Figure 3.1). Oscar and Tomtom are situated between two points of emergence where dyes were recovered 4.5 hours and 45 minutes respectively after time of injection into rivulets observed to sink underground. It is a reasonable supposition therefore, that both are fed from the same area of subsoil recharge waters.

A thin, patchy soil has developed on the limestone and pockets of alluvial clays, sands, silts and gravels ranging in age from Tertiary to recent (Powell, 1992). As this region was south of the glacial advances, no glacial till is present. The vegetation is a deciduous forest that, although cleared once in the last century, has recovered and displays little human interference today. Continuous climate data were obtained from a rain gauge at the site and from the meterological station at Louisville Airport, 50 km to the east. There, the 30-year mean annual temperature is 13.4°C and mean annual precipitation is 1110 mm. The seasonality in temperature is very evident with a January mean of approximately 0°C and 29°C in July. Maximum precipitation is in the spring while the summers are dry. Potential evapotranspiration for this region is approximately 900mm while actual evapotranspiration is probably 800 mm. From December to February there are snowfalls, but these are either removed by snap winter thaws and rains or in the spring melt in March. For the period December 1995 to February 1996, a total depth of 600 mm of snow was

measured. This quantity was melted and included in the precipitation amounts used in this study.

3.4 Methods

Site selection and water sampling.

Previous work at Marengo Cave (Toth 1996, van Beynen et al. 1998) suggested that the two most reliable points for collecting water samples within the cave were two growing stalactites known as "Oscar" and "Tomtom" (Figure 3.1). They did not dry up even during the summer or fall, the periods of lowest soil moisture. The volume of water coming into the cave was measured by cave staff, and placed into acid-washed glass collection vessels at weekly intervals. The holding vessels were kept in the cave to prevent growth of algae and photodecay of the organic substances in the cave water. However, the collection vessels were often full at the time of replacement and only minimum estimates of dripwater volume could be determined. After the middle of February bottles were replaced twice per week. Unfortunately, due to the busy tourist season, there is a break in the measurement records of the volume of water from the end of May to the last week of August. However, once full, the bottles were still replaced. For the analysis to be undertaken at McMaster University, equal portions of each week's water were homogenized into 4 L to represent drips for each season. The collection dates for the 4 L of seasonal water were 22 February (winter), 17 May (spring), 13 September (summer) and 12 December (fall).

For comparison with the cave sites, one surface location was chosen that was a short distance upslope of the stalactite positions and lay on a transect between the two points of dye injection traced to the cave underneath. When a 1 metre deep trench was cut into the slope, a clear A- and B- horizon soil profile was revealed. The A horizon (dark brown soil-humus layer) had a thickness of 20 - 25 cm and the B horizon (yellow to white clay) had a thickness of 60 cm. The A and B horizons will be referred to as "soil" and

"clay" respectively. One 40 mm PVC collector pipe 3 metres in length was embedded orientated upslope in the lowest part of each horizon. Along the top half of each pipe slits were cut every 10 mm to allow percolating water to enter the pipe, and it was attached to plastic tubing which was buried and drained into a separate 90 L plastic drum (Figure 3.1). The drums were buried at 1 metre to minimize the freezing of the water and 6 metres down slope to facilitate gravitational drainage. The drums were stirred and 4 L was removed at the close of each season (see above) and taken with the cave water samples to McMaster University for analysis. The remaining water was emptied from the drums and they were cleaned for the start of the next collection period.

3.5 Isolation of Humic Substance Fractions

A flowsheet of the analytical processes is given in Figure 3.2. Four litres of water from each location was concentrated to approximately 250 ml, using a Buchi Rotory evaporator, with the bath kept below 40 °C to avoid degradation of the humic substances (p.c. Rick Bourbonierre). The soil water was adjusted to the same pH as the cave water (pH = 8) using 0.1 N NaOH and all solutions were split into equal portions, one half being used for FA-HA separation, the other for molecular weight fractionation.

Both halves of each solution were filtered using Whatman GF/F glass fibre filters (0.45 μm) to remove the particulate organic carbon (POC). To determine the concentration of the POC, one pre-weighed filter of each sample was dried in an oven and weighed. The second filter was flushed with MilliQ water to remove the POC for fluorescence spectroscopy. The POC filtrate solution, the total dissolved organic matter (TDOM), allocated for FA-HA separation was adjusted to a pH of 2 with 4N HCl and left for 24 hours. The precipitated HA was then filtered from the solution with the Whatman filters (Leenheer, 1985). The filters were rinsed with 0.1 N NaOH to resolubilize the HA. The filtrate was now operationally defined as FA. To collect any FA that may have been trapped with the HA, the HA solution was again acidified, filtered and the filtrate added to the

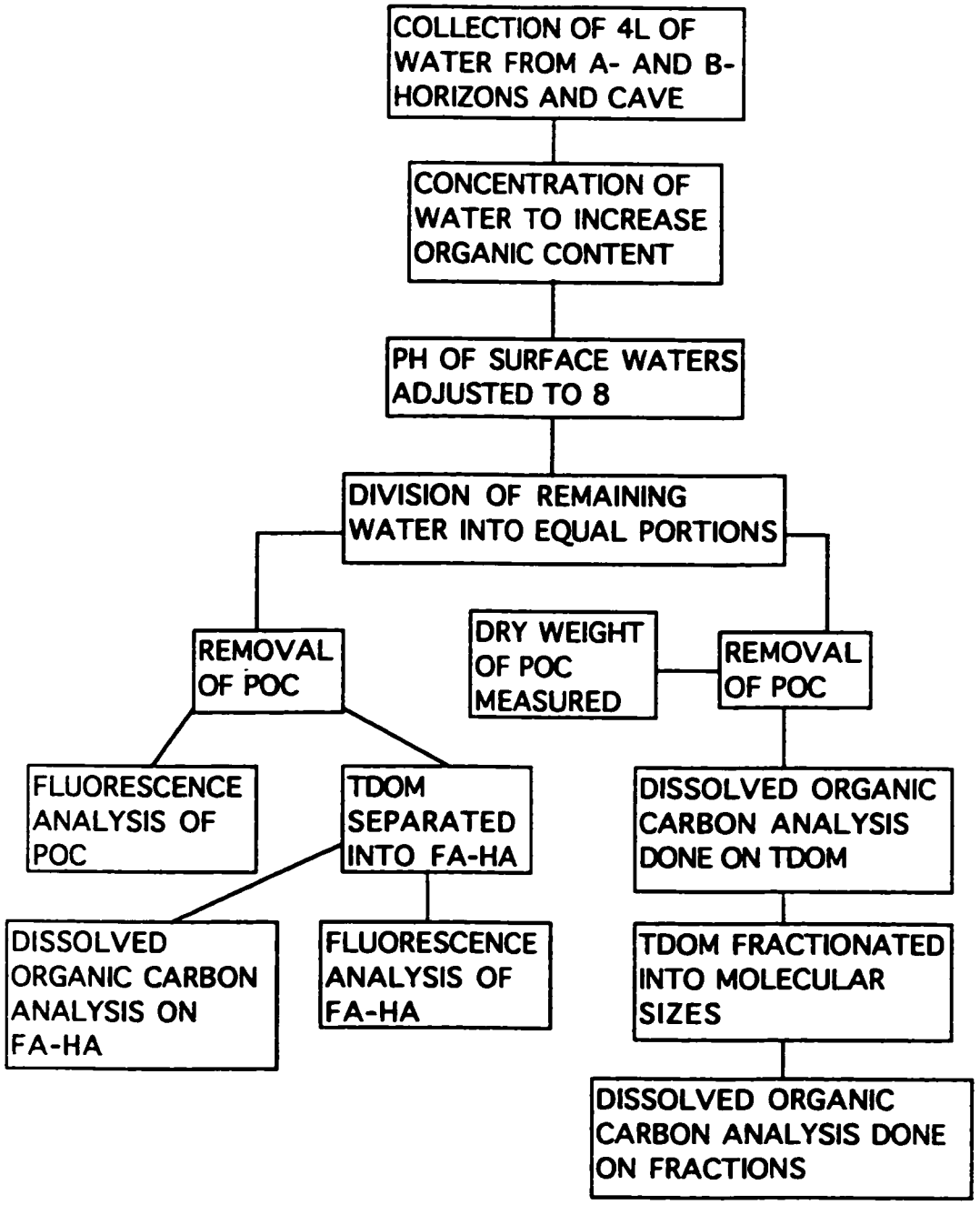


Figure 3.2. Overview of analysis undertaken on water samples

earlier FA separate. Remaining HA was then flushed from the filter with the 0.1 N NaOH. All fractions were refrigerated to prevent any organic decay.

Dissolved Organic Carbon

Dissolved organic carbon (DOC) was determined for the TDOM fraction, FA, HA and the size fractionation solutions with a Dohrmann DC-180 Carbon Analyzer. The inorganic carbon was removed from the solutions by acidification to pH 2-3 with 4N HCl and purged with argon gas for five minutes. Samples were analyzed three times to ensure reproducibility, and results were only accepted if replication fell within 5%. To achieve a realistic measure of the actual amount of organic compounds in each fraction, the DOC values had to be adjusted for two factors. First, they were adjusted for the concentration effect because the solutions were concentrated from 4 L to 250 ml. Second, they were adjusted to compensate for the percentage that carbon accounts for in the organic compounds of each fraction. These percentages are approximately 50% of the total in FA (Suffett and MacCarthy, 1989) and 63% in HA (MacCarthy and Malcolm, 1989). Because the great majority of the TDOM is FA, the 50% value was used to adjust this fraction as well.

Absorbance Spectroscopy

The absorbance spectra of FA and HA extracts were collected using a Perkin-Elmer UV/Visible Lambda 6 spectrophotometer. The samples were scanned over a wavelength range of 200 - 600 nm.

Fluorescence Spectroscopy

A Perkin-Elmer LS-5 fluorescence spectrophotometer was used to record the fluorescence spectra for each FA , HA and POC sample. Slit-widths of 5 nm were used for the 2 monochromators with a default scan speed of 60nm/min and the scale factor was 1. MilliQ water (DOC $\approx$ 0.5 ppm) and the empty cuvette were analyzed each day to test for

stability and cleanliness of the apparatus. The fluorescence intensity of all these tests was within the background noise of the machine at 0.1-0.3 units. To allow semi-quantitative comparison of fluorescence intensities between solute samples, a predetermined quantity of Rhodamine WT dye (1 ml of 1 ppm standard) was added to subsamples of the fractionated solutions after their pH was adjusted to 10. All fluorescence spectra of the fractions were measured at pH 10 particularly because it enhances spectral definition of FAs (Mobed et al., 1996). The maximum intensity of the Rhodamine WT standard also occurs at this pH (Smart and Laidlaw, 1977). A blank solution with the dye was used to produce a standard fluorescence intensity for the dye and the spiked sample solution intensities normalized to this standard (Goldberg and Weiner, 1991). This procedure accounted for machine drift and intrasample irregularities. Both excitation and emission spectra were measured for all the fractions, with numerous scans made to find the bands of peak excitation and emission wavelengths. The POC was stirred before each scan to minimise settling of the particles.

Ultrafiltration

After removal of POC, the filtrate was separated into organic fractions by ultrafiltration, using MSI centrifuge tubes for the 100 and 10 fractions, and Spectrum Spectra Por 6® dialysis bags for the 1kD fraction. The process yielded the four fractions, 0.45 μm -100 kD, 100-10kD, 10 - 1 kD and less than 1 kD. DOC was measured on each fraction by the method noted above. The purpose of this experiment was to help characterize the organic substances, to verify the FA - HA separation results and to determine if organic substance assemblages varied seasonally.

3.6 Results and Discussion

3.6.1 Seasonality in Organic Substances in Surface and Cave Waters

A) Concentration and speciation of dissolved organic matter

Figure 3.3 shows the concentration of the total dissolved organic matter (TDOM) in the surface and cave waters. Little seasonal variability occurs in the TDOM of the surface

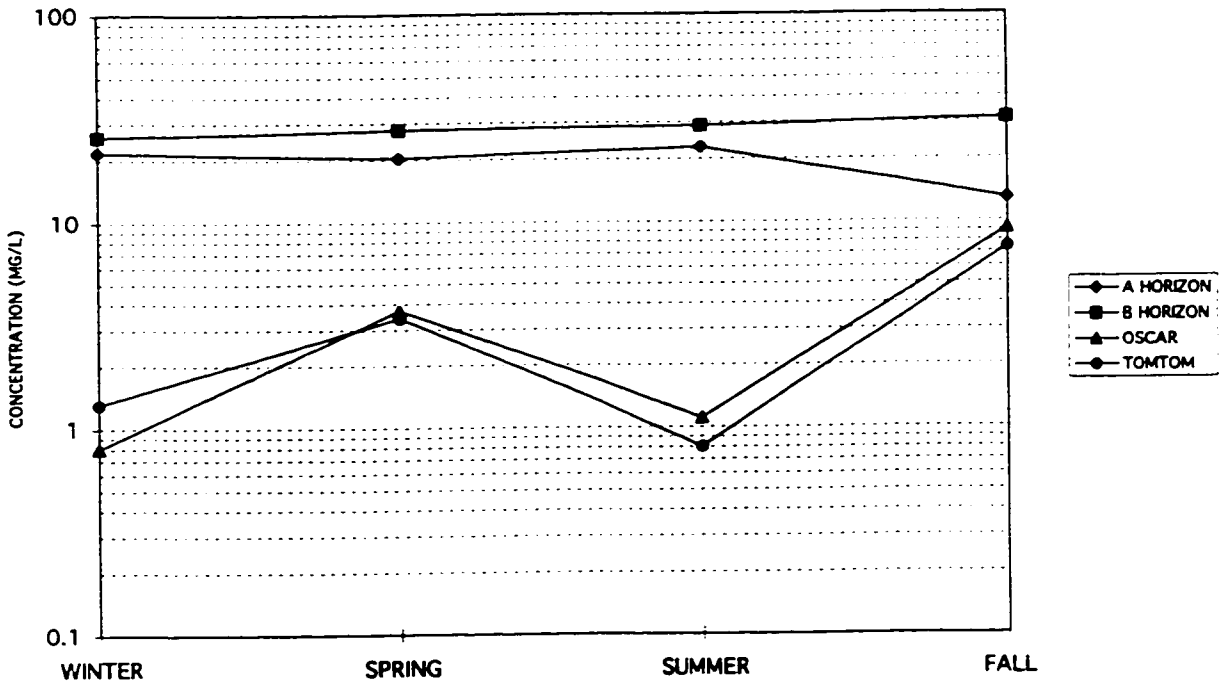


Figure 3.3. Seasonal variability in total dissolved organic matter

waters with concentrations ranging between 20 to 30 mg/L except for the A-horizon when it falls to 14 mg/L. Cave waters, while having <50% of the TDOM of the surface waters, display much greater seasonal changes in concentration, with the spring and fall having the highest values. Spring flushing of the soil is believed to produce the increase in TDOM of that season (van Beynen et al. 1998) and lower recharge to the drip sites accounts for the fall's augmented concentrations. Tomtom and Oscar display very similar behaviour to each other, as do the surface waters.

Seasonal variability in the relative concentration of the different organic fractions was detected in all of the waters (Figure 3.4). The largest differences occur in the POC content, with the four sites displaying maximum concentrations at different seasons. In the soil, the summer produces the highest levels, the winter for the clay, spring for Oscar and the fall for Tomtom. The summer POC value at Tomtom was contaminated with calcite that precipitated from the water onto the filter during the separation of POC. The other sites did not have this problem. The calcite was very white in appearance and consequently is believed to have contained very little organic matter (estimated at 5-10%).

There is little seasonal variability in FA or HA in the surface waters (Figure 3.4). FA values at the two cave sites are almost indistinguishable from each other but vary through the seasons more than the surface waters, with the spring and fall periods having the greatest concentrations. During the fall the clay waters also have their highest concentration of FA, about four times that of the soil water. FA concentrations show the filtering processes of the soil and bedrock as the percolation waters flow to the cave (Thurman 1985, Jardine et al. 1989) because the soil water FA concentrations are usually at least double those of the cave waters (Figure 3.4). However, in the fall FA levels in the soil water and at Oscar become comparable. Such filtering is more apparent in the HA where concentrations in the drips are consistently about 10 times lower than those in surface waters, showing remarkable sensitivity to the adsorption process. This adsorption may

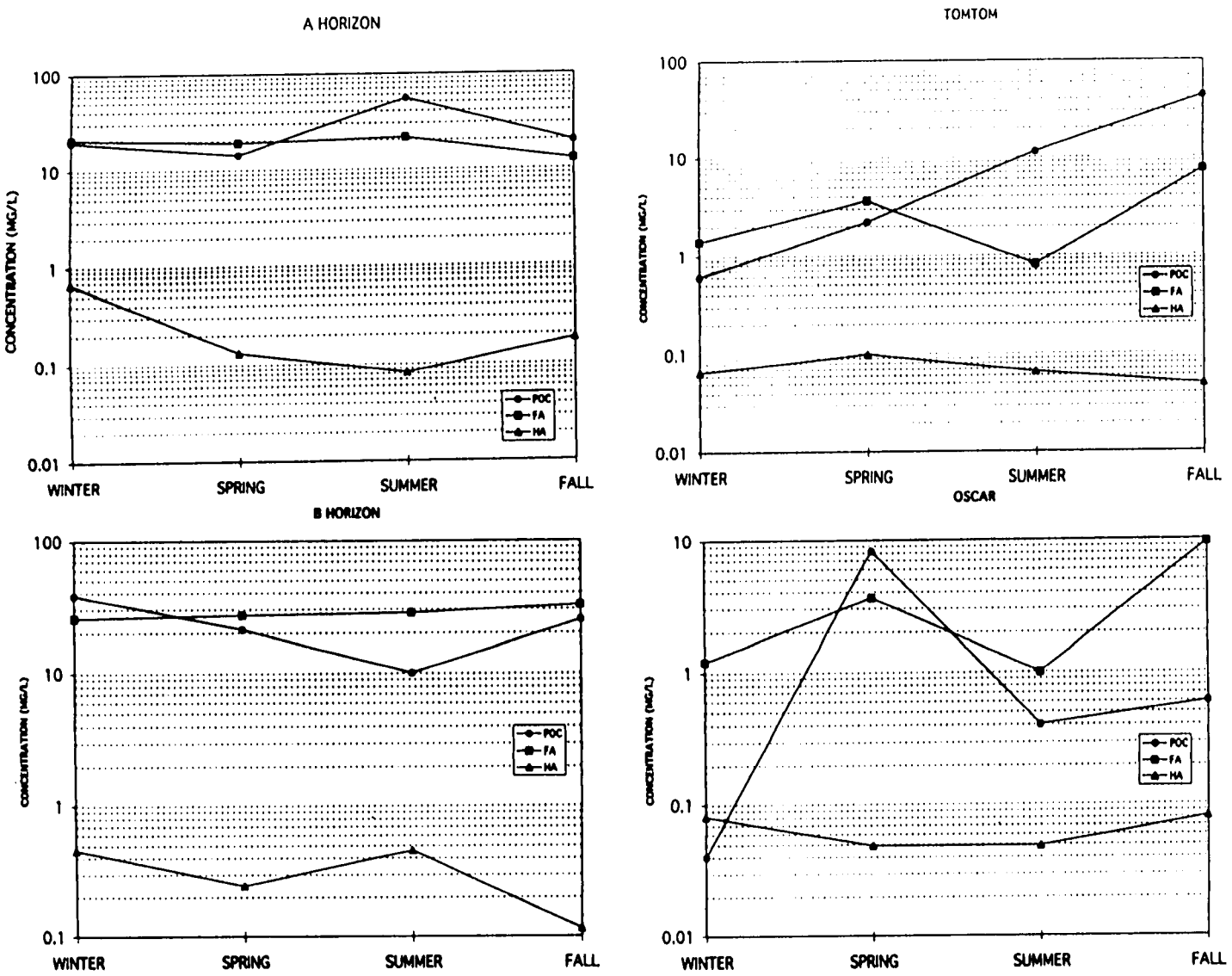


Figure 3.4. Seasonal variations in concentrations of POC, FA and HA for surface and cave waters

occur because of the hydrophobic nature of HA (Drever and Vance, 1994). Concerning seasonal variability, winter values of the surface waters show the greatest concentrations of HA, although in the clay waters the spring also has high levels. Concentrations of HA in the cave waters are so low compared to surface waters that any seasonal differences are probably not significant.

The variability of POC and FA in cave waters is caused by two factors, both of which are related to climatic conditions. Spring produced high total yields of organics because of the flushing of organic substances from soil - clay layer after the winter thaw (Thurman, 1985). The fall's high concentrations are produced by a "concentration effect". As a consequence of lower precipitation and highest evapotranspiration in the summer and early fall recharge is at its lowest levels. This results in a lower dilution of the available organics in the clay to be washed into the cave compared with the rest of the year when conditions are wetter. The lower dilution yielded higher concentrations in the clay and consequently in the cave.

Figure 3.5 shows the relationship between precipitation and the amounts of water collected at the two drip sites for a sample period in the summer and fall. During the winter and spring, the bottles were always completely filled each week, and thus no detailed relationship between the two variables can be determined. Only when precipitation and recharge drop to their lowest level in the fall were the drip volumes reduced to the point where a clear relation could be seen. It has been noted already that the fall was the period of highest organic concentrations in the cave, and Figure 3.5 clearly shows the reason: that the volume of water entering the cave closely reflected the precipitation levels and consequently led to lower dilution levels of the available organic compounds.

B) Molecular weight distributions of dissolved organic matter

These findings that FA is dominant in the dissolved fraction are confirmed by the

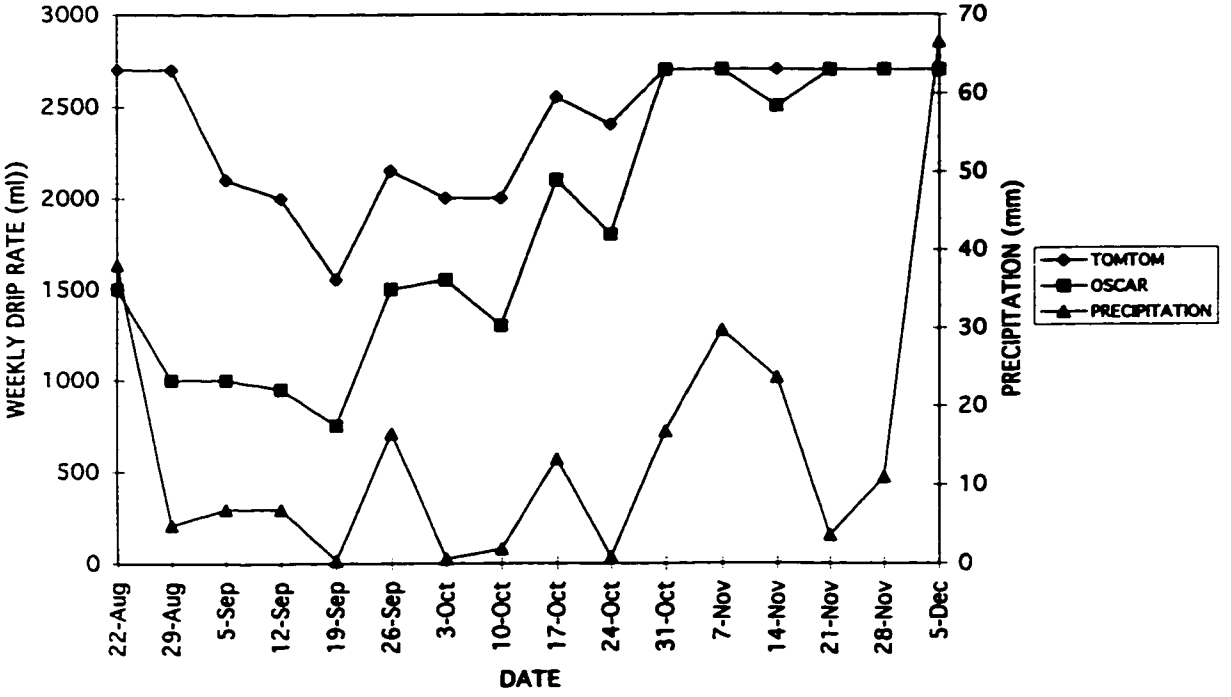


Figure 3.5. Precipitation and cave drip rates

abundance of the small molecules obtained in the size fractionation results (Figure 3.6). In the cave waters, the <1kD fraction is dominant, except in the fall when the 1-10 kD fraction is equally important. A striking result is the virtual absence of the 1-10 kD fraction in all the cave waters except during the fall whereas it is very prominent in the clay and soil water, except in the A-horizon during the summer. This fraction must be strongly adsorbed during its transit through the rock on the way to the cave. Alternately, it is possible that this fraction is formed by higher molecular weight complexes with Ca as pH rises during the passage through the rock. The larger molecular weight fractions measured in the spring and summer at the cave sites are puzzling because Figure 3.4 does not exhibit an abundance of HA during this period, with HAs supposedly being the larger molecules. This raises some question about the molecular weight assignment of HAs. In all the sites, there is much variability in the presence of the 10-100 kD fraction. An unusual aspect of this fraction is that it is present in reasonable abundances in the A-horizon for most of the year but hardly present in the clay water. During the winter it is present at Tomtom but hardly at all at Oscar. Only in the spring is it abundant in both cave waters. Subsequently, Tomtom and Oscar experience a decrease in this fractions concentrations. Why this variability occurs throughout the year is still uncertain.

Absorption Spectrometry

The absorbance, excitation and emission spectra were studied to obtain a different, independent measure of the seasonal variability of the different types of organic substances at the various sites (Figure 3.7). The absorbance was measured from 200 to 600 nm. The similarity between the different seasons and the fairly constant absorbance values peak wavelengths demonstrate that absorbance in this instance is not an important factor in altering the fluorescence excitation and emission spectra. Self absorption generated by high concentrations in solutions (Mobed et al. 1996) shifts the excitation and emission wavelengths of the organic compounds to longer wavelengths (Senesi, 1990).

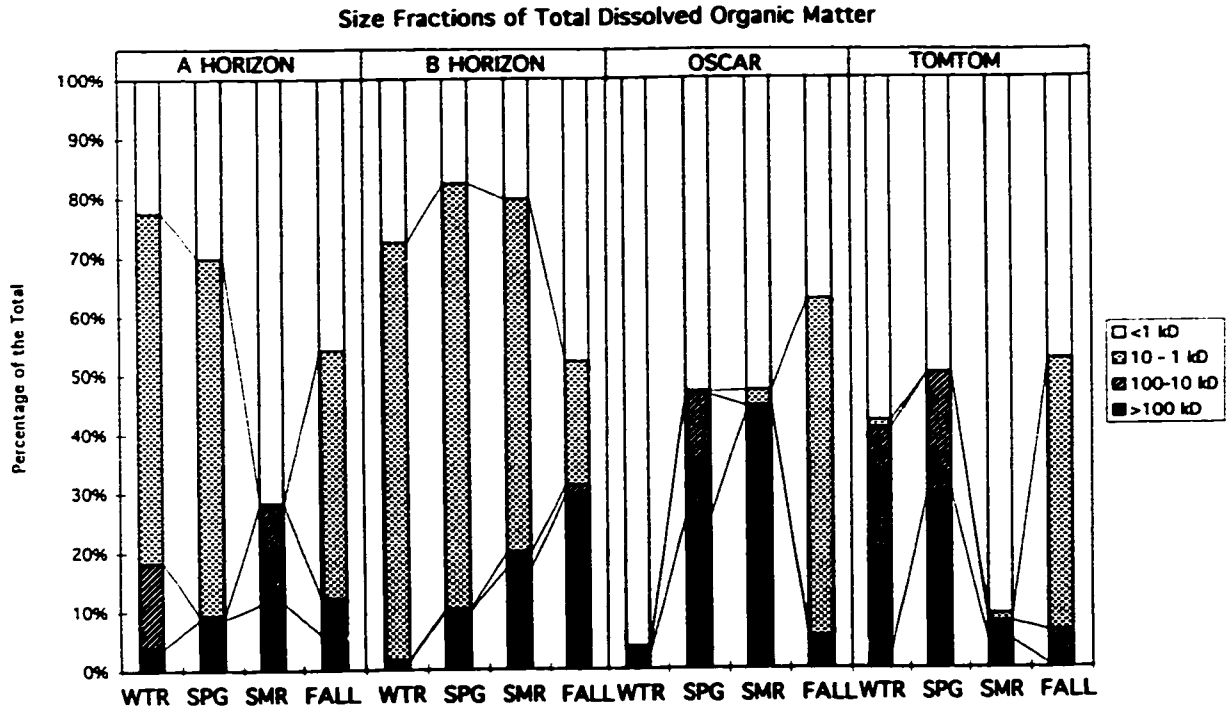


Figure 3.6. Size fractions of total dissolved organic matter (after POC removed) for surface and cave waters

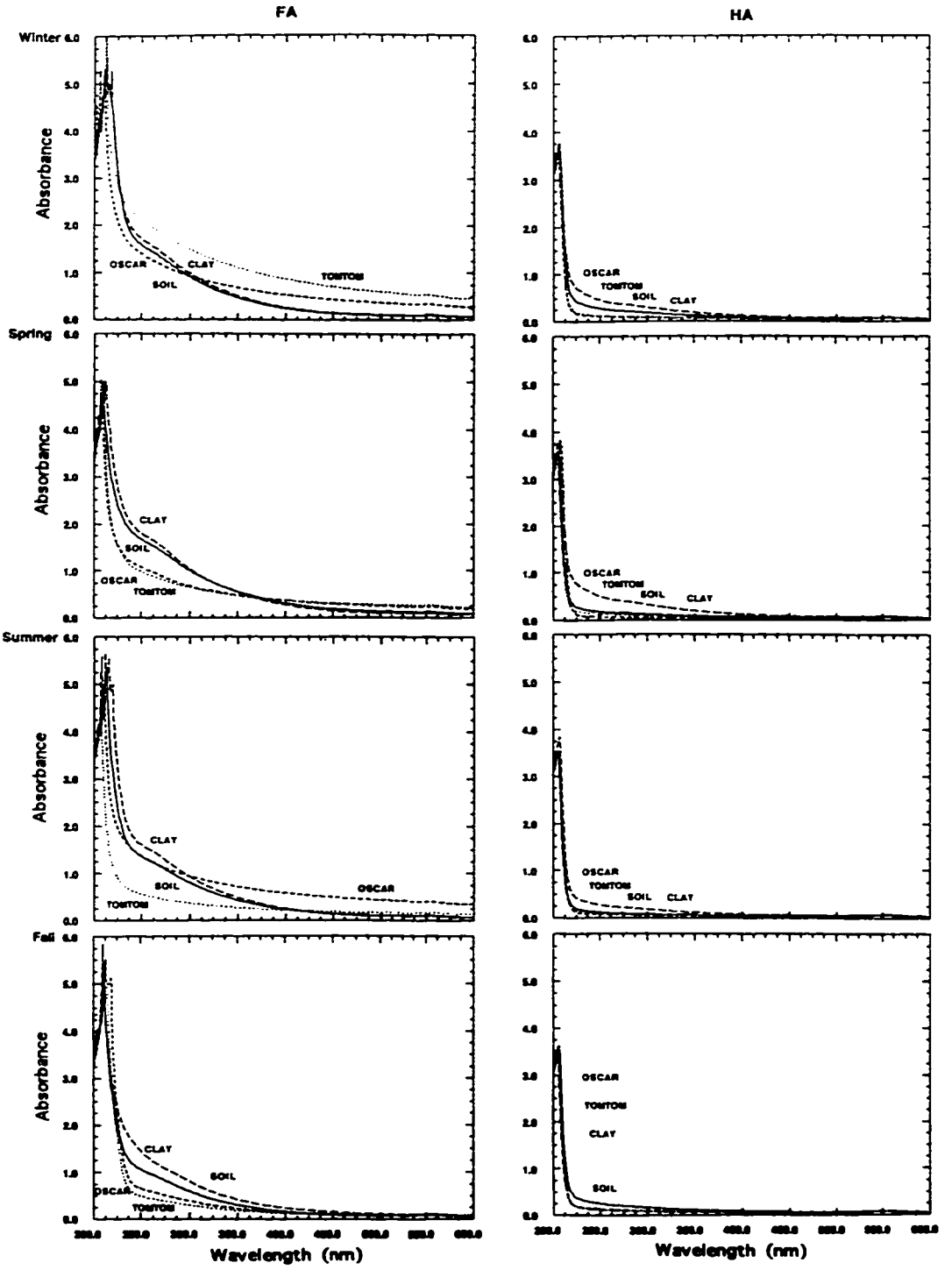


Figure 3.7. Absorbance spectra of fulvic and humic acids for surface and cave waters

Figure 3.7 demonstrates that FA and HA in all the waters show a peak between 210 - 220 nm, which is characteristic of aromatic compounds. Slight differences in the wavelength of maximum absorbance are caused by the addition of COOH and OH groups to various positions on the aromatic ring (Bloom and Leenheer, 1989). FA, unlike HA, displays structural differences between the cave and surface waters, with a shoulder centred on 260 nm in the latter but not in the cave waters. Such a shoulder has been noted in FA before, although the reason for its presence has not been ascertained, it may suggest the presence of more than one chromophore (Bloom and Leenheer, 1989). For all the waters, HA does not exhibit any shoulder and therefore appears to be simpler in molecular composition than FA. Another difference between surface and cave waters for FA is that the cave waters absorb strongly in the visible range in winter and summer. Simple quinones are known to absorb in the visible wavelengths, but only very weakly (Bloom and Leenheer, 1989). However, if there are no quinones in the surface waters, it is difficult to envisage where they may have come from in the cave water. It is also possible that the longer wavelength absorption is due to complexes of organic molecules bridged together with Ca ions from dissolution of the bedrock. Such a mechanism may be operating because, as noted, it can also explain the virtual disappearance of the 1-10 kD molecules in the cave samples. On the other hand, organic matter is present in the limestone bedrock and small amounts of this could be liberated by the acidic percolation waters. However, such a contribution at a significant level might be discounted because the organic concentrations in the cave waters would then be more nearly constant throughout the year: Tomtom and Oscar exhibit clear variability in organic matter.

Fluorescence Spectrometry

a) Excitation

Preliminary fluorescence measurements showed 425 nm to be the prominent emission peak at numerous excitation wavelengths for both the FA and HA solutions. It was therefore chosen as the fixed wavelength for the excitation scans shown in Figure 3.8.

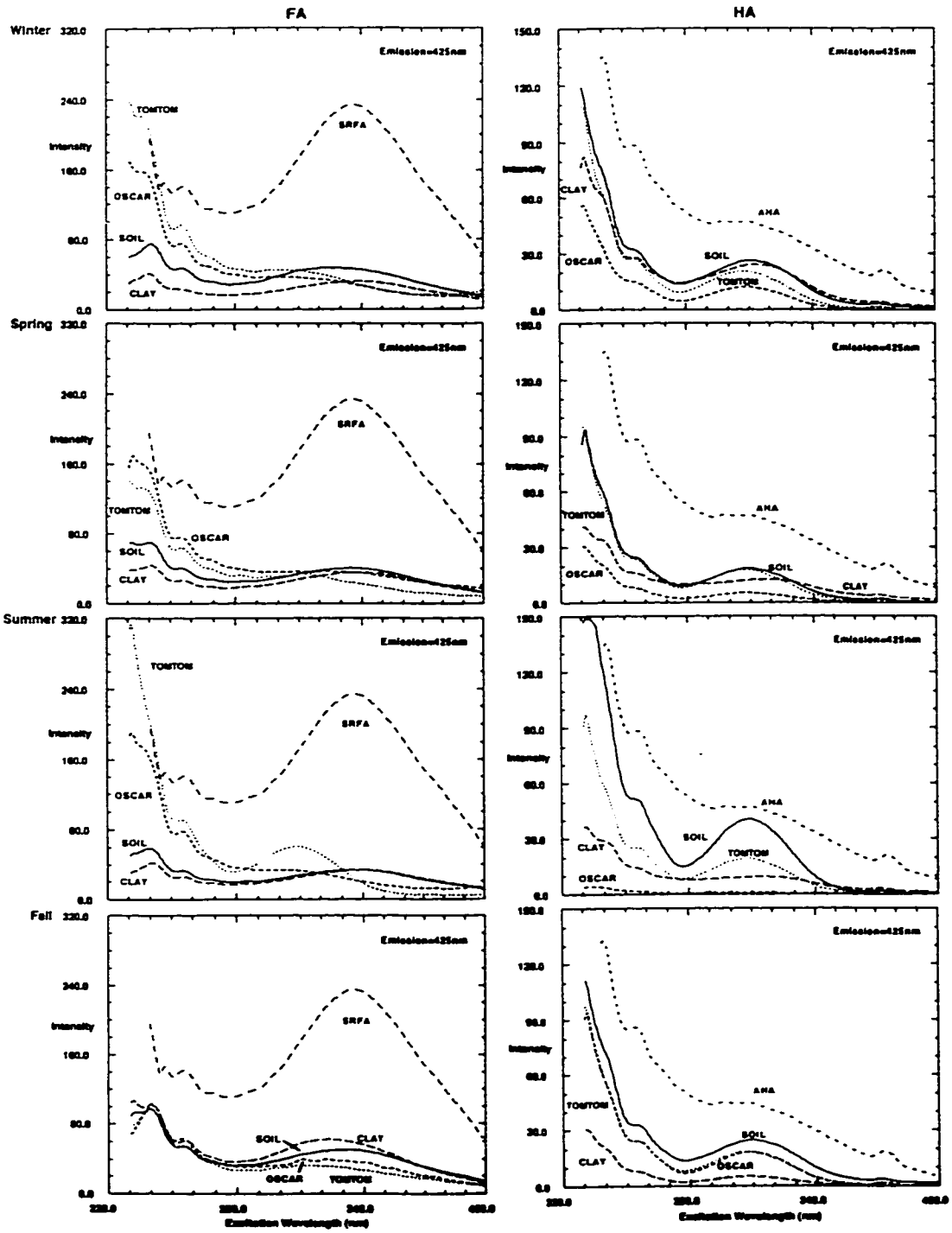


Figure 3.8. Excitation spectra of fulvic and humic acids for surface and cave waters. SRFA and AHA are included for comparison purposes.

The widely used international standards, Suwannee River FA (SRFA) and Aldrich HA (AHA) are included for comparison. Both these standards had concentrations of 10 ppm, but their intensities have been magnified for ease of comparison with the surface and cave waters. The FA excitation spectra show shorter peak excitation wavelengths in the cave waters (300 nm) during the winter and spring, when compared to the surface waters and to SRFA (330-340 nm). However, during the fall, the cave peak excitation wavelengths increase to more closely resemble the surface waters and SRFA, although wavelengths are still somewhat shorter. In contrast, excitation spectra of the HA fraction in all the waters show strong similarities to each other and to AHA (310 nm). All samples, whether they are FA or HA, have an excitation peak or shoulder centred on 255 nm.

For FA, the most significant change in the excitation peak intensity wavelength occurs in the fall when there is a lengthening of the peak intensity wavelengths in the cave waters. One possible explanation of this feature is self-absorption generated by absorbance of the exciting UV light at higher concentrations of FA, producing a shift towards longer wavelengths in both excitation and emission wavelengths (Mobed et al., 1996). Such a supposition can be discounted by the absorbance results (Figure 3.7), however, which showed no differences in absorbance between the various sites or seasons. A second possible explanation is related to the presence of larger molecules which fluoresce at longer wavelengths than smaller ones (Senesi 1990). Figure 3.6 does show that the fall is the only period in which FA of cave waters contains an abundant 1-10 kD fraction, contrasted with the dominance of the <1 kD fraction for the rest of the year. For all seasons but the fall, there is a striking difference in spectra between cave waters and surface waters. This is consistent with the observed dominance of smaller organic molecules for most of the year in the cave waters.

Excitation data for HA (Figure 3.8) display closer similarity between the surface and cave waters than is seen in the FA data. For all the sites and seasons, the common

excitation peaks are at 255 nm and 310 nm. The clay water has a slightly longer peak at 320 nm for all the seasons except the fall. Adopting the proposition that larger molecules cause longer wavelengths, Figure 3.6 does show that the clay possesses a greater dominance of the 0.45 μm-100 kD fraction than the other solutions. Hence, seasonal differences in the size fractions of the waters can cause differences in the excitation spectra generated.

b) Emission

The most common excitation peak wavelength for all the solutions in Figure 3.8 was at 255 nm; accordingly, this was the fixed wavelength for the emission measurements of FA-HA fractions in Figure 3.9. The cave waters fluoresce at shorter wavelengths than the surface waters and SRFA, once again suggesting the dominance of smaller molecules (Senesi, 1990). In the fall, the presence of the 1-10 kD fraction produces a longer wavelength fluorescence which more closely resembles that of the surface waters. Until the fall, cave water FAs fluoresce more strongly than the surface waters, a phenomenon that is attributed to the dominance of higher molecular weights and to higher concentrations in the surface waters (Figure 3.4a and 3.4b). Larger molecular weight compounds and high concentrations produce self absorbance which decreases the fluorescence intensities (Mobed et al., 1996). The differences in emission spectra of the cave and surface waters could also be caused by organic substances contributed by the dissolved bedrock, as noted above; a major problem with this hypothesis is that the fall does not exhibit the same spectra as the rest of the year and, even during the summer, Tomtom shows the same fluorescence as the surface waters. If the hypothesis was correct, the fluorescence of the cave waters should be similar for the whole of the year, not just part of it.

Once again, there are strong similarities between the HA emission wavelengths of the surface and cave waters. However, all the sites are now emitting at shorter wavelengths than AHA, a feature probably caused by a lesser dominance of larger molecular weight compounds in these waters when compared to AHA. HA molecular size ranges from 2 to

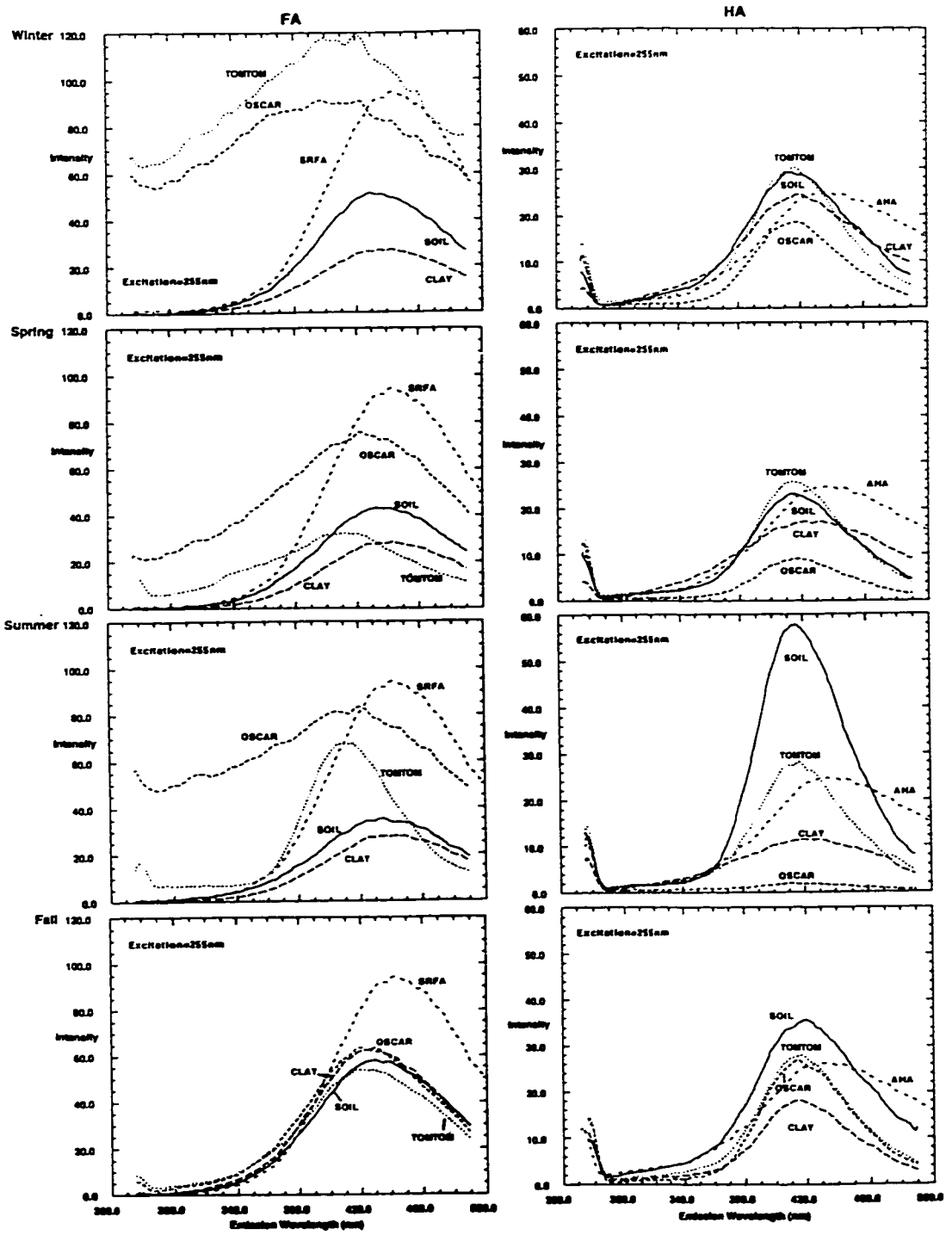


Figure 3.9. Emission spectra of fulvic and humic acids for surface and cave waters. SRFA and AHA are included for comparison purposes.

300 kD (Suffett and McCarthy, 1987). The clay exhibits a longer fluorescence than the other sites, which is probably caused by the prevalence of these higher molecular weight species during the spring and summer, times of most active production of humic substances.

The fall emission spectra strongly resemble those obtained by White (1997) which show a peak emission fluorescence centred on 420 nm produced by organic molecules removed from samples of calcite speleothems. The dominant molecular weight contributor to this fluorescence was the 5-10 kD fraction. This fits well with the dominance of the 1-10 kD molecular weight fraction in the fall at Marengo.

c) POC fluorescence

To enable comparison with the fluorescence of the HA and FA, the same fixed emission wavelength of 425 nm was selected (Figure 3.10). Two common excitation peak wavelengths were centred on 240 and 255 nm for both FA and HA. Excitation spectra are identical from the winter to the summer and only increase in intensity in the fall. The high POC concentration in the fall for Tomtom (Fig 3.4a) explains such a result but the high intensities generated by Oscar were not expected because of its low POC concentrations for this time of year.

Emission spectra were obtained using an excitation wavelength of 240 nm instead of the 255 nm used for FA and HA because the data showed 240 nm to be the dominant wavelength of excitation and the cave waters did not always display the 255 nm peak, e.g. in the winter. It is somewhat surprising with such similar POC excitation spectra for all the waters that emission spectra of cave and surface waters (Figure 3.10) were so dissimilar. The surface waters produce an emission peak at 445 nm, approximately 90 nm longer than the cave waters, a result most evident in the winter and summer. It appears that the cave water POC has much lower average molecular weights than the surface waters (Senesi,

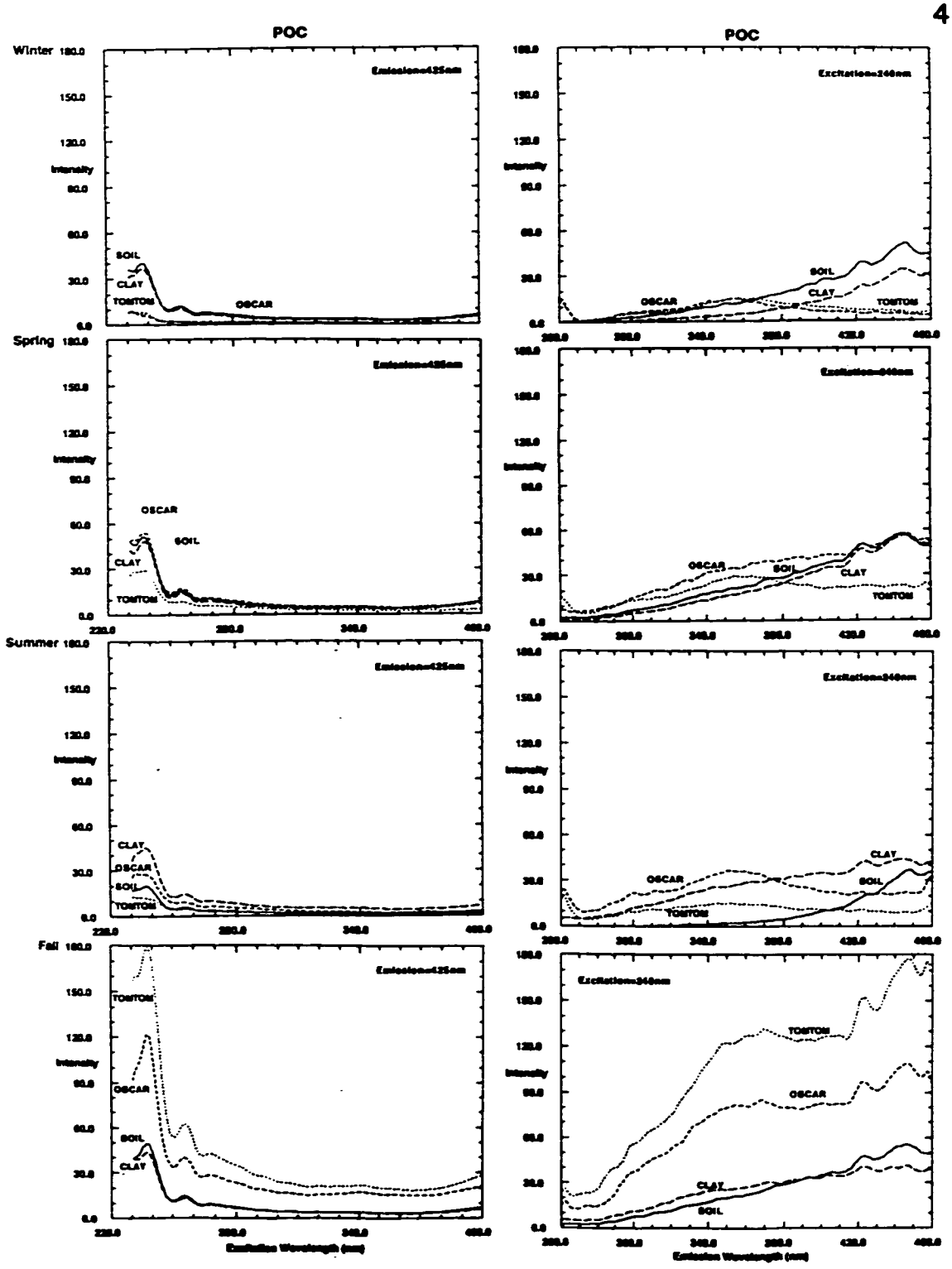


Figure 3.10. Excitation and emission spectra of particulate organic carbon for surface and cave waters

1990). In the fall, when the POC increases in the cave water, the fluorescent emission peak of the smaller molecular weight compounds is still evident but is not as dominant as the peak at 445 nm. Only during the spring at Oscar does the fluorescence of POC in the cave water resemble that of the surface waters, a result attributed to POC being flushed from the soils with the spring rains. Possibly only lower molecular weight compounds (<100 kD) may be washed into the cave during most of the year, whereas in the fall, there is an abundance of fresh POC produced by the decay of the leaves from the deciduous forest above the cave which is flushed into the cave waters. The main weakness of this hypothesis is that the surface waters have the same fluorescent fingerprint the whole year. A second hypothesis is that this variability is generated by the low precipitation of the fall, leading to the "concentration effect".

3.6.2 Seasonal Weather Changes and Responses in Soil and Cave Waters

The next objective of this study is to determine whether changing surface conditions generate any response in the cave. Such changes can be of temperature, precipitation or recharge that transmit their influence translated through the surface waters to the cave. Influences could be exerted on the organic concentrations in the waters and consequently on the fluorescent intensities. Accordingly, scatter plots were produced to check the relationships between seasonal temperatures, precipitation levels, fluorescence emission peak intensities, total organic matter (TOM = POC +FA+HA) and dissolved organic carbon (DOC), a surrogate for concentration of FA and HA in the solutions. The reason for use of both DOC and TOM is that POC may not become trapped in the calcite of the speleothem even though it is present in the cave water. Its presence may depend on the sizes of the crystals in the speleothems. A study of POC in calcite speleothems (van Beynen et al., 1998) showed that not all have significant abundances of this larger organic matter, although they do have both FA and HA. Due to the low number of data points, the scatter plot displays were preferred to correlations: therefore, any relationships portrayed by the lines of best fit drawn on the plots are not necessarily significant in a strict statistical sense.

Figure 3.11 shows x-y scatter plots for these relationships between TOM, DOC, fluorescence intensity and climate. The only plots where all the sampling sites have clear relationships with the weather are for DOC. The patterns in the surface waters suggest that increases in temperature promote augmented DOC concentrations. With increased temperature at the surface, more water is lost to evapotranspiration, therefore the DOC levels in the water will increase.

For DOC and precipitation the cave and the B horizon show noticeable negative relationships. The A horizon is weakly positive. The fall is the driest period at Marengo Cave, when the amount of water that flows through the clay and into the cave decreases, thereby augmenting the concentration of dissolved organic carbon in solution. The base of the clay is the final soil zone above the cave and therefore the relationship that exists there between DOC and precipitation may be carried through into the cave, as appears to happen in this case. The soil A horizon probably has more rapid flow of water, dries out more quickly and therefore requires more precipitation to flush DOC from its matrix. Conversely, the clay with its finer grains retards water flow and dries out less quickly, a feature that will affect the concentration of DOC coming from its matrix. However, all these hypotheses are very tentative because of the low number of data points. Clearly more sampling is required before firm conclusions can be made.

It is reassuring to observe that for at least the DOC - temperature and precipitation relations that two drip sites in the cave show the same trends (Figure 3.11). This has important implications for paleoenvironmental work because it is desirable that changing environmental conditions have the same effect at different locations in a cave if speleothems are to be removed for fluorescence analysis and averaged chronologies produced from them.

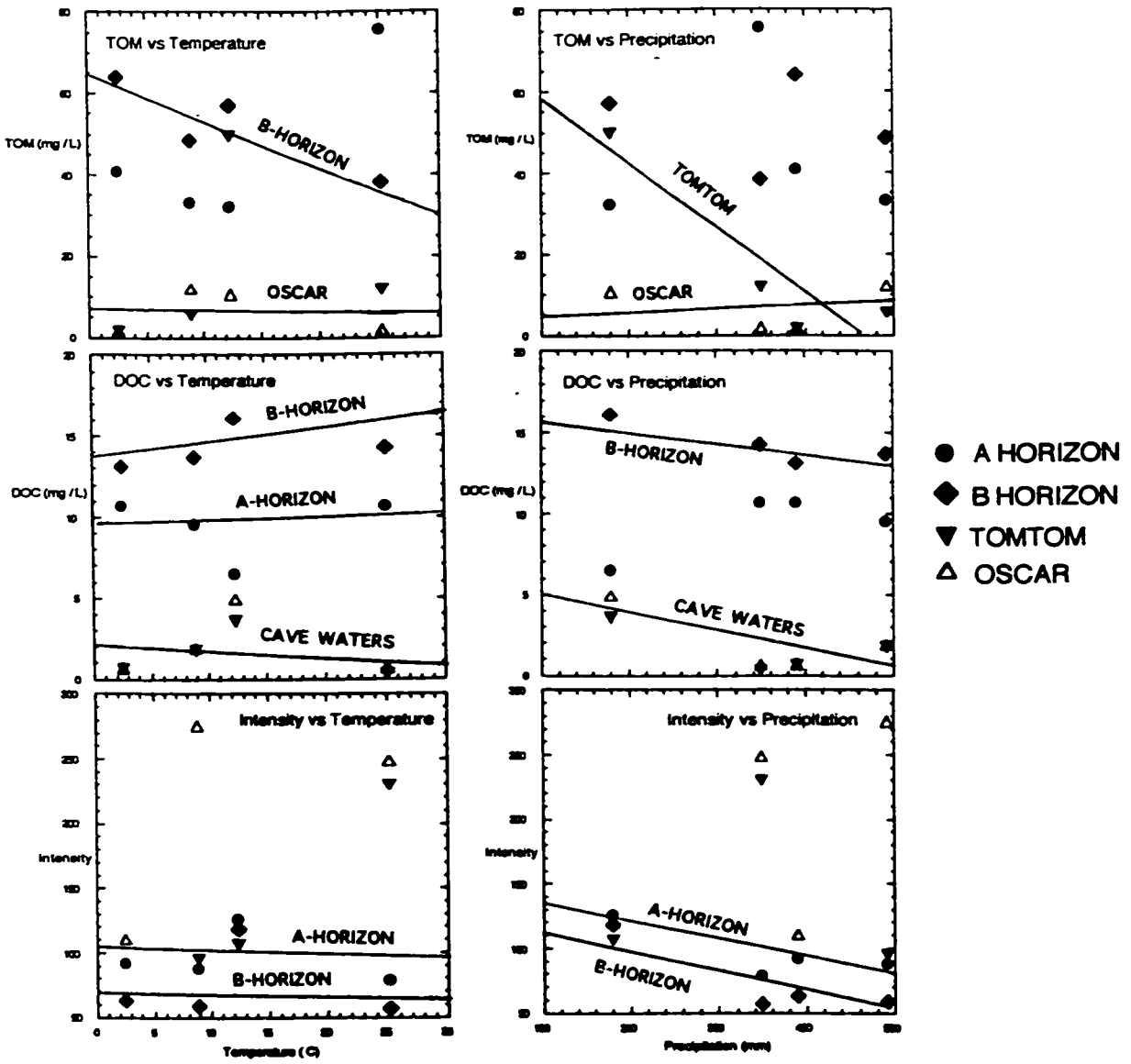


Figure 3.11. Scatter plots of dissolved organic carbon, total organic matter and fluorescence intensity vs climate. Lines of best fit added to graphs are done so by eye and do not represent any statistically significant relationship

3.6.3 Differences Between Cave and Soil Organic Substances

Figures 3.4 and 3.6 show that there are differences in organic substances produced as water travels from the surface to the cave. In addition to reduction in concentration by 60-80% there are significant changes in the distribution of the organic species. In the cave waters, HA is significantly less abundant (≈10 times) compared to the surface waters, which is probably due to its hydrophobic nature (Drever and Vance, 1994, Jardine, et al., 1989). This result is given added weight by the lower abundance of intermediate molecular weight compounds present in the cave water. HA molecular sizes range from 2 to 300 kD (Suffett and McCarthy, 1987), and only in the fall season do lower molecular weight compounds of HA (contained within the 1-10 kD fraction) reach the cave. The change in molecular weight distribution may be partly produced by Ca ions complexing the 1-10 kD fraction into larger molecules as pH rises with the percolation waters passing through the rock, as suggested above. This explains the virtual absence of this fraction and the presence of some of the larger fractions instead.

A comparison of the fluorescent intensities at peak emission wavelengths gives some indication of the dominant types of organic compounds present. Figure 3.6 showed the dominance of larger compounds in the surface waters when compared to the cave and results presented in Figures 3.8 and 3.9 seem to confirm this. Figure 3.12 depicts the differences in fluorescence between the two realms, with the cave waters fluorescing at shorter wavelengths and more intensely. Mobed et al. (1996) note that higher concentrations of organics can cause increased self absorption, which may generate such shifts. However, from Figure 3.6, the absorbances of the soil A and clay waters are very similar, which contradicts this suggestion. The importance of lower molecular weight compounds is supported by the work of Senesi and others (1991), who show that low molecular weight compounds with abundances of hydroxyl, methoxyl and amino groups can produce this enhanced fluorescence at shorter wavelengths compared to the carbonyl

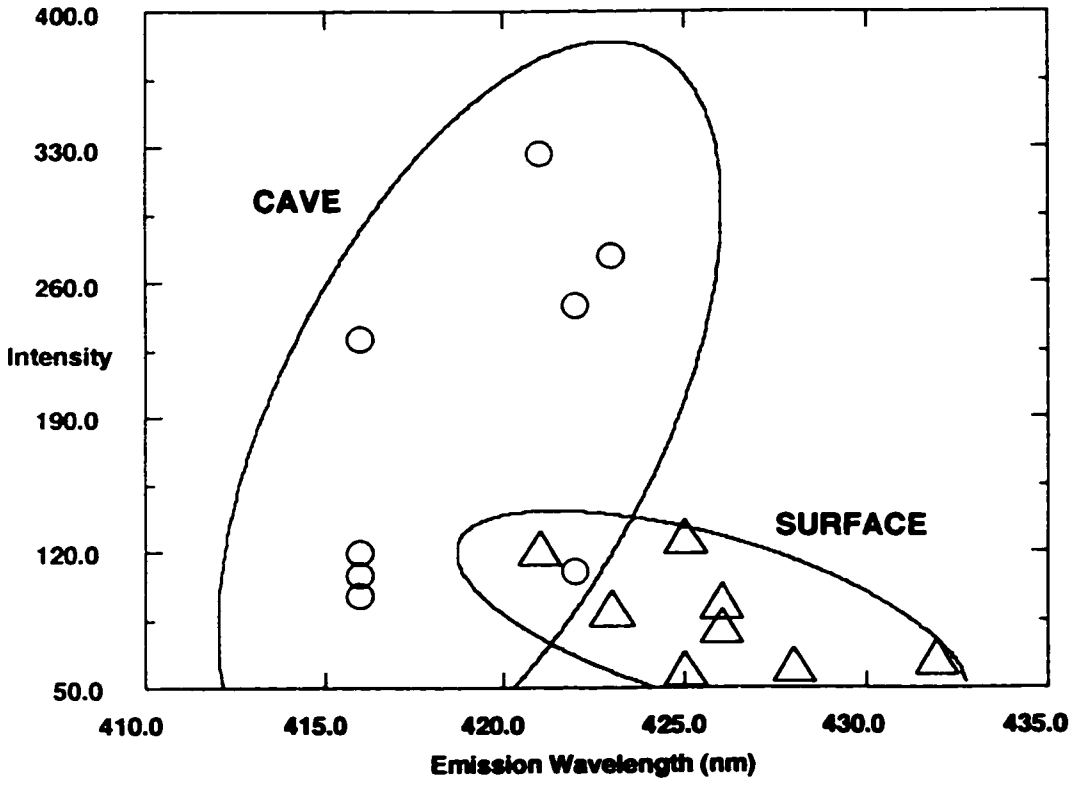


Figure 3.12. Comparison between surface and cave water fluorescence emissions and intensities

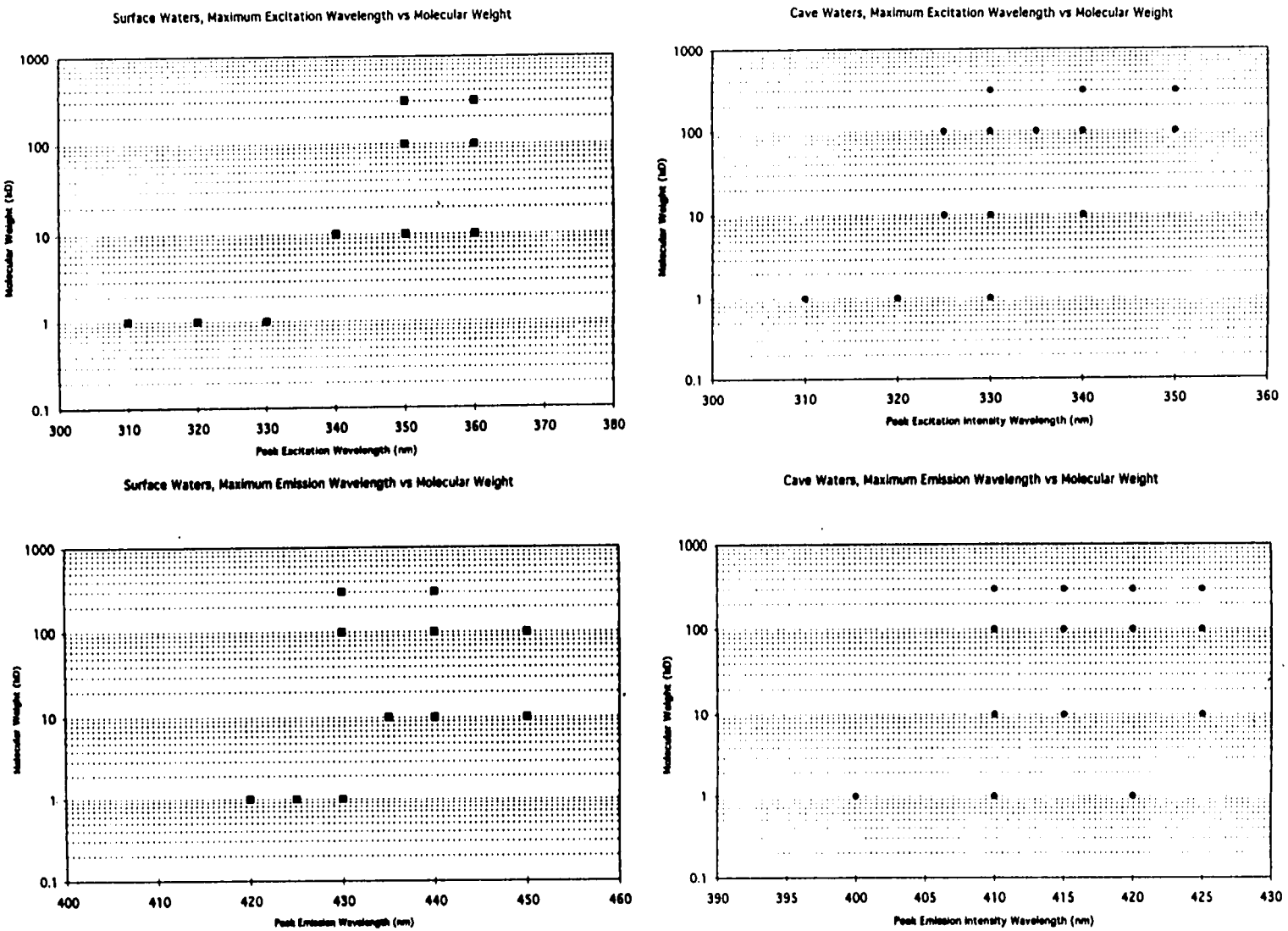


Figure 3.13. Scatter plots of excitation - emission peak intensity wavelengths and molecular weight fractions for surface and cave waters. Note that the axes do not have the same scales. The molecular weight fractions are represented as follows: 300 kD (<0.45 μm), 100 kD (<100 kD), 10 kD (<10 kD) and 1 kD (<1 kD)

and carboxyl groups of the larger molecules which reduce the fluorescence intensities and emit at longer wavelengths. Hence, the cave waters may be characterized by greater abundances of hydroxyl, methoxyl and amino acid groups than the surface waters. Further studies of NMR and IR spectra of these materials would be needed to test this proposition. However, we can partly confirm it by a comparison of molecular weight and wavelength of maximum excitation and emission (Figure 3.13). This figure shows the change in maximum fluorescence excitation and emission wavelengths as larger molecular weight molecules are progressively removed from the water. There is a decrease in the maximum intensity excitation and emission wavelengths with the removal of the larger molecular weight compounds. Such a shift is particularly evident in the excitation diagrams for both the surface and cave waters. The TDOM fraction, here presented as molecular weight at 300 possesses longer excitation and emission peak intensity wavelengths than the other extreme, the <1 kD fraction. Although concentrations of organic substance concentrations are higher in the TDOM fraction, the presence of the carbonyl and carboxyl groups may also contribute to the longer fluorescence (Senesi et al., 1991). The same difference in fluorescence wavelengths is also noticeable when comparing the cave and surface waters because the cave waters always fluoresce at shorter wavelengths.

3.7 Conclusions

The main aims of this study were to ascertain how a changing surface and soil environment may result in variable fluorescence within a speleothem in a cave below. It is not understood what interactions occur between the base of the soil and the drip point in the roof of the cave. A summary of the findings of this paper are as follows:

1) There was no seasonality in dissolved organic matter in the A- or B- horizons of the soil.
2) Striking seasonality appears in dissolved organic concentrations at both cave drip sites.
3) FA dominates over HA in both the surface and underground waters. There is little seasonal change in this relationship.

4) There is a striking change in the molecular weight partitioning between soil and drips.

5) There are clear differences in the fluorescence emission maximum and intensity between surface and drip waters, which are partly explained by the shift in the molecular weight changes between the surface and cave.

6) Ca complexes may form in the rock-water interface zone as pH rises, which could affect absorbance and molecular weight distribution of the cave waters.

Chapter 4: Causes of Colour and Fluorescence in Speleothems

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Abstract

When illuminated with UV light, speleothems fluoresce between 410-460nm. Here we are attempting to determine the nature of the fluorophores, thought to be either trace elements or organic acids trapped in the calcite. Fluorescence of solid speleothems was measured as well as that of organic species extracted from the calcite, to quantify their contribution to the observed fluorescence of the speleothems. All speleothems and extracts gave similar spectra with broad emission maxima centred around 410-430 nm, and two excitation maxima at approximately 255 nm and 330 nm. The organic compounds were partly characterized using fulvic acid (FA) - humic acid (HA) separation and molecular size fractionation. Trace elements, determined by neutron activation analysis, do not appear to be responsible for the observed spectra. Organic acids, particularly FA, were found to be the dominant fluorophore in the calcite. Darker speleothems, although having higher concentrations of FA and HA than light speleothems, had lower emission intensities, due to self-absorption. Average POC, FA, HA and TOM concentrations for the dark speleothems were twice that of their light counterparts. There is some correlation between vegetation type above the cave and the type of organics present in the calcite.

4.1 Introduction

Speleothem is a generic term given to stalagmites, stalactites and flowstone which are calcite or other mineral cave deposits. They display two important types of optical

properties. When viewed either in reflected or transmitted visible light, they display varying degrees of colour hue and intensity. Secondly, when illuminated by ultraviolet light, they emit fluorescent light in the visible range. Both of these effects are presumably connected to the presence of minor constituents in these calcitic deposits since chemically pure calcite is neither coloured nor fluorescent. Some of these properties have been attributed to the presence of trace elements in the calcite, but since the early work of Gilson and MacCarthney (1954), it was suggested this fluorescence was caused by organic substances, a supposition supported by Gascoyne (1977), Lauritzen et al. (1986), and White and Brennan (1989). Lauritzen et al. (1986) in particular demonstrated that much of the colour must be due to the presence of organic substances (humic compounds) dispersed in the calcite. Similarly, Shopov et al. (1994) argued that the fluorescent properties of speleothems were also due to the presence of organic compounds trapped in speleothems. The purpose of this paper is to study more closely the relationship, if any, between abundance and type of organic substances in speleothems and their colour and fluorescence.

Calcitic cave deposits contain paleotemperature records as derived from oxygen isotopes (Schwarcz, 1986, Gascoyne, 1992) and paleovegetation records as seen in the carbon isotope records (Dorale et al., 1992). These records can be dated with U-series analysis to a precision of ±1% up to 350Ka (Li et al., 1989). Broecker et al. (1960) using 14C dates found laminae in parts of speleothems that were annual in resolution. Such laminae are differentiated through changing colour of the calcite and are composed of a couplet of light and dark calcite. Their presence has also been revealed using fluorescence microscopy (Baker et al, 1993, Shopov et al, 1987, 1994). Production of annual laminae within calcite is to be expected: organic matter in litter and soil decomposes due to activity of microorganisms and percolation waters then transport the products through the soil and rock to the cave. Throughout the passage, organic matter may be altered by further

decomposition or removed by filtration processes occurring in the mineral medium and rock, but some remains in solution. A complicating factor is that some ancient organic substances may also be obtained from the dissolution of the limestone bedrock. Upon reaching the cave, degassing of CO_2 from aqueous solution may lead to calcium carbonate saturation and calcite or aragonite precipitation. Calcite has been shown to be an efficient adsorber of organic matter, especially of lipid material and amino acid substances (Suess, 1970, Carter, 1978). Mitterer (1968) found that organic matter may even induce $CaCO_3$ precipitation by concentrating calcium ions.

The principal subjects of this paper are the precise sources of the fluorescence in speleothems and the relationships between fluorescence and colour hue and density, chiefly the chemical differences between light and dark calcite. Organic substances are not the only natural entities which might fluoresce in speleothems. Rare earth elements such as samarium, dysprosium, europium, terbium and manganese have been shown to produce fluorescence when coprecipitated with calcite; in particular, europium and dysprosium fluoresce at the same wavelengths as the organic acids (Mason and Mariano, 1990). The calcium ion can be replaced in the lattice by these smaller divalent and trivalent fluorescing cations through substitution (Terakado and Masuda, 1988). In addition to these principal foci, we hoped to begin to establish the effects of differing climate, vegetation and soil above a cave upon the colour and fluorescence of speleothems precipitated within it.

Organic substances in soil waters may be divided into three components, which are operationally defined by their solubility in acid-base (Hayes et al., 1989). Fulvic acids (FA) are soluble in all conditions of acid-base; humic acids (HA) precipitate when the solution becomes acidic (as pH approaches 2); humin is insoluble in any solution, although it may be mechanically flushed from soils. It is believed that the residence times of these compounds in soils may range from decades to hundreds of years for FA and thousands of

years for HA (Schlesinger, 1977). The molecular weights of FA tend to be smaller than HA (Suffet and MacCarthy, 1987). Further differentiation of the FA is possible by partitioning them into hydrophilic and hydrophobic acids, bases and neutrals (Leenheer, 1985). Their structural complexity has hindered their further characterization (Ertel, 1988); however, major functional groups identified thus far include carboxyls, phenolic hydroxyls and alcohols (Visser, 1983). NMR studies have revealed ketone/aldehyde, carboxyl, aliphatic and aromatic hydroxyl groupings. Spectroscopic studies by Wilson and others (1987) and Wershaw (1985), have confirmed these findings.

The typical molecular weight of FA is 800-1000 daltons. HA are found to range greatly, from 2000 to 300,000 daltons (Suffet and MacCarthy, 1987). Their differentiation can be undertaken by gel permeation chromatography or ultrafiltration (Wershaw and Aiken, 1985). Much work has been done on the fluorescence of these substances. Suwannee River Fulvic Acid (Miano, 1988) is often used as a fluorescent standard for FA. It has a characteristic maximum excitation peak at 320 nm, a maximum emission peak at 425 nm and an average molecular weight of 2310 daltons (Chin et al., 1994). Humic acid has longer excitation and emission wavelengths, with respective peak intensities at 480 nm and 540 nm (Hayase and Tsubota, 1985). Aldrich HA is a commonly used standard for humic acid; it is larger than Suwannee River FA, with a molecular weight of 4100 (Chin et al., 1994).

4.2 Research design and procedures

Where alternating light and dark annual calcite bands occur in speleothems they are usually at a microscopic scale. To investigate their differences, larger samples that exhibit more uniform colour are desirable. We selected a set of twelve speleothems from a collection at McMaster University, ranging in colour from translucent white to opaque and nearly black. Their fluorescence was measured in the solid calcite and in solution. Solutions were fractionated into the FA, HA and particulate organic carbon (POC). The

hydrophilic and hydrophobic components were then separated for measurement of their individual fluorescence and DOC. Subsamples of the solid calcite were subjected to neutron activation analysis to determine whether there could be any significant trace element or REE contributions to the fluorescence spectra. These procedures are summarised in a flowchart, Figure 4.1.

4.3 Provenance of the sample speleothems

In addition to colour hue and density, an important criterion for selecting the speleothems was the variety of climate and natural vegetation found at the site of collection today . A considerable natural range was obtained. The samples are from the following locations:

Crowsnest Pass (49°30'N, 114°30'W) and Bow Valley (Ratsnest Cave, 51°45'N, 115°30'W) in the Rocky Mountains of Alberta-British Columbia. The settings range from boreal forest to alpine shrub tundra. Winters are cold and there is a strong spring thaw freshet. Samples - CNP, CNPLB and CNPDB (light and dark layers from the same speleothem) , RNCP and RNCF2.

Jewel Cave, Black Hills, South Dakota (43°44'N, 103°55'W). The setting is subhumid today, at the lower limit of ponderosa forest. There is a well-marked spring thaw. Sample - JC11.

Cold Water Cave, Iowa (42°3'N, 90°39'W) is beneath a deciduous forest but close to the tall grass-prairie transition. There is a spring thaw freshet. Sample - CW4.

McFail's Cave, New York (38°22'N, 86°20'W) is located in the Carolinian forest zone with milder winters than the previous sites but with Spring and earlier snap thaw events. Samples - MF1 and MF2.

Ogof Ffynon Ddu, Wales (52°N, 3°30'E) is in a temperate maritime setting beneath a deciduous (ash, oak) forest. Sample - OFD.

Rebecca's Cave, Cayman Brac (19°44'N, 79°48'W) has a tropical maritime climate with a summer rainfall bias. There is scrub vegetation on phytokarst above the cave. Sample - RCB.

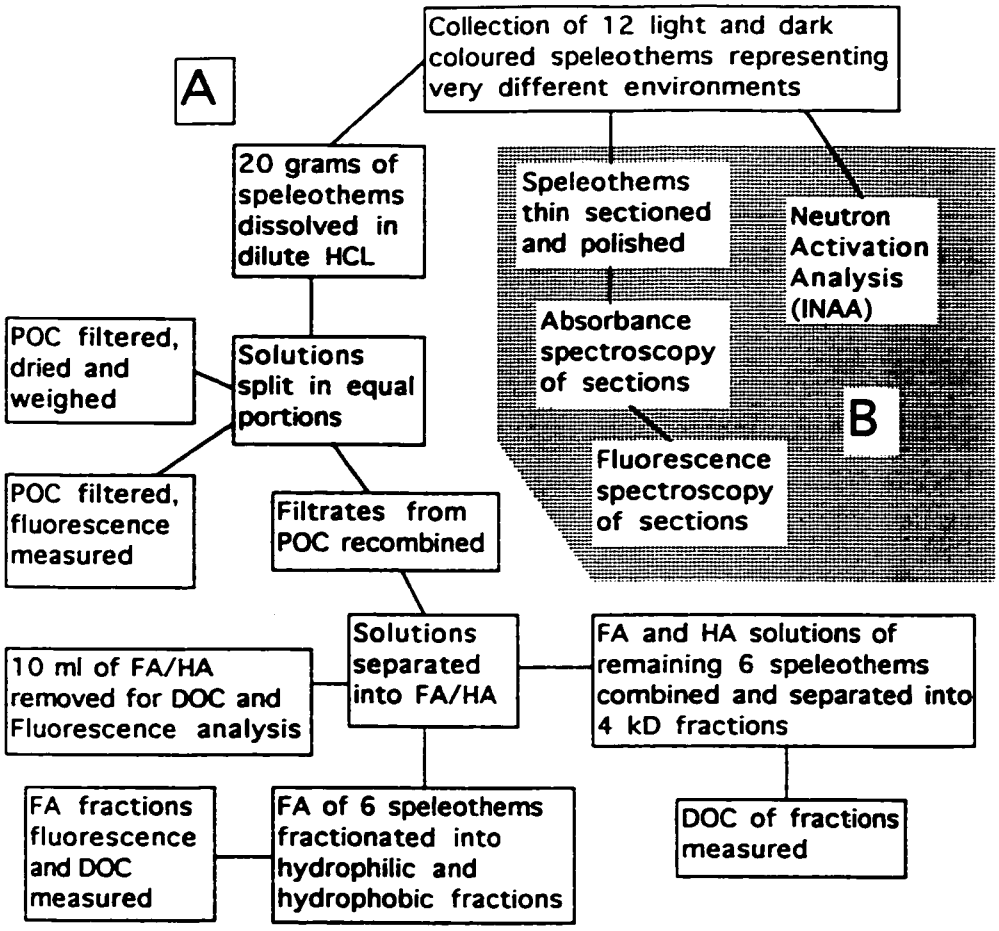

Figure 4.1. Flowchart of analysis undertaken on speleothems: a) Dissolved speleothems, b) Solid speleothems

Kimberley Ranges, Western Australia (26°S, 120°E) represents the warm arid limits for speleothem deposition today, with desert-savannah vegetation and sparse summer rains. Sample - WAH.

Further details of the locations and colours of the sample speleothems are given in Tables 4.1 and 4.2. The speleothems mentioned in Table 2 are arranged according to colour gradation, lightest to darkest. Six light and six dark coloured speleothems were selected.

4.4 Analytical Methods

Isolation of Humic Substances

20g of powdered calcite from each speleothem was added to 500 ml of MilliQ water and dissolved by adding sufficient 4N HCl to dissolve the calcite, releasing the humic substances. The pH of the resulting solution was adjusted to approximately 8 with NaOH so that HA would not precipitate, and the solution split in two equal portions. Both portions were filtered using Whatman GF/F glass fibre filters (0.7 microns) to remove the POC. To examine both the fluorescence and the concentration of the POC, one filter was then flushed with MilliQ water to remove most of its POC and the other was dried in an oven and weighed (the filter having been dried and pre-weighed). After POC removal, both filtrates were recombined for FA-HA separation.

HA was precipitated from the recombined filtrate by adjusting pH to 2, was left to stand for 24 hours and then filtered with Whatman GF/F filters (Leenheer, 1985). Filters were then rinsed with 0.1 N NaOH to resolubilize the HA. The remaining filtrate contained organics now operationally defined as FA. To collect any extra FA that may have been trapped with HA, the HA solution was acidified to pH of 2, filtered a second time and this filtrate added to the earlier FA solution. HA was then washed from the filter with the 0.1 N NaOH. All fractions were refrigerated to prevent any organic decay. 10 ml of the FA - HA solutions was removed for fluorescence spectroscopy and DOC analysis. The

Table 4.1: Locational Climate Descriptions

Site	mean annual temp. (°C)	mean daily Jan. temp. (°C)	mean daily July temp. (°C)	mean total precipit. (mm)	mean cave temp. (°C)
Crowsnest Pass	3	-10	15	1000	1.8
Cold Water	8.5	-7.5	23	820	8.5
Ratsnest	8.4	-6.5	22.3	263	8.4
Jewel	6.6	-4.8	18.1	525	9.4
McFails	7.5	-4.7	19.1	960.4	7.5
Kimberley	24.1	30.4	17.8	284	24.1
Cayman Brac	25.5	22.5	28.5	1025	25.5
OFD	8.3	2.5	14	2200	8.3

Table 4.2. Colour of the Solid Speleothems

	Sample	Colour (Munsell Soil Chart)	Description
Light	WAH	N 8/0	Grayish White
	MF1	5Y 7/1	Light Gray
	CNPLB	10YR 7/1	Light Gray
	RNCF2	5Y 8/1	Light Gray
	CW4	5Y 8/2.5	Light Gray\Pale Yellow
	RNCP	5Y 8/2.5	Light Gray\Pale Yellow
Dark	MF2	10YR 6/1	Brownish Gray
	OFD	5YR 4/8	Reddish Gray
	JC	5YR 5/3	Dull Reddish Brown
	RCB	5R 4/4	Dull Reddish Brown
	CNPDB	7.5R 3/2	Dark Reddish Brown
	CNP	7.5R 1.7/1	Reddish Black

remaining FA/HA solutions were then divided; the FA portion of three light and three dark speleothem solutions was used for the FA fractionation; and the FA-HA solutions of the remaining six speleothems were recombined and used for size fractionation. The final volume of the solutions for FA was approximately 800 ml and 150 ml for the HA.

FA Fractionation

The fulvic acid fraction, at low pH was treated like a water sample to separate the FA into six subfractions according to the method of Aiken et al. (1992) as modified in the laboratory at Canada Centre for Inland Waters (CCIW). This method involves separating hydrophobic components using XAD-8 resin and hydrophilics using XAD-4 resin. The density of the FA solution obtained as described above was higher than normal water samples and the sample was diluted with E-Pure water 2:1 to avoid problems due to floating resin beads. Briefly, at low pH, hydrophobic acids (HPOA) are retained on the XAD-8 resin and are subsequently eluted with 0.1N NaOH. Hydrophobic neutrals (HPON) remain on the XAD-8 resin after base elution. The organic matter (DOM) which passed through the XAD-8 resin is defined as hydrophilic. The hydrophilics are fractionated into hydrophilic acids (HPIA), which adsorb to XAD-4 at low pH and are eluted by 0.1N NaOH, hydrophilic neutrals (HPIN), which pass through both resins at low pH, and "XAD-4 acids" (Aiken et al., 1992), which remain on the XAD-4 resin after base elution. Thus by these procedures, the total soluble organic matter from the speleothems is fractionated into these six subfractions: HA, HPOA, HPON, HPIA, HPIN, X4AC. All of these fractions are quantifiable by measuring dissolved organic carbon (DOC) values at various points of the fractionation scheme (modified from Richmond and Bourbonniere, 1987). In all cases DOC was determined using a Dohrmann DC-190 Carbon Analyzer with platinum on alumina catalyst at 900° C. Standards and blanks were run daily to determine the daily system blank which was use to correct all sample results.

Dissolved Organic Carbon

Dissolved organic carbon measurements were made with a Dohrmann DC-190 carbon analyzer AT 9000 on all the dissolved fractions, namely, the bulk FA, bulk HA, hydrophilic and hydrophobic acid, basic and neutral FA. Inorganic carbon was first removed from the samples by the carbon analyzer through acidification with 20% H_3PO_4 and purging with nitrogen.

Calculation of Concentration of Organic Matter Fractions

The DOC values for FA and HA were adjusted to one litre so that all the solution volumes were equal. However, DOC analysis only measures the organic carbon content of the solution, but carbon accounts for approximately 50% of the total in FA (Suffett and MacCarthy, 1989) and 63% in HA (MacCarthy and Malcolm, 1989). To provide a more accurate estimate of total amount of organic matter in the FA and HA, these proportions were used to correct the DOC values. The resulting values now represent the total amount of organic carbon in 20 gm of calcite. Finally, all these values (in mg/ 20 gm calcite) were divided by 20 to convert them to total weight percentage of organic matter in the calcite for each fraction.

POC was measured on the dried preweighed filter paper and the amount doubled because only half the total amount was dried on the filter. The POC concentrations for the fluorescence spectroscopy are reported in ppm units because they represent the concentration of POC in the immersion oil solution. Half of the total POC was resuspended and homogenised in 10 ml of the immersion oil (discussed below) from which ppm values could then be calculated.

Preparation of Solid Speleothems for Absorbance and Fluorescence

The solid speleothems were cut along their growth axes and finely polished to a

thickness of 2 mm ±10% with silicon carbide (grain size ~25-30 μm) to produce smooth surfaces which prevented scattering of the fluorescent excitation beam. Each polished section was taken from the same location in a sample as the 20 g of calcite used for the fractionation work. The strength of the calcite was such that no backing or reinforcement was needed.

Absorbance Spectroscopy

Absorbance spectra of the solid speleothems only were measured with a Perkin-Elmer UV/Visible Lambda 6 spectrophotometer over the range 200 - 600 nm to characterize the concentration and nature of organic substances in the speleothems. Pure calcite is known to have negligible absorption in this region (Machel et al. 1991).

Fluorescence Spectroscopy

A Perkin-Elmer LS-5 fluorescence spectrophotometer recorded the fluorescence spectrum for each solid calcite sample and solution. 5nm slit-widths were used for the monochromator, with a default scan speed of 60nm/min. MilliQ water (DOC ≈0.5 ppm) and the empty cuvette were analyzed each day to test for stability and cleanliness of the apparatus; fluorescence intensity of all tests was within the machine background of 0.1-0.3 units. To allow semi-quantitative comparison of fluorescence intensities between solute samples and subsamples of the various fractionated and unfractionated dissolved speleothems, Rhodamine WT dye (1 ml of 1 ppm standard) was added to each sample after adjusting pH to 10. All fluorescence spectra were measured at pH 10 as this provides the best discrimination between organic species, particularly for FAs from different sources (Mobed et al., 1996). The maximum intensity of the Rhodamine WT standard also occurs at pH 10. Rhodamine WT was selected because it is a fairly stable dye and its fluorescence did not interfere with the fluorescence of the organic substances (Smart et al, 1978). A blank solution with the dye was used as a standard for fluorescence intensity and all spiked sample solution intensities normalized to this standard (Goldberg and Weiner, 1991). This

procedure compensates for machine drift and intrasample irregularities. Both excitation and emission spectra were measured for all fractions, with numerous scans being made to find the wavelengths of peak excitation and emission.

Size Fractionation

The remaining FA-HA speleothem solutions not used for the FA fractionation were recombined and separated according to their molecular sizes by ultrafiltration. An initial size fractionation had already been made with the POC having been removed, hence all the remaining fractions were <0.7 μm. The six speleothems used in this analysis covered the colour range from light to dark: Ratsnest Cave (RNCP, RNCF2), Crowsnest Pass (CNP), McFails Cave (MF2), Kimberley Ranges (WAH), and Ogof Ffynon Ddu (OFD). MSI centrifuge tubes were used for the 100 kD and 10 kD fractions and Spectrum Spectra Por 6 dialysis bags for 1 kD. The fractionations yielded size divisions of 0.7 μm-100 kD, 100-10 kD, 10- 1 kD, and <1 kD; to quantify these fractions, DOC values were measured by the method reported above.

POC Fluorescence Analysis

Because POC was insoluble in water, it was necessary to devise a method of suspending it in a fluid medium for fluorescence measurement. A fluid of higher viscosity than water was needed to keep POC in suspension, and with fluorescence emission and excitation peak wavelengths differing from POC. Fluorescence spectra of the solid speleothems indicated the wavelength range over which the POC would fluoresce. Cargille Immersion Oil (Type FF) was described by the manufacturer as having a viscosity of 170 cST and virtually no fluorescence, thus appearing to meet these criteria. However, fluorescence spectroscopy of the oil recorded a large peak in excitation at 258 nm and a smaller one between 280 and 290 nm. The lowest intensity of the oil was at an excitation wavelength of 350 nm: accordingly, this was used for the excitation of POC. It is a similar excitation wavelength to that used for the FA characterization and to that found in the solid

speleothems. The emission peak wavelength of the oil was well below 400 nm and did not fluoresce in the region where the solid speleothems fluoresced. The analytical procedure was to evaporate the water from the POC, which was then resuspended in the more viscous immersion oil and spiked with the dye in the same manner used for the solutions. 50% of the total amount of POC was suspended in the oil. Fluorescence intensities of the spiked samples were normalized to the fluorescence standard as described above. The solutions were stirred between each fluorescence run to prevent settling of the larger POC particles.

Neutron Activation Analysis

Trace element concentrations in the speleothems were determined by instrumental neutron activation analysis (INAA) in the McMaster University Nuclear Reactor, using a Ge (Li) detector (de Soete et al. 1972, McKlveen, 1981). The following trace elements were measured: As, B, Ba, Br, Ca, Cl, Co, Cr, Dy, Fe, Ga, I, K, La, Mg, Mn, Mo, Na, Nd, S, Sb, Sc, Sm, Ti, Th, U, V, W, and Zn..

4.5 Results and discussion

4.5.1 Causes of fluorescence in speleothems

4.5.1.1 Organic Compounds

The fluorescence of FA, HA and humins must be quantified to some degree before any interpretation can be made of the fluorescence of the speleothems. FA and HA are soluble at different pHs, hence both compounds can be examined spectroscopically. Humin, however, is insoluble at any pH; as a consequence it is difficult to quantify its fluorescence spectra, and thus it tends to be ignored in the literature. But as humin is insoluble, it is doubtful it would be entrained in the percolation waters and transported to the cave. POC and colloids, however, may be present in the cave feedwaters along with the soluble FA and HA; these compounds may resemble humins.

a) Standards for Organic Compounds

Figure 4.2 shows contour plots of the fluorescence of two humic substance standards, Suwannee River Fulvic Acid (SRFA) and Aldrich Humic Acid (AHA), in solution at pH 6.5 and at a concentration of 10 ppm. Both standards lack any sharp peaks in either excitation or emission wavelengths. SRFA displays two peak excitation wavelengths, at approximately 250-260 nm and 320nm, and one peak emission wavelength at 430nm. AHA shows a similar pattern less clearly but still has a shoulder of excitation centred on 250-260 nm excitation peak and 430nm emission peak. The smaller peak centred on 280 nm excitation and 260 nm emission is the Raman peak of water. This is only apparent in the HA because of its lower fluorescent intensities, which are approximately half of the FA; although at the same concentration as SRFA, AHA fluoresces less strongly. Senesi (1990) showed that with increased molecular weight, broadening occurs in the emission peak and there is a decrease in fluorescence efficiency, both of which are attributed to greater proximity of aromatic fluorophores to each other and deactivation of excited states by internal quenching in higher weight molecules.

b) Fluorescence of Speleothems

With this information about the fluorescence of standard organic compounds, the fluorescence of the speleothems can now be better understood. Figure 4.2 shows two examples of the fluorescence fingerprints of solid calcite, CW4 being a light-coloured sample from Coldwater Cave and JC11, a dark sample from Jewel Cave. CW4 fluoresces more strongly than JC11 and the two spectra differ quite markedly. JC11 has both longer excitation and emission peak wavelengths. More detailed interpretation of the differences between the light and dark calcite will be given below.

Comparison of the fluorescence of the speleothems with the FA and HA standards suggests that there are some similarities between SRFA and AHA and CW4. All have centres of peak emission at 430nm, shown by Miano et al (1988) and Mobed et al. (1996)

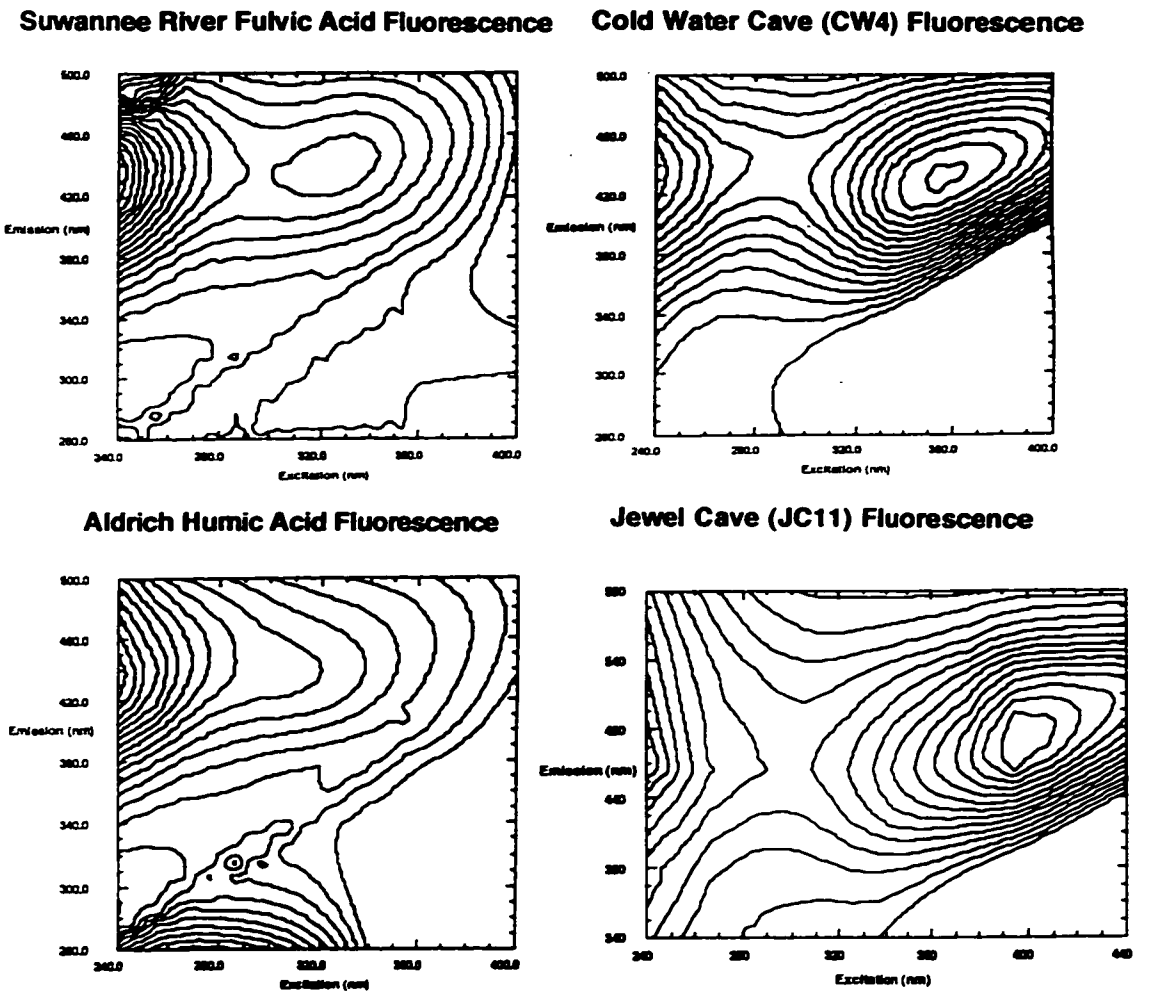

Figure 4.2. Fluorescence of standards for fulvic and humic acid, plus two speleothems: note the different scales on axes

to be indicative of FA fluorescence. However, the spectrum also resembles that of AHA. The latter does not display the distinctive excitation peak of the speleothem samples, but it does share the same emission peak at 430nm as CW4. The slightly longer wavelength excitation peak of CW4 is probably due to the higher concentration of organic substances in the calcite and its own absorption effect. Mobed et al. (1996) describe such an effect and show that it is caused by inner filtering produced by self absorbance. At higher concentrations, the incident light is absorbed by the molecules themselves, thereby removing the shorter excitation and emission wavelengths, an effect known as inner filtering or self absorbance. This effect is most pronounced when the fluorescence spectrum of JC11 is compared. It is a particularly dark calcite presumed to have high concentrations of organic substances; it displays much longer wavelength excitation and emission peak intensities, with the excitation peak centred on 400nm and the emission peak at 490nm.

In Figure 4.3 are shown the emission fluorescence spectra of the solid speleothems and the solutions. The spectra of the solid samples are similar but there is a clear distinction between the light and dark specimens, with the lighter speleothems fluorescing more intensely and at shorter wavelengths than the dark samples. Such a result can be explained by the self absorbance effect described in the previous paragraph. The emission wavelength peak centre between 430 and 450 nm and longer. A reversal of the solid speleothem results occurs when the speleothems are dissolved. Solutions of the darker speleothems now fluoresce more intensely. It appears that the self absorbance effect has been diminished because the organic molecules are at lower concentrations in solution than in the solid calcite. Peak intensity emission wavelengths are all centred between 410 nm and 430 nm. Spectra of the FA fraction are similar to that of the total dissolved organic matter (TDOM) solution with common intensity peaks at 425 - 430 nm, although as with the TDOM, intensities do vary. The darker speleothem FA fraction intensities vary from sample to sample but not greatly between light and dark coloured speleothems and certainly not as

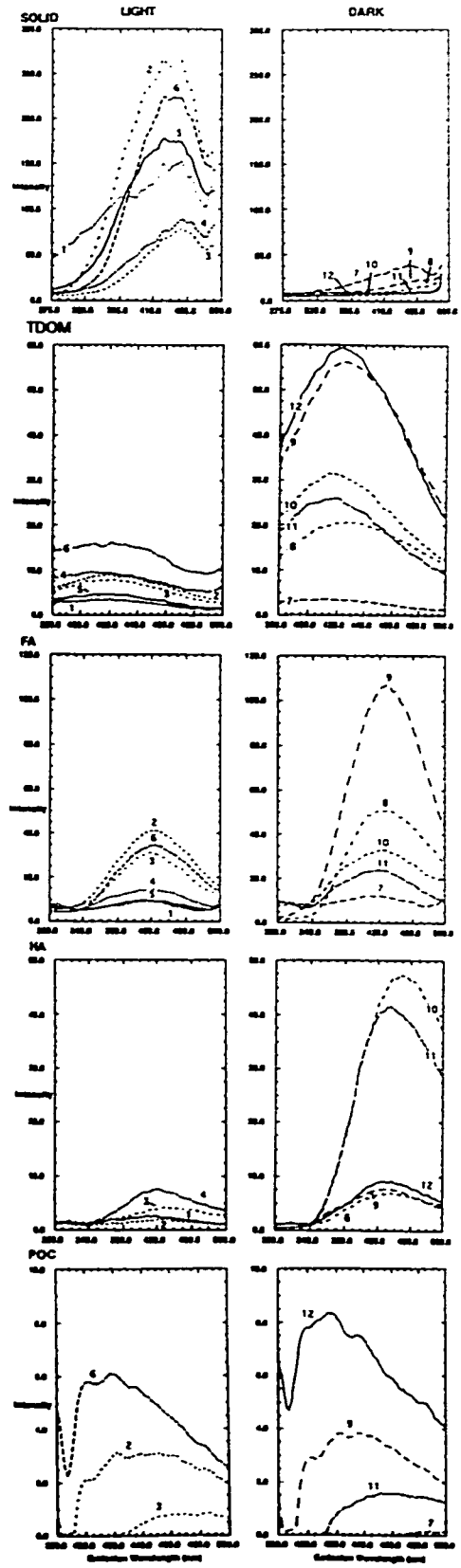

Figure 4.3. Fluorescence of various fractions of the speleothems: the numbered lines correspond to WAH (1), RNCP (2), MF1 (3), CNPLB (4), RNCF2 (5), CW4 (6), MF2 (7), OFD (8), JC11 (9), CNP (10), CNPDB (11) and RCB (12). Excitation wavelength is 255 nm, except for POC-350 nm

markedly as for the TDOM solutions. The HA fraction possesses a very similar emission to the FA fraction, although its intensities are lower. The POC spectra reveal great variability in both intensity and maximum emission fluorescence wavelengths. Whether such variability is produced by differences in concentration which could influence absorbance and consequently fluorescence intensity and wavelength will be discussed in subsequent sections.

c) Concentrations of the Fractions

Before discussing the specific fluorescences of each fraction of the organic components present in the speleothems, their quantities should be identified as these may help to explain the different intensities and spectra. Figure 4.4 gives concentrations in each speleothem in ppm per gram calcite. Concentrations for POC range between 67 ppm for RNCF2 to 1070 ppm for MF2. FA values are much lower, yet the ranges also differ greatly from only 0.208 for CNPLB to 5.346 ppm for JC11. Finally, HA concentrations are very low but also display variability, ranging from 0.028 ppm for OFD to 0.146 ppm for JC11. The most striking feature is the dominance of POC over FA and HA in all speleothems. The McFail's Cave samples have particularly high abundances as do the Crows Nest Pass speleothems. At such high proportional concentrations, we might expect to observe a quenching effect on the fluorescence of the speleothems, due to self-absorption, contributing to the lower intensities noted in the solid dark speleothems (Figure 4.3). However, the lighter speleothems, which fluoresced more strongly in the solid, also display high proportions of POC. Conversely, certain samples tend to have higher relative abundances of FA, especially RNCP, RCB, OFD and JC11. HA does not appear to be very prominent, except in CNPDB, CNP and JC11. The relative abundances of FA and HA are more clearly displayed in Figure 4.5. Generally HA is at least five times less abundant than FA. Such a result must have important implication for the contribution of HA to the fluorescence of the speleothems. Although not shown in Figure 4.2, SRFA has twice the fluorescence intensity of AHA measured at the same excitation and

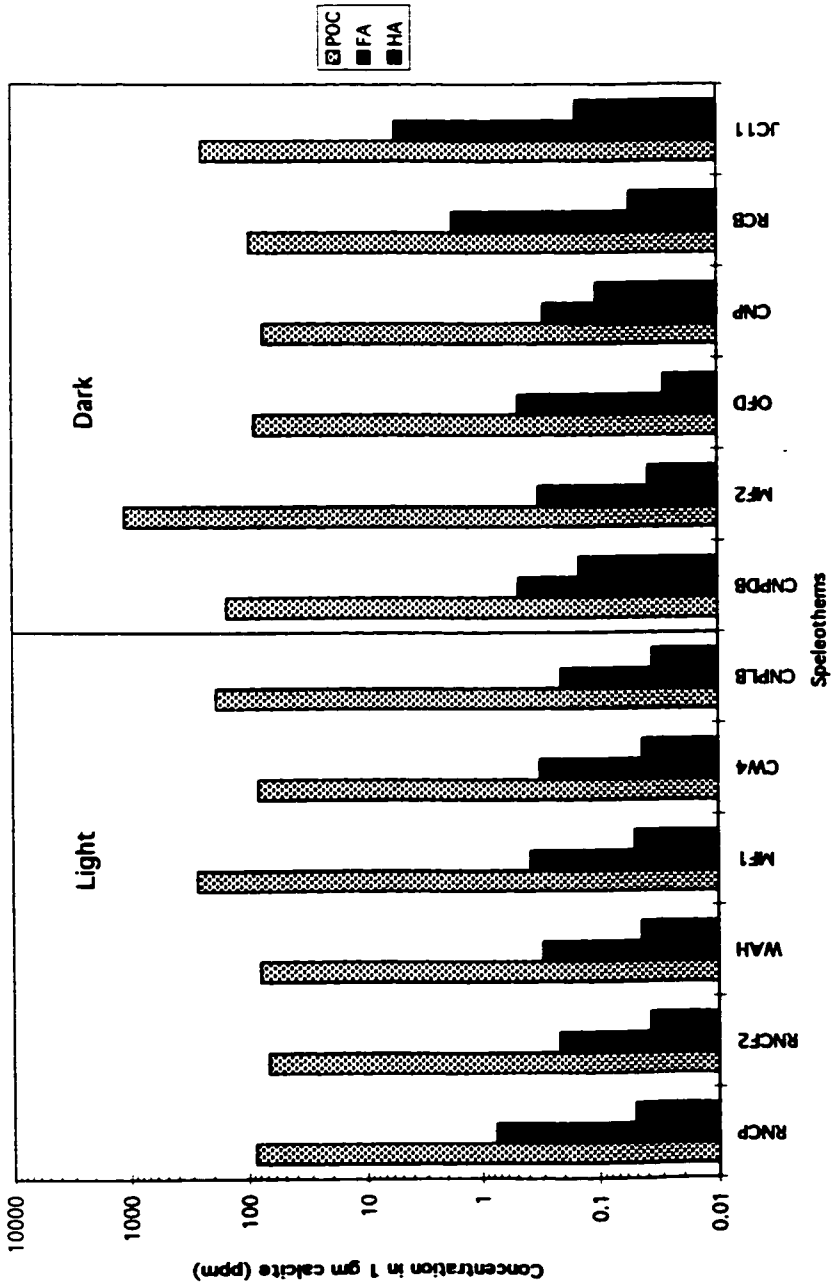

Figure 4.4. POC, FA and HA concentrations in the speleothems

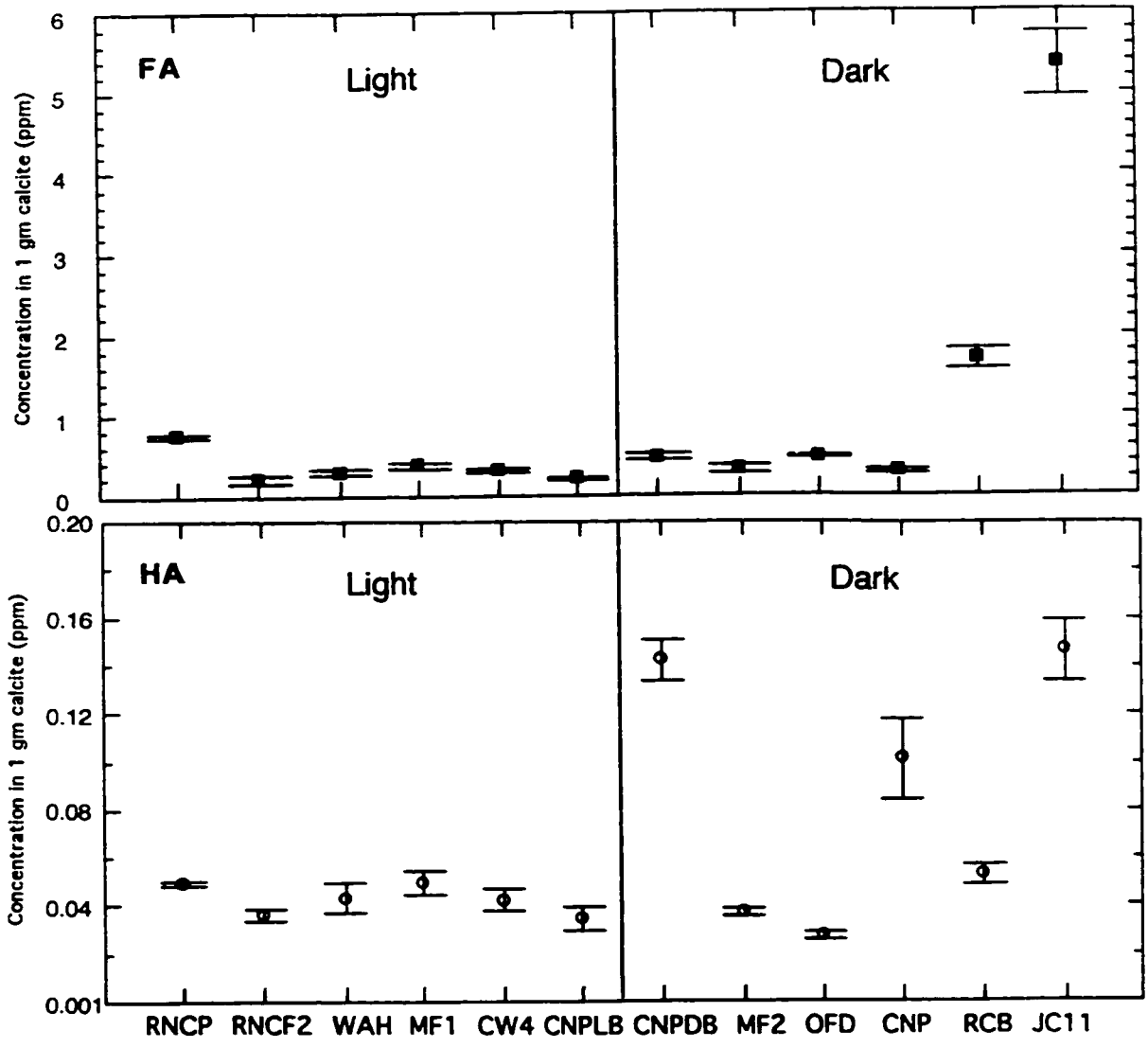

Figure 4.5. Concentrations of FA and HA in speleothems

emission wavelengths, with both having concentrations of 10 ppm. As FA has twice the fluorescence at the same concentration as HA, and the speleothems possess at least five times the quantity of FA to HA, then FA must be the dominant fluorophore due to its concentration. There is also a slightly higher average concentration of organic compounds for the dark speleothems compared to the lighter samples.

d) Fluorescence of the FA, HA and POC Fractions

To allow easier comparison between the three fluorescing organic components, FA, HA and POC, the spectra of the light and dark speleothems were averaged after they had been normalised by dividing the entire spectral intensities of each sample by its maximum intensity (Figure 4.6). The normalisation process retains individual sample spectra variability. Figure 4.6 shows a comparison of the FA and HA standards with the speleothems; the "light" and "dark" lines are the averaged fluorescence spectra for the twelve light and dark speleothems used in this study.

Speleothem FA yields a fluorescence spectrum slightly different from that of SRFA, both excitation and emission peak wavelengths being shorter. In the excitation spectra, the dark speleothems show a prominent plateau between 300 and 350 nm which encompass the peak intensity wavelength (340 nm) of SRFA. The light speleothems resemble the dark samples' excitation spectra. Emission peak intensities are centred between 410 and 420 nm, 20 nm shorter than in SRFA, which suggests that the cave deposits probably contain smaller molecular weight FAs than the standards. The basis for this suggestion is the work of Senesi (1990) who showed that larger organic compounds fluoresced at longer emission wavelengths than smaller organic molecules.

The HA fraction of the speleothems (Figure 4.6) behaves similarly to Aldrich HA (AHA) in both light and dark calcite. They all share the same excitation peak at 255 nm, although the dark samples do not have the same plateau between 300 to 320 nm as do the

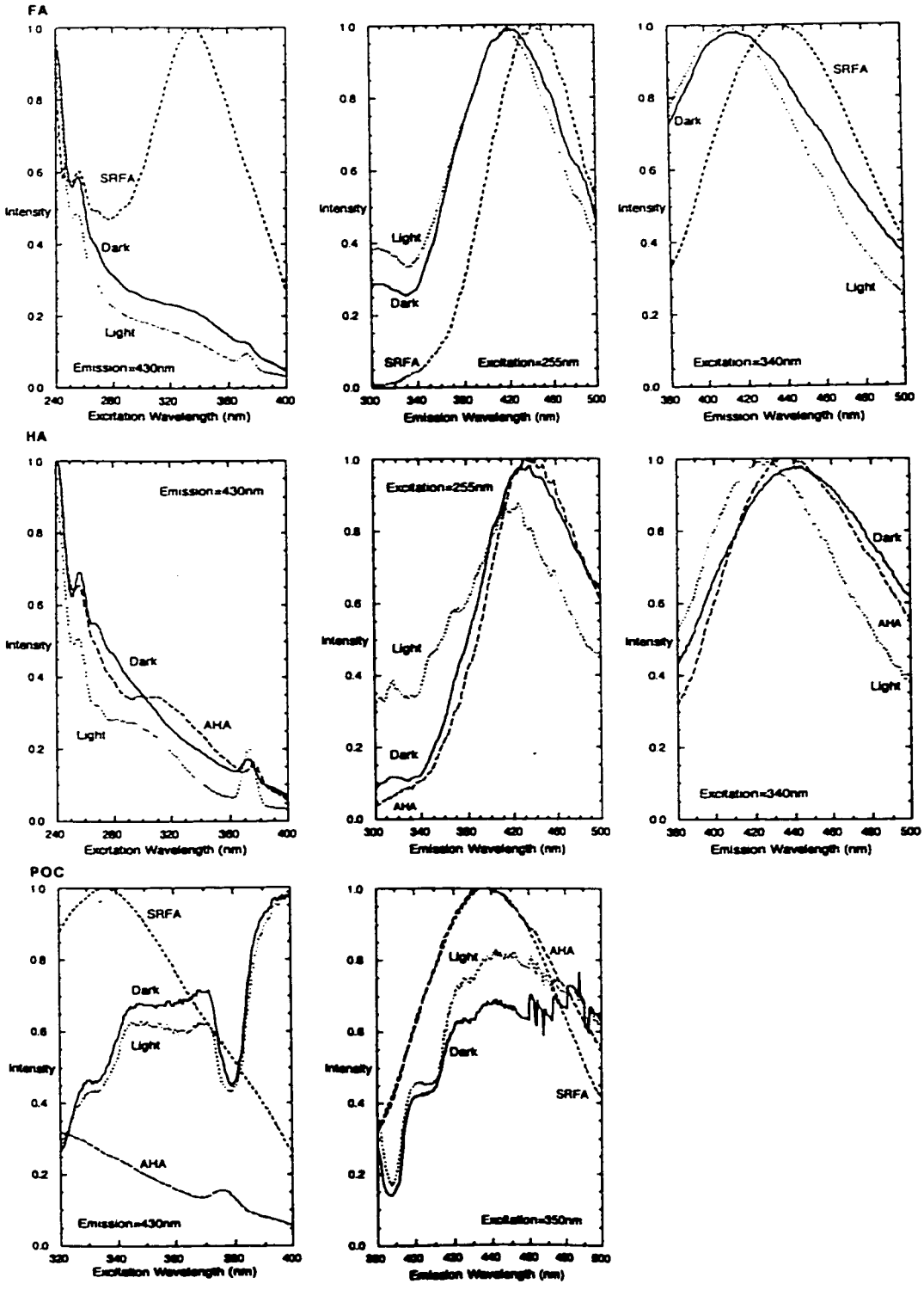

Figure 4.6. Normalised and averaged excitation and emission spectra for FA, HA and POC extracted from speleothems compared to Suwannee River FA and Aldrich HA

light samples and AHA. Emission fluorescence spectra of speleothems and standards are very similar at an excitation of 255 nm, peaking in intensities at approximately 430 nm. However, with an excitation wavelength of 340 nm the light and dark coloured speleothems differ in maximum emission fluorescence wavelength by 20 nm. Both higher concentrations (Mobed et al, 1996) or larger organic molecules (Senesi, 1990) could account for this difference, and Figure 4.5 shows that the dark speleothems generally have higher concentrations of HA than their lighter counterparts.

Figure 4.7 shows a comparison between the maximum fluorescence wavelengths for emission and excitation for the solid speleothems and their FA, HA and POC fractions. The FA and HA standards are also included. The solid speleothems exhibit a wide scatter of points although a distinction can be made between the light and dark coloured speleothems and they encompass the peak fluorescence wavelengths of the standards. FA and HA both possess tighter groupings and as with Figure 4.6 fluoresce at shorter wavelengths than SRFA and AHA. The HA fraction does show a difference between the light and dark speleothems as did the solid speleothem fluorescence. The POC fraction is quite different from the other two fractions, but it does possess the longer excitation and emission wavelengths of some of the solid speleothems. As mentioned earlier, this is probably because both possess high concentrations of organic matter.

The above discussion of the fluorescence spectroscopy concentrated on the similarities, or lack thereof, of excitation and emission wavelengths between the organic fractions extracted from the solid speleothems and the FA and HA standards. However the intensities have not been examined and whether FA is the more dominant fluorophore than HA or POC. The answer has been alluded to in the description of the concentrations where FA is far more prevalent than HA and the standards' fluorescence showed that SRFA fluoresced twice as much at the same concentration as AHA. The question remains whether the speleothems depict the same phenomenon. Figure 4.8 shows the peak fluorescence

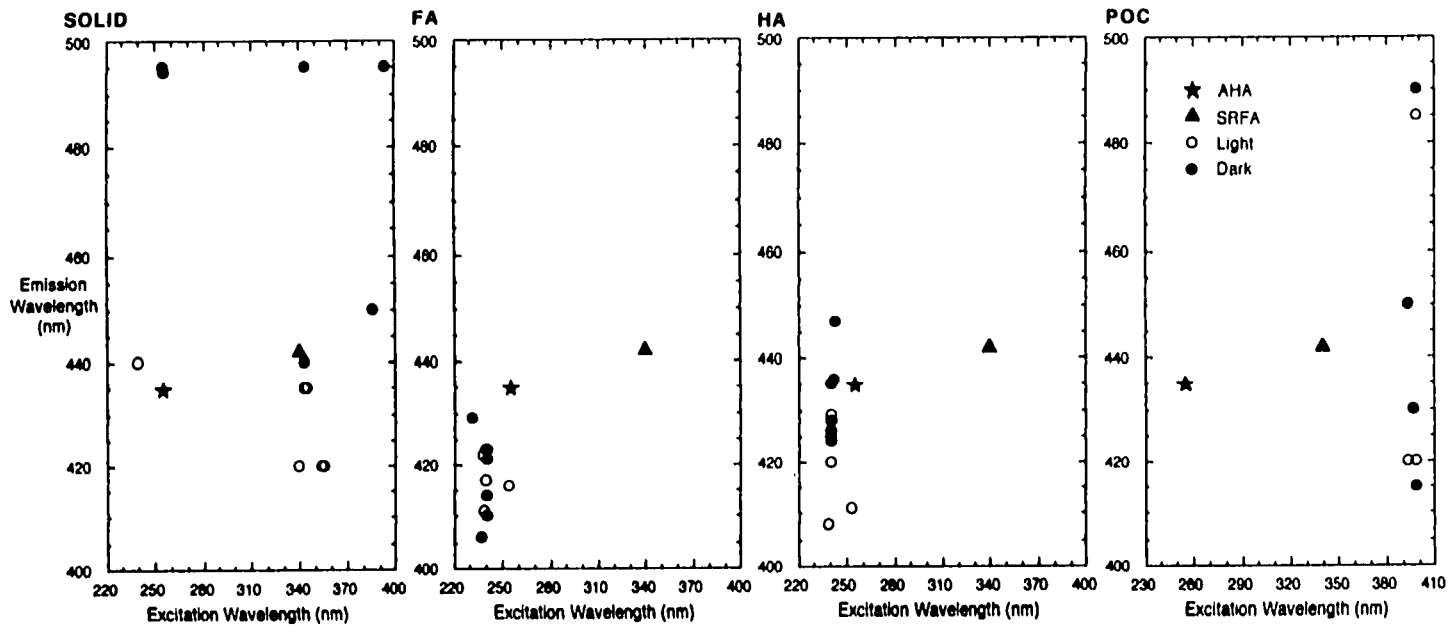

Figure 4.7. Maximum excitation and emission fluorescence wavelengths for the solid speleothems and their various fractions. SRFA and AHA are included for comparison purposes. As for all the subsequent figures, the shaded symbols are those of dark speleothems and the unshaded symbols represent the light coloured speleothems

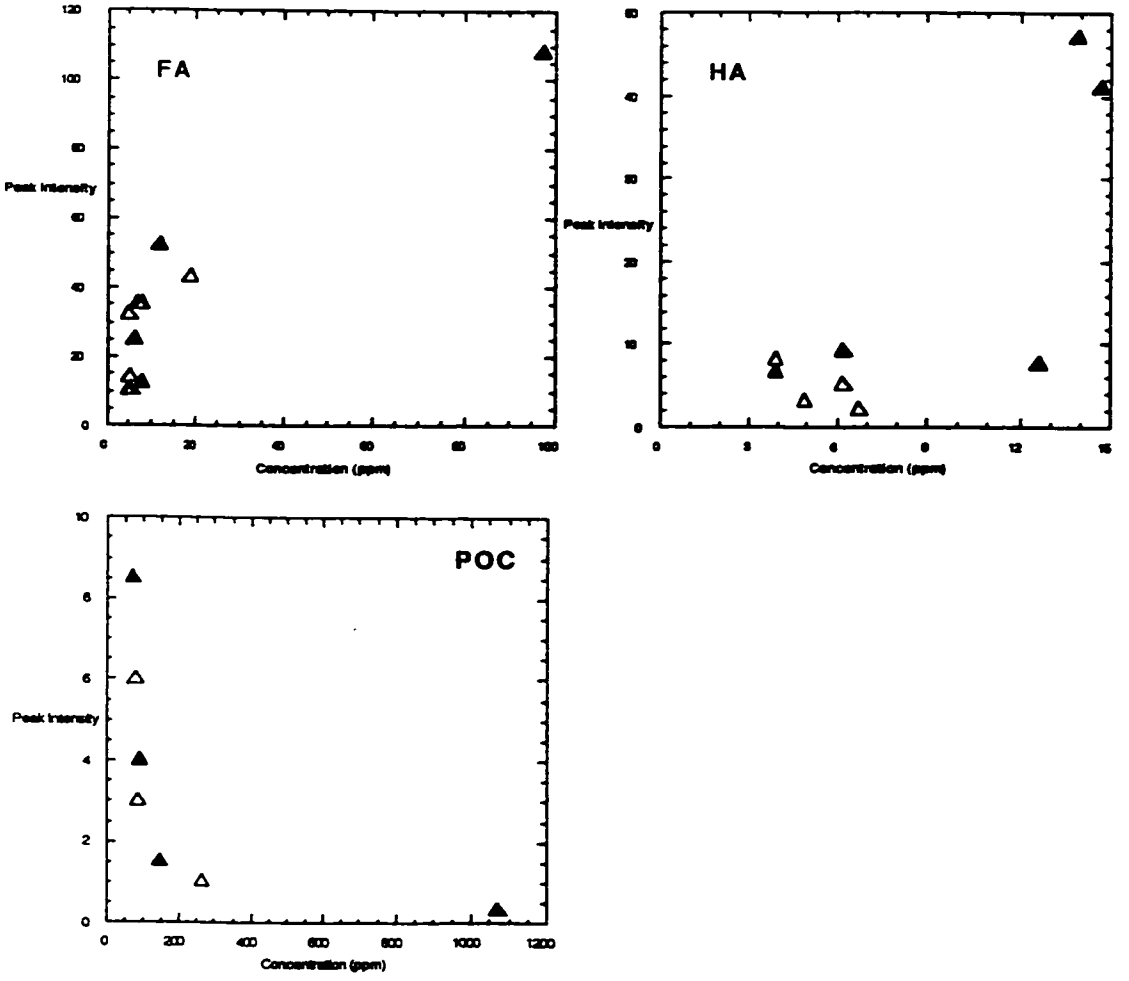

Figure 4.8. Relation between fluorescence intensity (arbitrary units) and concentration of FA, HA and POC in respective solutions. These are the DOC values of the solutions for the total organic fraction of FA, HA and POC. Clear and shaded symbols represent light and dark speleothems respectively, in this and subsequent figures.

intensities generated at measured concentrations (using the carbon analyzer for FA and HA and weighed filter paper for POC). Both FA and HA have weak positive relations between intensity and concentration. At the low concentrations of FA and HA, a positive correlation between concentration and fluorescence intensity would be expected (Senesi 1990). More importantly, the FA and HAs have similar concentrations and yet the FAs generally fluoresce at twice the intensity of the HA, confirming the SRFA and AHA result. POC is at much higher concentrations, and the self absorption effect (Mobed et al. 1996) is therefore very noticeable with an apparent exponential decay in fluorescence.

One final test of whether FA was the dominant fluorophore was undertaken by comparing the solid speleothems' fluorescence with relative concentration of FA to HA (Figure 4.9). A positive relation between these two variables would suggest that FA is indeed the most significant fluorophore in the calcite. This does appear to be evident in Figure 4.9 although the light and dark speleothems show divergent trends. The light speleothems display a rapid increase in fluorescence with only small increases in concentration of FA while the darker samples show a more gradual increase.

POC fluorescence depicted in Figure 4.6 yielded a peak excitation intensity at 350 nm when the emission wavelength was fixed at 430nm, the value of the peak emission obtained from the dissolved speleothems (Figure 4.3). The shorter excitation wavelengths were not shown because of the very strong excitation peaks observed in the immersion oil used for suspension of the POC. Their strength would totally outweigh that of the POC's shorter excitation wavelengths. Emission measurements revealed spectra similar to the solid or dissolved samples. POC of both the light and dark calcite show a peak emission wavelength of approximately 420 nm, which compares well with the 430nm of the FA and HA in the dissolved speleothems. However, the most important feature is the very low fluorescence intensity of the POC and also the broad, featureless form of the emission spectra, producing a white fluorescence. Fluorescence of POC has not been reported in the

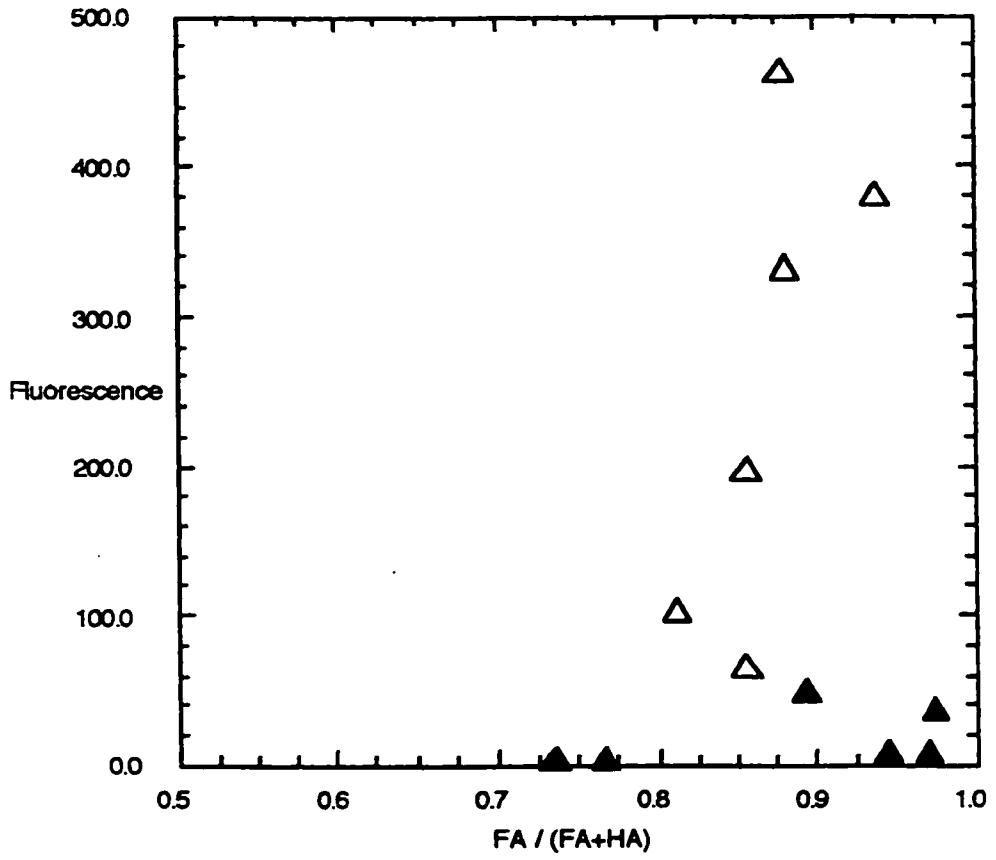

Figure 4.9. Relation between each speleothem's relative proportion of FA in the TDOM and fluorescence

literature hitherto. If POC is trapped in solid speleothems, in low abundances it may add to their white fluorescence, but in high concentrations (as in MF2), fluorescence may also be quenched, due to absorption of UV by the POC.

The averaging process used in Figure 4.6 does not entirely show the whole truth of POC. Figure 4.3 displays a trend towards longer excitation wavelengths and lower intensities in samples with higher POC. Such a result may be explained by the occurrence of colloidal matter in the speleothems. Skjemstad and others (1993) showed that aggregates of humic substances and soil-clay particles can inhibit fluorescence. Such microaggregates are silt -clay particles bound by organic matter or the organic matter is adsorbed onto the surface of the clay. This would explain the higher concentrations yet lower fluorescence of the operationally defined POC recovered from some of the speleothems. These aggregates or colloids may become trapped on the growth surfaces of the calcite speleothems.

e) Fractionated FA

With FA being the dominant fluorophore, some characterization of what compounds make up this fraction is useful. Of the samples subjected to the FA fractionations, only JC11 and RCB had high enough DOC concentrations to provide meaningful results. Figure 4.10 depicts the percentage of total DOC accounted for by the hydrophilic and hydrophobic fractions. The chemical characteristics of each of these fractions is defined by Leenheer (1981). For both speleothems, the dominant FA fraction are the hydrophilic neutrals or the carbohydrates. For JC11, the next most significant component are the hydrophilic acids, which are comprised of a mixture of various hydroxy acids. RCB has a higher abundance of the hydrophobic acids than hydrophilic acids and these include a vast array of different functional groups. The smallest group in both samples are the hydrophobic neutrals, which incorporate hydrocarbons and longer chain length lipids containing carbonyls, including fatty acids.

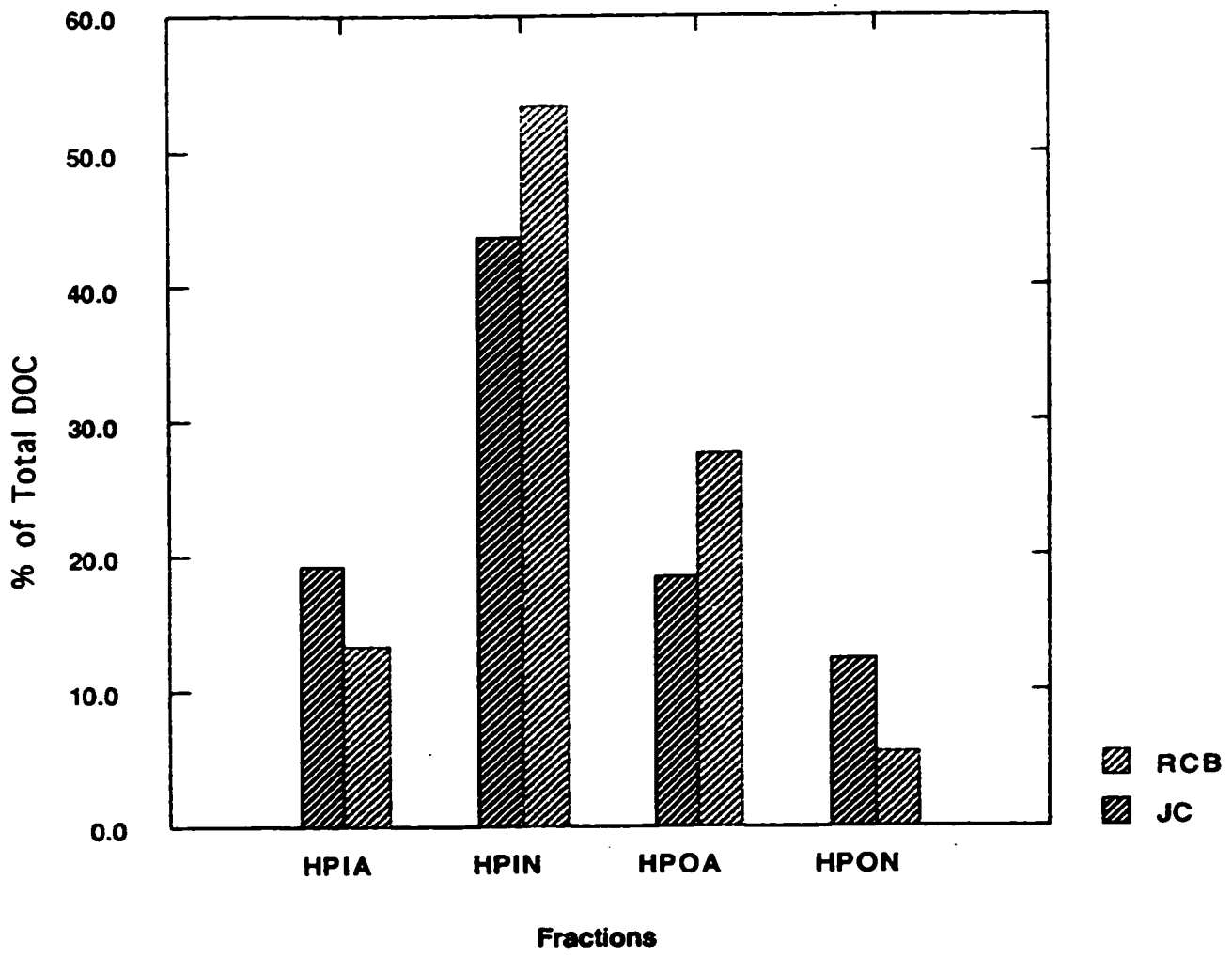

Figure 4.10. FA fractionation results for JC11 and RCB

If the two categories are grouped together, then the hydrophilics are much more dominant than the hydrophobics. Such a result is expected because there are plenty of opportunities for most of the solute hydrophobics to be removed from the percolating waters as they travel through the soil and micro-fissures in the bedrock. The clay filtration will remove most of the hydrophobics (Thurman, 1985).

f) Size Fractionation

Ultrafiltration of the organic compounds in the solutions was undertaken to determine the molecular weight distribution of the fluorophores. As noted earlier, fulvic acids typically have molecular weights of 0.8-1 kD whereas humic acid sizes may range greatly, from 2 kD to 300 kD (Suffet and MacCarthy, 1987). If FA or smaller organic compounds are the dominant fluorophores, a dominance of the less than 1 kD fraction could be expected.

Total DOC values of the six bulk speleothem solutions and their fractions were measured. Figure 4.11 is a summary of these data, showing each fraction as a percentage of the total DOC. It demonstrates one important finding from this work, that in all the six speleothems at least 50% of the soluble organic substances have molecular weights less than 10 kD (MWCO). For RNCP, RNCF2 and OFD the dominant fractions are the FAs (<1 kD), ranging from 47% to 73% of the total dissolved organic carbon in the speleothems.

However, Figure 4.5 would suggest a more clear dominance of FA than is depicted in Figure 4.11. A possible explanation for this result could lie in the procedures used in the removal and separation of the FA and HA from the solid speleothems. This process involved acidification to free the organic compounds from the calcite matrix which would promote precipitation of the HA from the solution. Although the solution was adjusted to a pH of 8 before the separations occurred, what proportion of the HA failed to resolubilize is

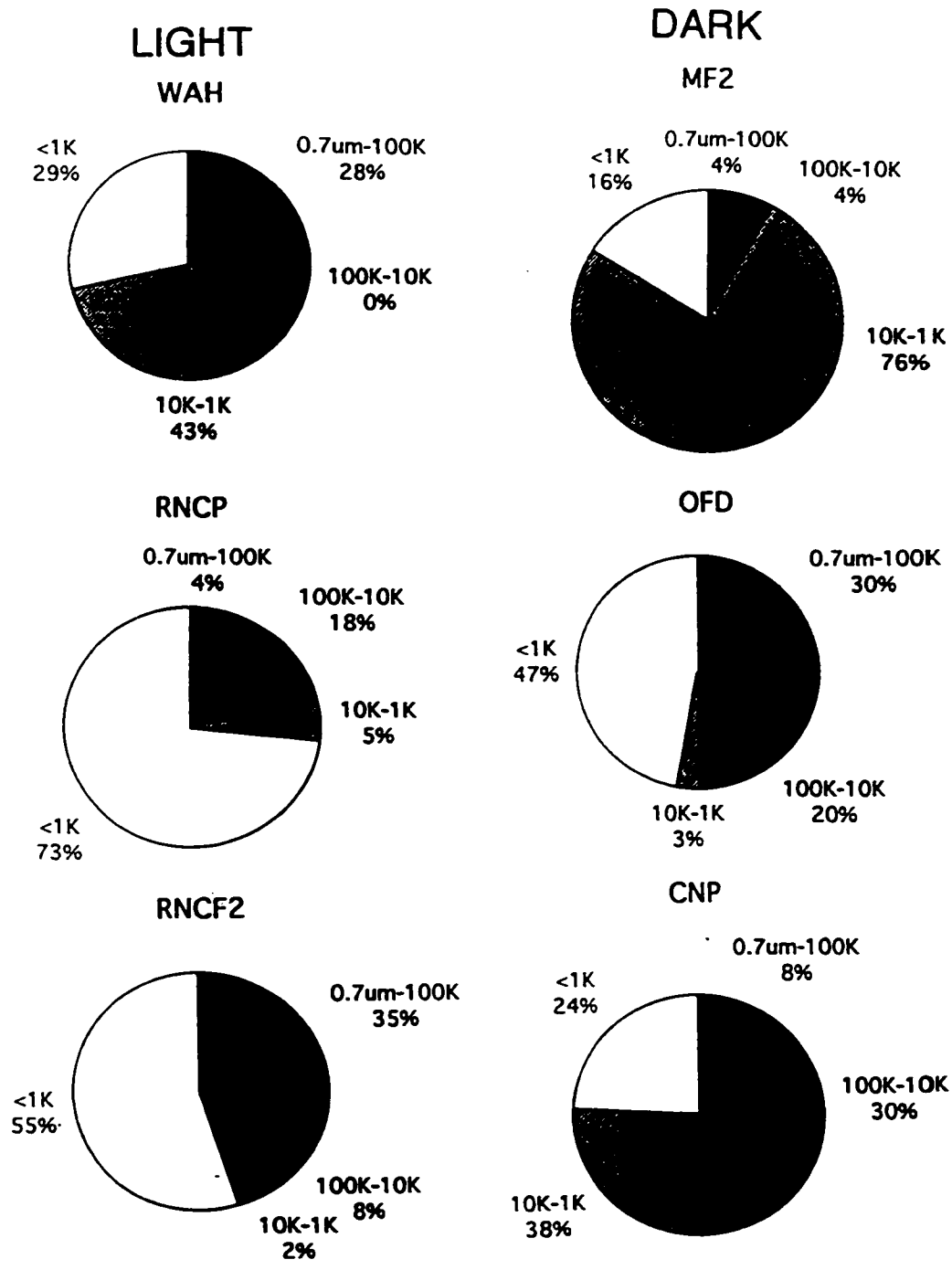

Figure 4.11. Molecular weight size fractionations using ultrafiltration

uncertain. This could lead to erroneous results when measuring the total amount of HA in the speleothems. FA, which is soluble at all levels of pH, would not suffer from this problem. Therefore, Figure 4.11 could be taken as the more reliable estimation of the distribution of humic species.

Humic acids, the 1kD to <0.7 μm fractions, are the dominant components in OFD, WAH, CNP and RNCF2. However, smaller colloids (<0.7 μm) could also be contributing to the 0.7 μm -100 kD fraction because some have been found to be as small as 0.01 μm and therefore not trapped as POC (Sposito, 1989). Their presence may explain why the larger molecular weight fractions are significant in certain speleothems but the HAs are not. Colloids, once entrained, remain highly mobile and consequently could be flushed with the organic substances into the cave.

4.5.1.2 Trace Elements

The results outlined above strongly suggest that the fluorescence observed in speleothems is dominated by organic compounds. However, because trace and rare earth elements might also contribute, it was important to ascertain if they were present and in sufficiently high concentrations.

Table 4.3 reports all elements present in the sample speleothems at concentrations greater than the detection levels. None of those found in significant quantities fluoresce at the same wavelengths as the solid speleothems or the separated organic compounds. Mn has a peak excitation wavelength at 266nm, similar to the organic substances, but peak emission is at 580 nm (Munoz and Rubio, 1988). Zn and Na in crystals are non-fluorescent (p.c. W.B. White, 1998), while Cr have even longer excitation - emission peaks (O'Donnell et al., 1989). Fujimori and others (1989) found that U also fluoresces at longer wavelengths (510-650nm). From Table 4.3, this leaves only I and Br as potential

Table 4.3. Trace elements present in speleothems (concentration in ppm)

Elt.	Light						Dark						D.L.
	WAH	MF1	CNPLB	RNCF2	CW4	RNCP	MF2	OFD	JC11	RCB	CNPDB	CNP	
Ba					60		70		210				40
Br									11	7			2
Cr		0.6					0.7			0.4		12.8	0.3
I	90	310		30	20	40	220		180	110			10
Mn		2					12						1
Na	20	150	70	40	60	40	230	30	240	310	30	40	10
U	0.3	1.5	2.6	0.3	0.7	0.6	0.7	2.0	5.5	0.1	2.1	4.4	0.1
Zn	60		270	30	15			10			840	460	10

Abbreviations: Elt. - Element, D.L. - Detection Limits

fluorophores. Br and Ba were detected in only several of the samples and consequently can be discounted as a common contributor to fluorescence in speleothems. Iodine tends to have much longer excitation and emission wavelengths, 540nm and 1300-1700nm respectively (Macler et al., 1989) and can also be discarded. Eu could not be measured by the INAA technique. Eu^{2+} yields an emission peak at 416nm (Machel et al, 1991), close to that of the dissolved speleothems, but its fluorescent spectrum is quite different and hence it can be discounted as a significant fluorescent contributor.

Trace elements do not, therefore, appear to be potential fluorophores in our sample speleothems, further supporting the notion that organic substances are the only significant fluorescing components.

The high concentration of iodine in our samples is of interest in its own right. Ullman and Aller (1985) showed that environmental iodine is incorporated by marine organisms and released to accumulating sediments through organic decomposition. It is not surprising that speleothems may incorporate some iodine as their calcite is composed of $CaCO_3$ derived largely from the overlying rocks. Natural abundances of iodine (Becker et al., 1972) have been measured in limestone (<29 ppm), sandstone (<37.6 ppm) and calcareous shales (<38 ppm), all of which are above caves used in this study.

4.5.2 Causes of colour differences between lighter and darker speleothems

Annual laminae in speleothems are delineated by differences in the colour of calcite, a couplet of light and dark calcite. What need to be ascertained are the chemical differences between each calcite. As stated earlier, due to their microscopic size, annual laminae cannot be investigated, and therefore bulk samples of calcite were investigated instead. Although what is defined as light and dark calcite is subjective, every attempt was made to have a

clear distinction between these two groups. Only the McFail's Cave samples MF1 and MF2 are from the same cave and yet the former is light and the latter is dark. The samples CNPLB and CNPDB are the only ones to come from the same specimen: it contained bands of light and dark calcite that were thick enough to provide 20 gm of calcite for each analysis.

One possible quantitative measure of the difference between light and dark calcite is absorbance. Absorption spectra (Figure 4.12) show that the expected higher absorption of darker speleothems in the visible region is accompanied by correspondingly high absorption in the near UV. That is, the chromophores responsible for visible darkening of the speleothems are also absorbing much of the incident UV and could therefore be expected to reduce the fluorescent intensity through self absorption effects.

Absorbance peak wavelengths of the speleothems range between 240 and 300 nm. Such wavelengths, particularly between 270 - 280 nm, are where the electron transitions occur for the UV range for phenolic substances, aniline derivatives, benzoic acids, polyenes, and polycyclic aromatic hydrocarbons (Braun et al, 1988). As some of these are components of the humic substances which have been shown to be the primary cause of the fluorescence in the speleothems, it was anticipated that the peak absorbances would occur at these wavelengths.

To test the role of the organic matter in contributing to dark colour in speleothems, the absorbance was quantified by measuring the area under the peaks (mm^2) over the wavelength range 200 to 600 nm. This is plotted against various variables in Figure 4.13. Figure 4.13a shows a clear inverse correlation between fluorescence and absorbance as expected from our earlier observations; this shows quantitatively the difference between light and dark calcite but also the importance of self absorption, caused by the presence of

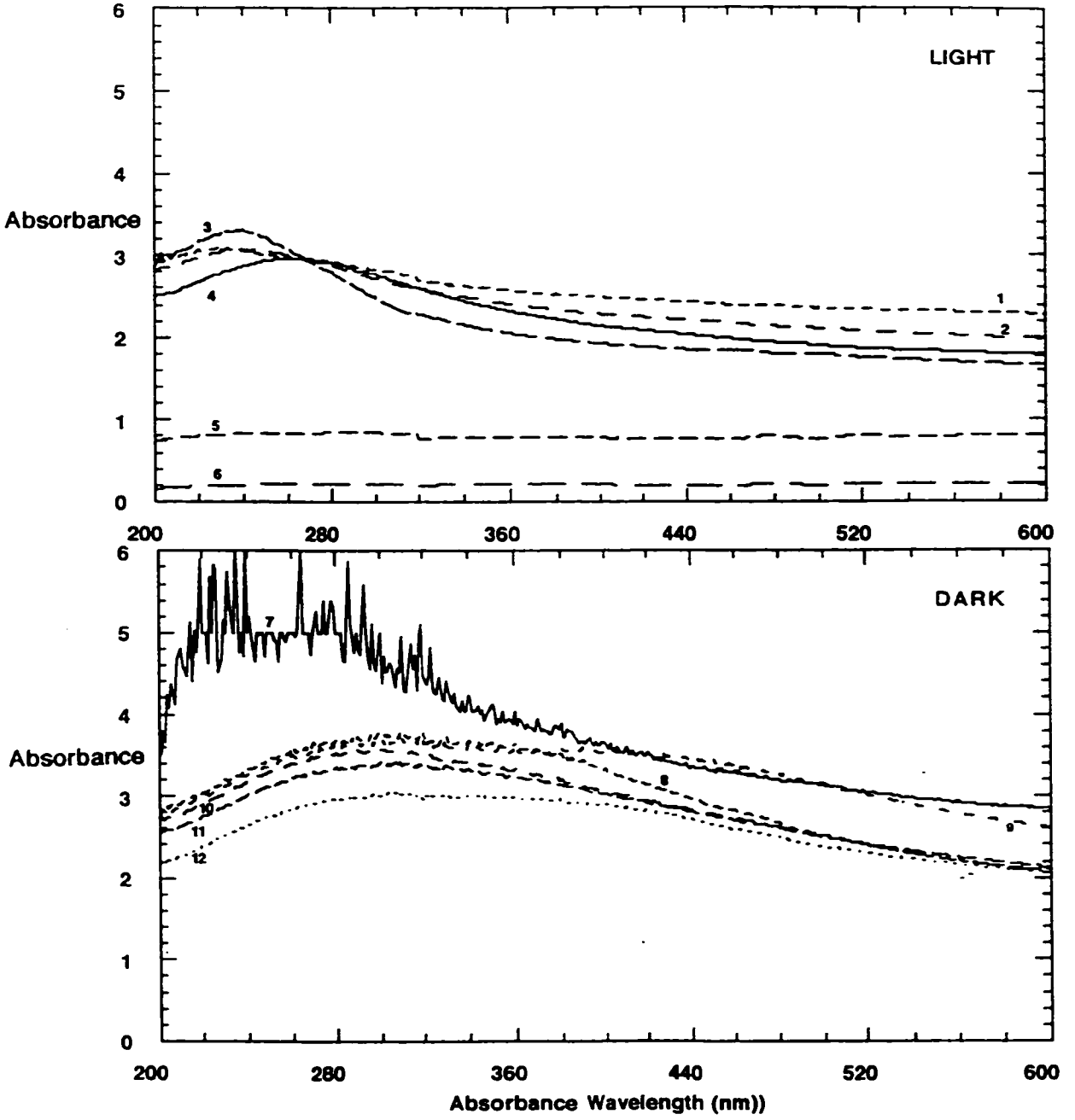

Figure 4.12. Absorbance spectra of all speleothems. The same numbers as Figure 3 are used for the speleothems.

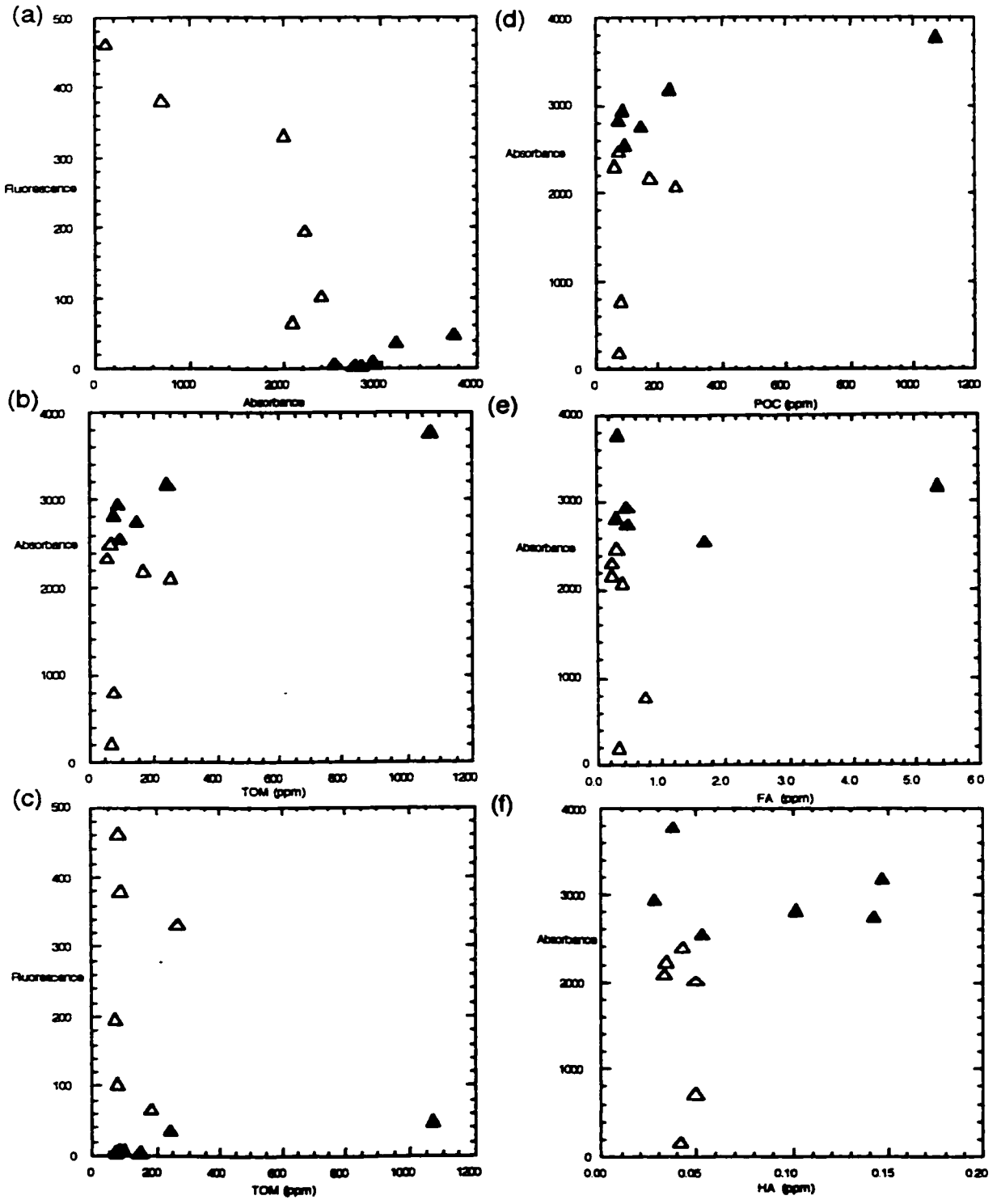

Figure 4.13. Relations between absorbance, fluorescence of the solid speleothems and their fractions organic concentrations.

organic matter, on quenching of fluorescence.

Figure 4.13b shows the correlation between absorbance and total organic matter (FA+HA+POC). This parameter was chosen because it is not clear which of the three organic components of the speleothem would be most responsible for the absorption. There is a weak positive correlation, therefore TOM could be considered a reasonable proxy for absorbance and colour. However, dark speleothems are not consistently higher in TOM (Figure 4.4).

Figure 4.13d-f demonstrates the relation between absorbance and the FA, HA and POC concentrations respectively. It is not immediately apparent which fraction is most responsible for absorption because they all resemble the TOM distribution of Figure 4.13b. However, the POC fraction resembles the TOM most closely, not surprisingly since, in most speleothems, POC is the dominant species and therefore is probably the principal chromophore. Such a conclusion is given added weight by the reversal noted in fluorescence intensities (Figure 4.3) when the solid speleothems have been dissolved and the POC removed. Before dissolution, the light coloured speleothems fluoresced more strongly than the dark speleothems, but after dissolution and filtering of the POC, the dark samples fluoresced more intensely. This reversal could be produced by the removal of the absorbing POC.

In Figure 4.13c the fluorescence of the solid speleothems is compared to the concentrations of TOM. A positive relation for low values of TOM could be expected, since zero TOM would be expected to correspond to zero fluorescence intensity. For the light coloured speleothems, however, even the lowest measured TOM values correspond to high intensities. There is a suggestion of a decreasing trend at higher TOM. On the other hand, the dark speleothems yield a slightly increasing trend in fluorescence with increasing TOM, but are in general much less fluorescent than the light coloured speleothems, for a given

range of TOM values. This suggests that organic substances responsible for dark colour in speleothems are also very efficient at quenching fluorescence, but their concentration is not well represented by TOM values. Much of the organic matter in speleothems seems to play no role in fluorescence, but does seem to play some role in the colour intensity (Figure 4.12b) as shown by the weak positive relation.

Figure 4.5 demonstrates that the concentration difference between light and dark calcite is only partially stronger in the HA than in the FA, three of the six darker speleothems having more than twice as much humic acid as any of the lighter specimens. For the FA, only JC and RCB among the darker samples contain significantly higher concentrations. This is confirmed to some extent by the absorbance results which show a weak positive relation between the organic substance concentrations (TOM) of the light and dark speleothems and integrated absorbance.

The size separation results also give an indication of the different size concentrations of the various light and dark coloured speleothems. Distinctions are made difficult by the small sample size and the lack of total unanimity between the light and dark samples as seen in Figure 4.9. Not all twelve samples could be analyzed as the other six speleothems were used for the FA fractionation. For the light samples, RNCP and RNCF2 both show the dominance of the smallest fraction, <1 kD, while OFD and CNP show higher concentrations of the larger molecular weight fractions. These larger molecular weight fractions are probably the HAs, and it was shown in Figure 4.5 that the darker speleothems have on average a higher concentration of HA. Regarding the two smallest size categories (<1-10 kD), it appears that all the speleothems have similar proportions ranging between 60-75% of the total soluble organic matter, with the exception of MF2.

4.5.3 Environmental influences

One goal of this research was to seek relationships between the organics in speleothems and the broad natural environmental conditions overhead in the local soils and vegetation. As noted, the twelve samples span the range from alpine tundra to tropical forest and desert transition, with temperate grassland and forest settings in between. It was hypothesised that differing amounts and proportions of FA, HA and POC generated in the source soils might be recognised in the solid speleothems and/or speleothem extracts beneath them.

Any correlations must be treated with caution at present for two principal reasons. Stevenson (1985) states that the lack of standardization in extraction, fractionation and purification procedures used by researchers makes it difficult to reach firm conclusions on environmental influences in soil extracts. Secondly, Drever and Vance (1994) claim that FA is the dominant organic acid in all soil solutions, therefore all speleothems should exhibit dominance of this substance regardless of their overlying environment. It should be added that attempts to distinguish the environmental influences are not helped by the broad spectrum of organic substances that all soils produce (Stevenson, 1985).

Comparison of FA, HA and POC in the speleothems (Figure 4.4) shows that POC is quantitatively the dominant fraction. There appears to be a clear division with Crows Nest Pass and McFail's Cave possessing >181 ppm POC in the calcite compared to the rest of the samples which have virtually identical amounts of approximately 80 ppm POC, with the exception of JC11 at 235 ppm. McFail's Cave lies beneath a Carolinian temperate forest with mild winters and warm summers. Crows Nest Pass is a very different ecosystem today with its alpine shrub tundra, but as it is quite close to the boreal treeline, it could well have been a boreal forest when its ancient speleothems were deposited.

If the vegetation did not change above the CNP samples, then the type of environment may not be as important a determinant of the concentration of POC as conditions in the underlying rock. Its influence could be two fold: first, the minimum apertures of matrix or fractures could be large enough not to filter much of the POC or, second, a thinner rock overburden might permit more POC to reach the caves.Our results suggest that the apertures along the feedwater canals must be greater than 0.7 μm in size and allow significant drip rates. To complicate matters, the speleothem with the thinnest overburden, RCB, has one of the lowest proportions of POC. It has only vestigial soils, which may lack the capacity to generate much POC. Absence of a clay horizon in a soil may also be important, as clay filters out most of the organics reaching the groundwater (Thurman, 1985).

Only one speleothem possessed bands thick enough to compare light and dark calcite, supplying samples CNPLB and CNPDB. CNP was an entirely different speleothem from this locality and serves as a control sample. The reason for the difference in light and dark calcite here appears to be the four times higher concentration of HA in the dark band (Figure 4.5). Otherwise, both have quite similar POC and FA concentrations (Figure 4.4). This speleothem has two thick pairs of both light and dark bands, therefore at two times its growth it switched from a period of high HA concentration to low HA concentration. Such a transition must be a reflection of a changing environment above the cave but unfortunately these phases have not been dated because the sample is greater than 350,000 years in age. Unsurprisingly, CNPDB exhibits much stronger fluorescence than CNPLB in the HA solution fraction. Other than this, there are no differences between these two portions of the speleothem. Such a finding has important implications for the difference between light and dark calcite in annual bands. CNP has slightly higher POC levels than CNPDB and CNPLB but, more important, it is a dark speleothem like CNPDB and displays similar high levels of HA. This confirms the findings at this cave concerning the differences between the light and dark calcite.

Variability between speleothems from one cave is not surprising. Both of the Ratsnest samples are light coloured speleothems, each displays similar fluorescence spectra in solution (Figure 4.3), with peak emissions centred at 425nm. However, the intensity of fluorescence of solutions of RNCP is greater than for RNCF2. This is related to the relative abundances of the various fractions; RNCF2 has equal portions of the larger and smaller fractions while RNCP has a very clear dominance the less than 10 kD fraction (Figure 4.11). Figure 4.5 also demonstrates that RNCP has twice the amount of FA in its counterpart. A possible explanation for such a result may lie in the variable flow paths of the feedwater in the soil and bedrock above the speleothems. RNCP may have a thicker clay layer which would filter more of the larger organic substances. Such a scenario is entirely possible in an epikarst environment.

Figure 4.5 provides a clearer illustration of the yields of FA and HA in each of the samples. Stevenson (1985) states that forest soils will have higher proportions of FAs while peats and grasslands will have more HAs. RNCP, RCB and JC11 all have high concentrations of FA and all are from forested environments. CW4 and OFD are also under a deciduous forest and although their total FA concentrations are not high compared to other samples, the differential in HA is just as noticeable. Rats Nest Cave and OFD are portrayed in Figure 4.11 as both having quite similar distributions of the smaller fractions of organic substances, with a particular dominance of the <1 kD. The CNP samples conversely have high proportions of HA and are from a peatland tundra environment. Figure 4.11 confirms this result because CNP contains a smaller concentration of the <1 kD fraction than RNC or OFD. JC and RCB also have high HA levels compared to the other speleothems, but more importantly these levels are low in relation to their FA concentrations when compared to other speleothems. WAH is the only semi-desert location and its organic substance assemblage in Figure 4.11 is quite different from all of the other environments.

Possible influences that the various environments may have on the fluorescence can be seen more clearly when excitation and emission peak intensity wavelengths are compared (Figure 4.14). In this figure the diverse environments are shown for the five fractions: solid, dissolved, FA, HA and POC. The variability can be attributed to two principal factors: the varying concentrations of organic substances in the speleothems and the different types of organic compounds produced in the diverse locales overlying them. The concentration effects on wavelength are prevalent at high levels of these substances (Mobed, 1996), but for the more dilute solutions of FA and HA where concentration is not a factor, small differences in fluorescence maxima are still evident. Although the peak excitation wavelength of 255 nm is still dominant with a secondary peak between 340-350 nm, peak emissions wavelengths are variable. The peak emission wavelengths vary between 415 nm for the total dissolved calcite of CNP and 490 nm for the POC of McFails's Cave.

The greater the spread of points, the more complex and numerous are the types of organic substances for a particular ecosystem. The scatter of points representing the fractions must be viewed with the influence of concentration in mind. Both the solid speleothems and the POC fraction have higher concentrations of organic substances than the TDOM, FA and HA fractions. These higher levels in the solid speleothem and the POC produce longer maximum intensity excitation and emission wavelengths due to self absorbance. Such a shift towards the red part of the spectrum produces a wider cluster of points for all the environments than would be present if concentrations were lower. Once this has been accounted for, there do not appear to be substantial differences between the environments; regardless of environmental diversity, the organic substances produce such broad and amorphous fluorescence spectra that distinction is scarcely possible. However, the tundra, boreal, desert and maritime environments do show a clear difference between the FA and HA. This confirms the size fractionation results (Figure 4.11) where both CNP (tundra) and OFD (maritime) had relatively high concentrations of larger fractions but also

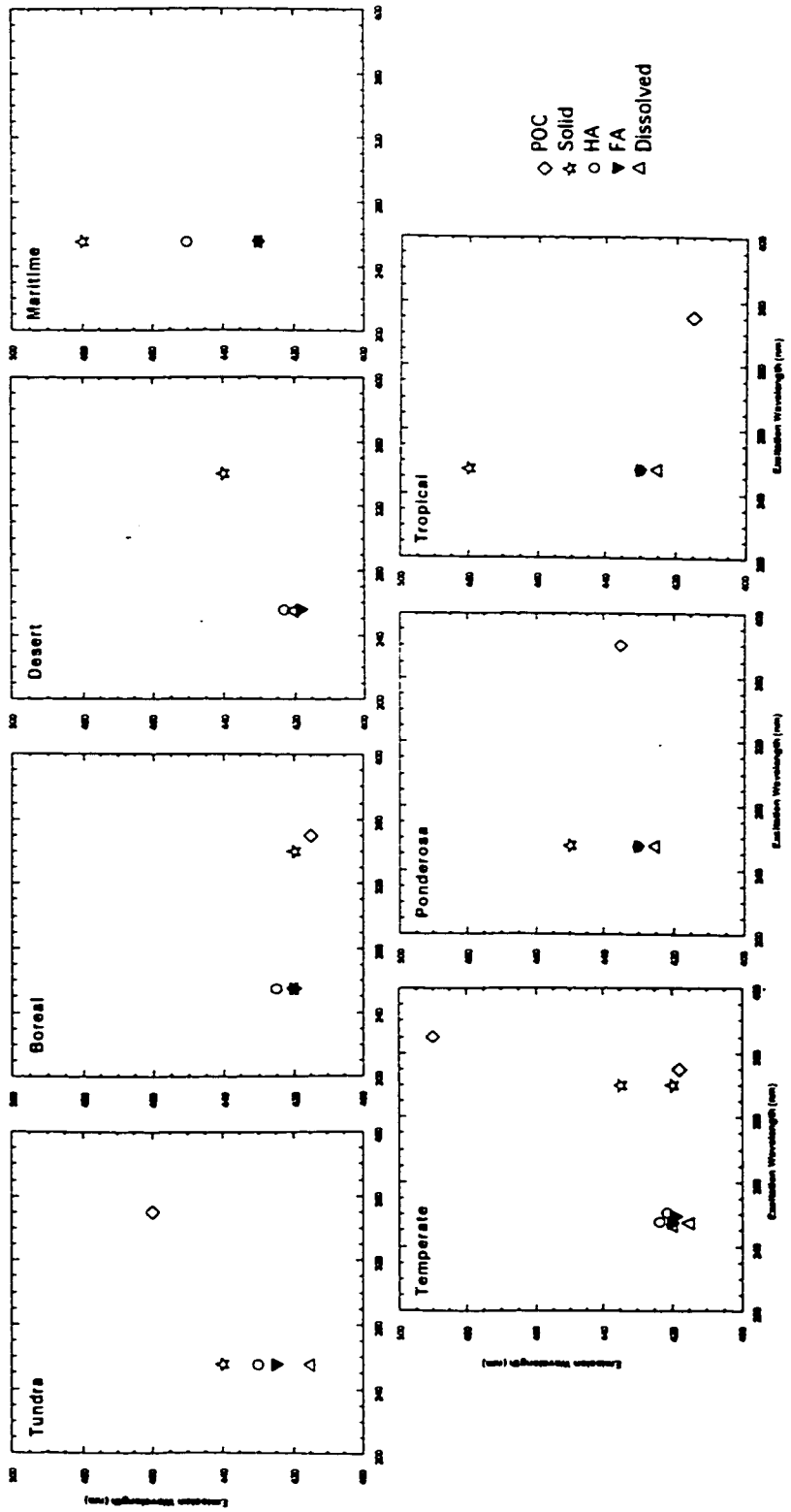

Figure 4.14. Environmental influences on fluorescence of speleothems and their organic fractions

that the HAs of these environments may be different from the temperate, tropical and ponderosa forests.

4.6 Conclusions

The purpose of this study was to investigate the relation between organic constituents in a broad sample of speleothems and their fluorescence and colour. From the results it appear that fluorescence is predominantly caused by humic substances and not by trace elements. FAs are approximately double in their fluorescent yield (per ppm) compared with HAs and FAs, because of their higher relative concentration appear to be the dominant contributors to fluorescence. Of the FAs, the hydrophilic neutrals are the most dominant fraction, followed by the hydrophilic acids. The POC present in the speleothems may have a quenching effect on the fluorescence, a finding suggested by the reversal of fluorescent intensities of light and dark calcite once the POC was removed when the speleothems were dissolved. However, the enhanced fluorescence can also be caused by the dilution of the FA-HA which in the solid calcite absorbed a great deal of the fluorescence.

Average organic concentrations (in 1 gm of calcite) in the speleothems are as follows: POC - 204 ± 268 ppm, FA - 0.893 ± 1.4 ppm and HA - 0.064 ± 0.04 ppm. The above results show that some darker calcite possesses higher concentrations of humic substances, but that it fluoresces less strongly than lighter calcite. Some dark speleothems also contains larger, more complex organic substances (HA) as well as the simpler compounds (FA) of the lighter calcite. Extrapolating these findings to the interpretation of annual laminae in speleothems, periods of high organic concentrations in cave feedwater will produce low fluorescence in the solid speleothem, such as late winter and the spring when humic substances are flushed from the soil (van Beynen et al. 1998). Conversely periods of low concentrations will yield high fluorescence in the speleothem, such as the summer and fall. Therefore the presence of annual bands can be determined using fluorescence microscopic photography.

The environmental influences on fluorescence were not clearly established. However, it appeared that forested environments have similar organic compound assemblages, which are different from the tundra or desert. Hence, the environment from which a speleothem comes will possess a certain assemblage of organic substances, dependent probably on the vegetation type. Such an influence will affect the optimum excitation wavelength to be used to attain the maximum emission of fluorescence from a speleothem selected for paleoenvironmental analysis of its laminae's fluorescence.

Pairs of speleothems from two particular caves (Ratsnest and McFails) exhibited some differences in behaviour, results that can best be explained by the nature of the epikarst above the cave which can produce different flow paths and soil thicknesses above the speleothems. However, the Crows Nest Pass speleothems were quite similar. Comparison of light and dark calcite from this cave showed that dark calcite (CNPDB) contained more HA than the light calcite (CNPLB), a result which adds weight to other conclusions about the chemical differences between light and dark calcite.

Chapter 5: Studies of Fluorescence in Annual Laminae of Modern Calcite Speleothems

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Abstract

The environmental significance of fluorescence produced by speleothems is a topic still very much in its infancy. The reasons for fluorescence variations are still being investigated. The purpose of this research was to ascertain if fluorescence can be used to delineate annual laminae in speleothems and if successful, to determine the environmental influences on the annual variability in fluorescence. Fluorescence was captured both photographically and using a Raman spectrometer. Annual bands were found in all the speleothem fluorescence signals using both techniques. Different speleothems from the same caves showed very similar fluorescence signals which suggests that the varying signal is being generated by environmental influences. The fluorescence was used to determine the widths of each lamina. These laminae widths, along with the fluorescence of each speleothem, was correlated with temperature and precipitation data. However, the only truely significant relationships were between increased fluorescence and laminae widths with augmented rainfall.

5.1 Introduction

A speleothem, such as a stalagmite, stalactite or flowstone, is a cave deposit composed of either calcite or aragonite. In 1960, Broecker and others used 14C dates of a

Holocene speleothem to investigate laminae, similar to the light and dark couplets of varves, within the calcite. Visually counting the bands they determined the laminae were annual in resolution. Little follow-up work was done on this phenomenon for many years, although speleothems came to be recognized for high precision paleo-temperature records using stable isotopes as proxies for temperature changes (Schwarcz, 1986) and U-series mass spectrometric dating to fit a timeline to these changes (Li et al., 1989).

When excited with UV light, many speleothems fluoresce. Gilson and MacCartney (1954) were the first to suggest that this fluorescence or luminescence was generated by organic matter contained within the calcite. Lauritzen et al., (1986) in an early study of the organic substances, concluded that fulvic acids were the dominant compound in the speleothems. White and Brennan (1989) reached the same conclusion when investigating fluorescence in speleothems. Shopov et al. (1987) were the first to report that annual couplets showed up in the fluorescence spectra of some speleothems and could also be recognized in samples where no couplets were visible in ordinary light. These findings renewed interest in annual banding in the speleothems. Baker et al. (1993) also found annual laminae in some modern stalagmites from Western Europe. Shopov et al. (1994) demonstrated that sequences of luminescence in laminae within certain speleothems correlated with sunspot activity and atmospheric ^{14}C cycles. Genty and Quinif (1996), investigating young speleothems in Belgium could not find any relations between their fluorescence and the standard climate variables above the cave during the period of their growth. They proposed that the thicknesses of the laminae, as measured by fluorescence, may be related to Ca concentration of the cave dripwater rather than to immediate climatic conditions; of course, Ca concentration in vadose speleothems has close associations with soil climatic conditions.

The difference between lighter and darker calcite, which defines the annual band or other couplet, was proposed to be caused by differences in organic substances (Gascoyne, 1977). However, work by van Beynen et al. (1998) found that there was no clear relation between colour and organic substance concentration. Only one speleothem showed increased concentrations of humic acids in its dark calcite layer. Although the principal fluorophore is fulvic acid, particulate organic matter (POM) and humic acids also contribute to fluorescence in the speleothems. However, POM in high concentrations could actually quench fluorescence intensity. Trace elements did not significantly contribute to the fluorescence measured in their wide sample of speleothems. This confirms Gascoyne's (1977) conclusions which also ruled out the presence of most common trace elements as contributors to colour and thus to colour banding.

Annual banding where there is continuous deposition of calcite or aragonite speleothems is generated by seasonal variations in the concentrations of organic substances (van Beynen et al., 1998). Each band may be a visible couplet of lighter and darker calcite, the light material corresponding to lower concentrations of organic substances trapped within the precipitating calcite. But many speleothems with fluorescent banding display no visible light-dark couplets at all. In studies at Marengo Cave, we found that two speleothem drip sampling sites displaying highly variable behaviour (they dried up after a few weeks of drought), yielded a pronounced Spring freshet enriched in organics. Two similar but perennial drips yielded the highest concentrations of the organics in solution during the lowest drip rate period, which was late summer/early fall. By contrast, Baker et al. (1997) found that over a longer term increased discharge into a cave in Bristol, England, was correlated with increases in luminescence.

Clearly there is complexity and local variation in the organic signals being incorporated into continuously deposited speleothem calcite. Therefore, the purpose of our new investigation is to compare pairs of neighbouring samples of similar appearance and

size, growing, a) where the maximum age of both samples can be reasonably approximated by independent means; b) where there is continuous dripwater feed (i.e. a humid climate) but strong thermal and biotic seasonality, and c) where there are comparably long meterological records of good quality to permit us to attempt correlation with climatic variables over decadal or centennial timespans.

5.2 Sample Sites and Speleothems

Channel Rock Cavern, Minneapolis, Minnesota (Figure 5.1) was discovered in 1935 when it was intercepted by a sanitary sewer (Spong, 1980). The natural cavern offered a convenient site for dumping sandstone tailings, which covered speleothems already present and provided a fresh stratum for new calcite growth. Drip water flow continued and produced pits within the tailings which became sealed by precipitating calcite from which clean new "inverted" stalagmites could grow. Two speleothems (Figure 5.1) 5 metres apart were collected for analysis in the summer of 1996. Both pits were approximately 20 cm deep and between 5 and 10 cm wide. The thickest portion of each speleothem was selected for analysis, 8.31 mm for sample Minne1 and 4.75 mm for Minne2. Both display a distinct marker horizon, a transition from dense, light coloured calcite to more porous, coarser dark calcite. For Minne1 this starts at 3.10 mm and Minne2 at 1.92 mm from its beginning of growth.

The cave is located beneath a roadway and residential area with a wide strip of parkland and large grassland areas and trees on the western bank of the Mississippi River (Figure 5.1). It is 18 m below the surface within Black Riveran strata of Middle Ordovician age, a series comprising the Platteville dolomitic limestone with interbedded shale, Glenwood shale and sandstone, and St. Peter sandstone formations (Spong, 1980). The main cavern is 265 m long with an average height of 7 m and width of 17 m. The climate above the cave is very strongly seasonal, with the January and July mean temperatures

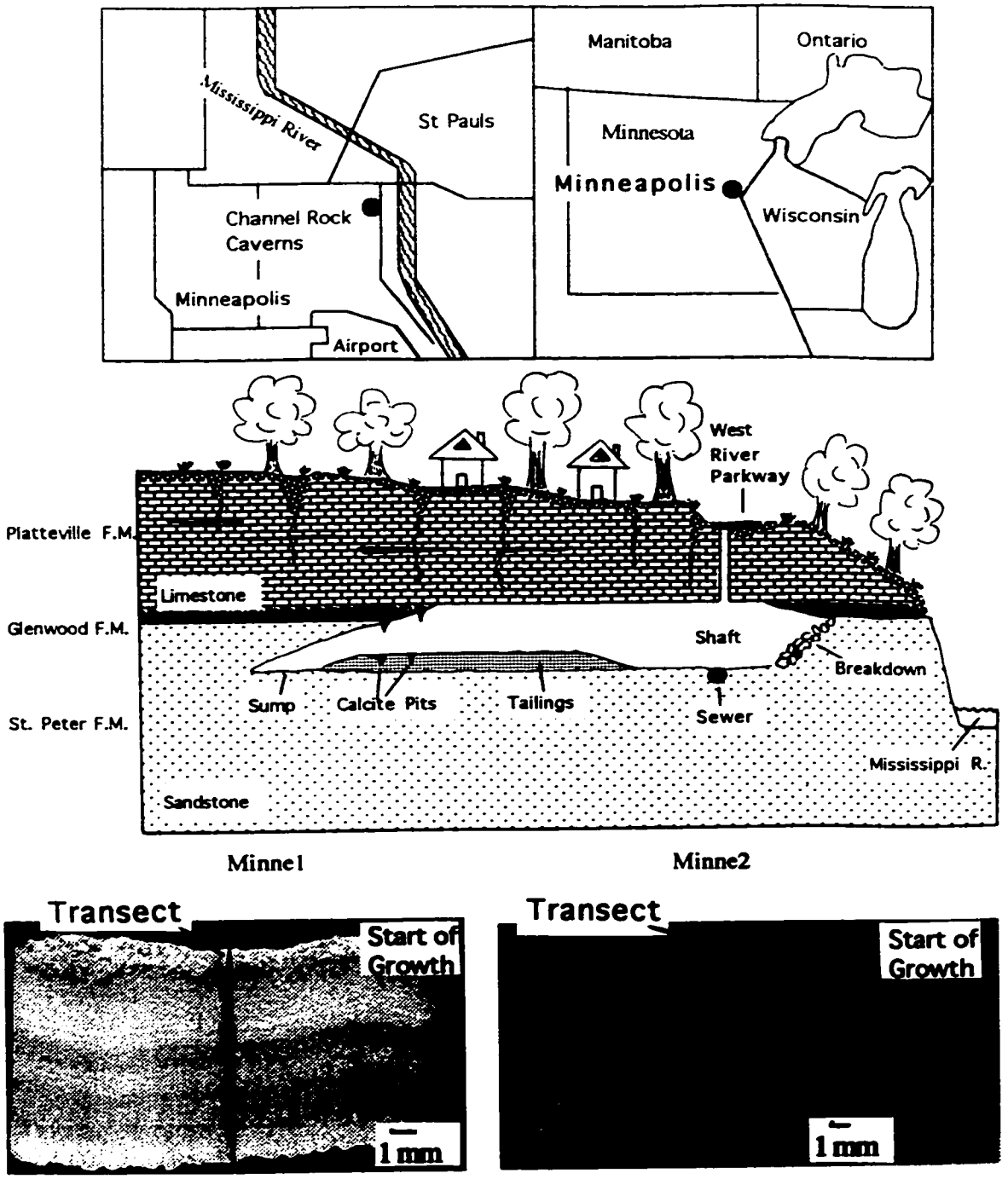


Figure 5.1. Location and Cross-section of Channel Rock Cavern Speleothems; Images of the Speleothems are Taken Under Plain Light

being -10.8°C and 23.0°C respectively and average total precipitation of 725mm for the last 35 years, although there is substantial variability (Figure 5.2).

Baradla Cave, Aggtelek, Hungary, is part of the Baradla-Domica system which straddles the Hungarian-Slovak border (Figure 5.3). The bedrock is massively bedded Triassic platformal limestones and dolomites. It is highly deformed by Carpathian tectonism which allows ready groundwater penetration. As a consequence, the cave is profusely decorated with calcite speleothems. Three specimens (AGG 1-3) were taken 10 m apart in an abandoned river passage at a depth of 40-60 m beneath the surface, which is mantled with terra rossas, rendzinas, luvisol and rendol soils. These soils are mostly less than 50cm thick. Natural vegetation of the region is oak, hornbeam, beech and ash. There are also mixed areas of grassland, spruce and pine (Zambo and Ford, 1997).

Samples AGG 1-3 were bright, white stalagmites growing upon the blackened stumps of three much larger stalagmites. Blackening was caused by smoky torches used in the earliest days of tourist showing of the cave. Most large, old speleothems close to trails in the first 2-3 km from the natural entrance at Aggtelek display this blackening. Breaking off stalactites and stalagmites to provide souvenirs for the visitors was also a common practice. Shortly after 1800 AD lanterns came to replace torches (p.c. K. Szekely, 1996) and it may be presumed that the discoloration of new calcite was much reduced.

The strong seasonality of climate for this location is depicted in Figure 5.4. The 30-year average January and July temperatures are -3.5°C and 18.5°C respectively. Precipitation also shows a seasonal variation with a summer maximum and an annual mean of 560mm. The mean annual actual evapotranspiration is less than 400mm.

5.3 Analytical Procedures

Each sample was cut perpendicular to its growth axis and polished with silicon

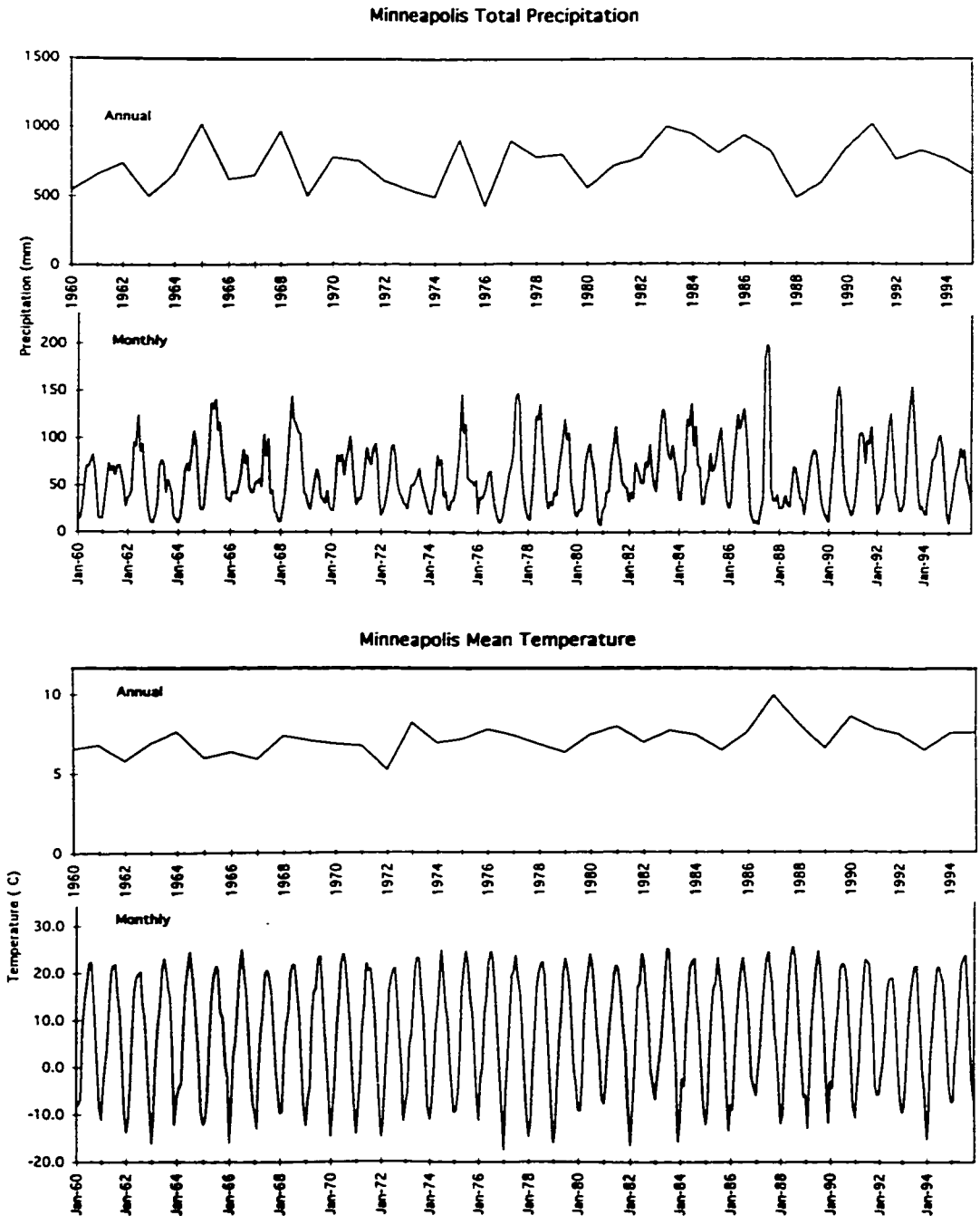
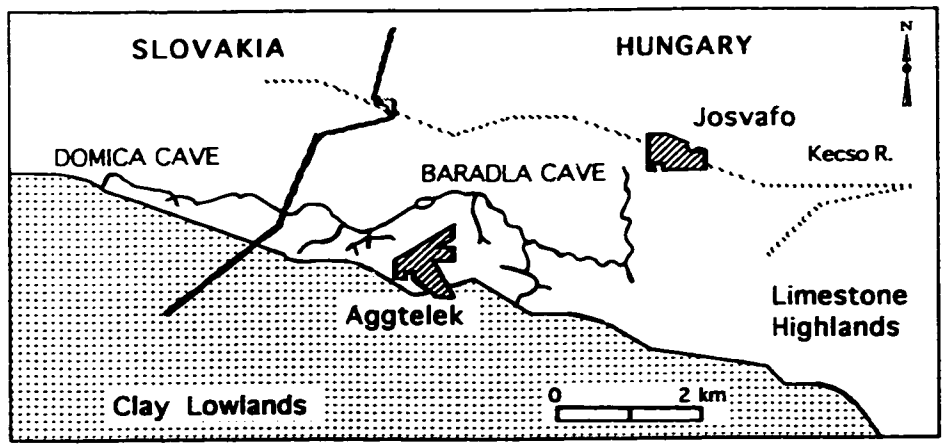
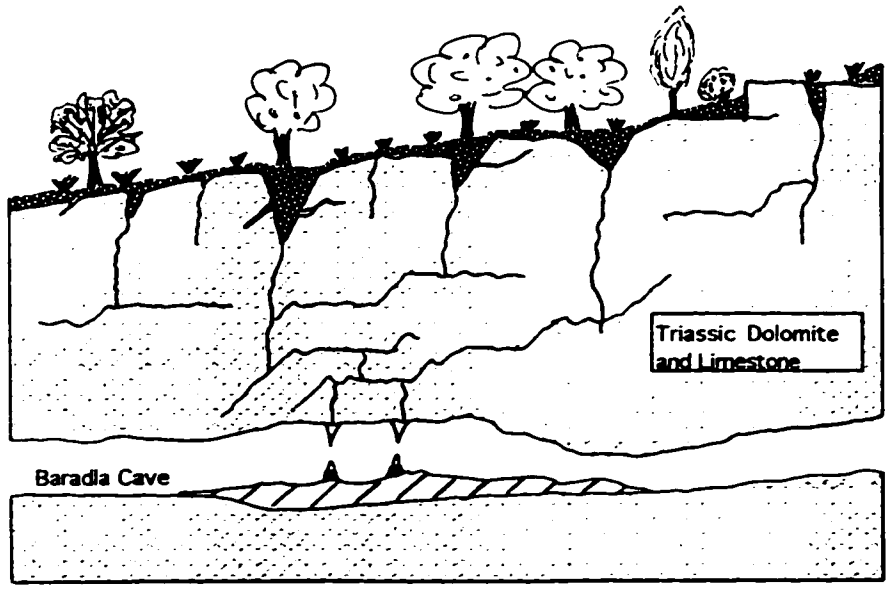


Figure 5.2. Seasonal and Annual Variability of Climate for Minneapolis International Airport for the last 30 years





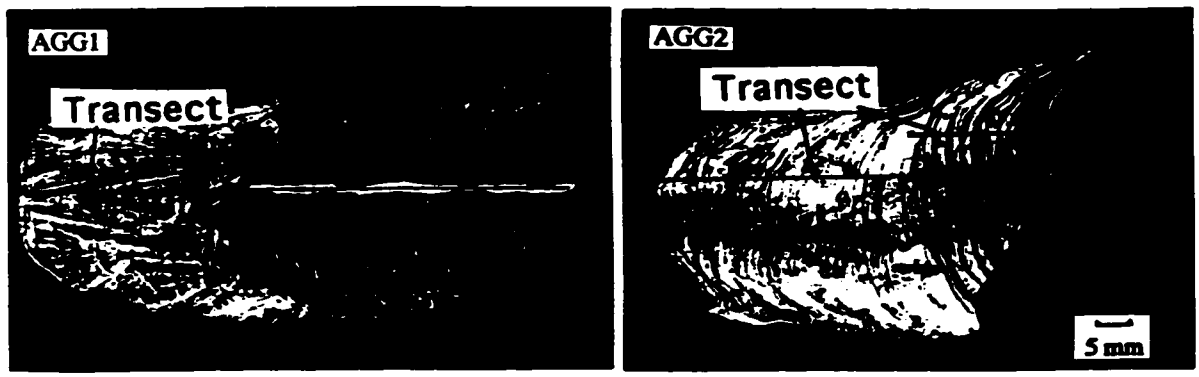


Figure 5.3. Location and Cross-section of Baradla Cave Speleothems; Images of the Speleothems are Taken Under Plain Light

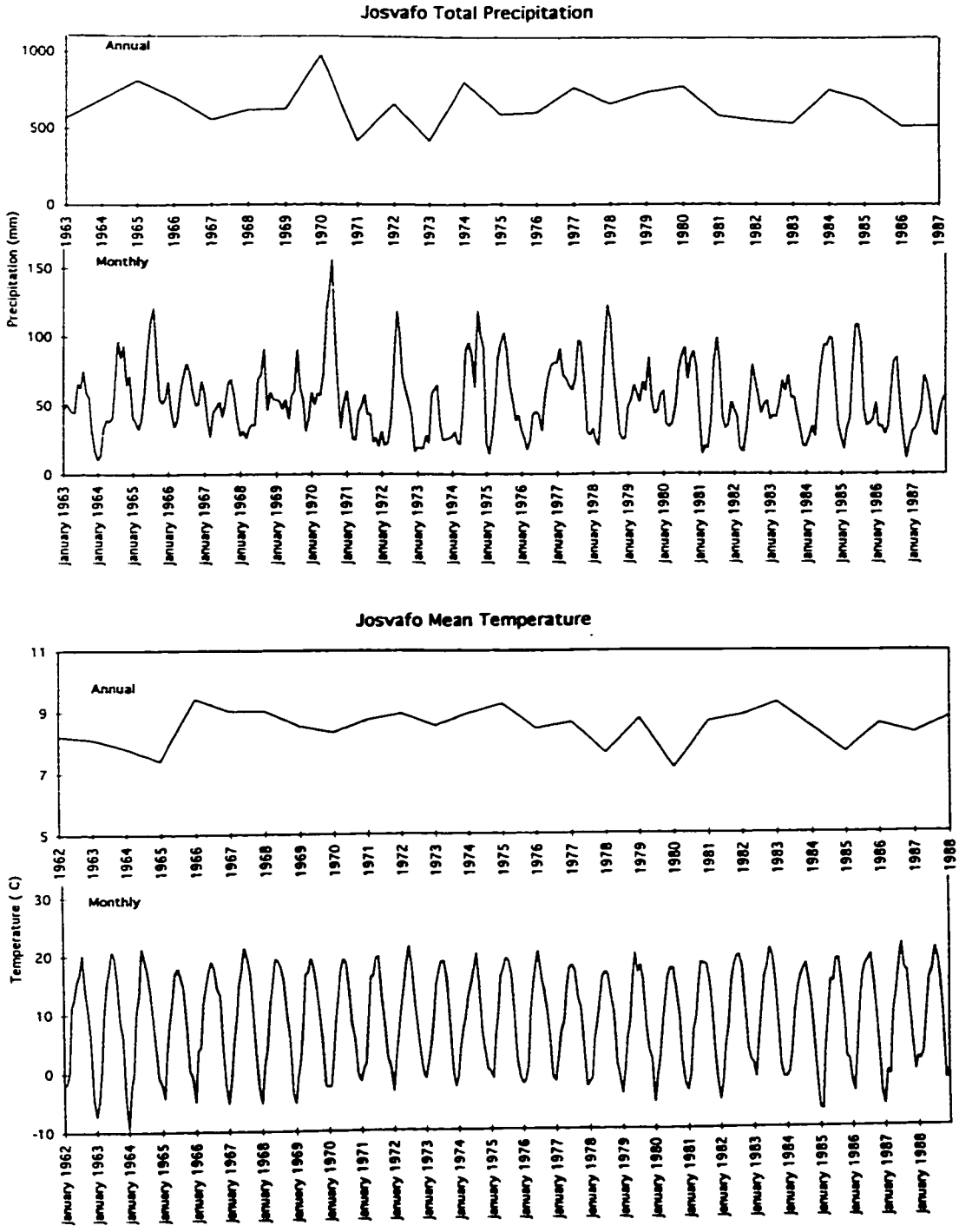


Figure 5.4. Seasonal and Annual Variability of Climate for Josvafo for the last 30 years

carbide (grain size 25-30 microns) to a uniform thickness of approximately 2mm. Every attempt was made to ensure that the growth bands were 90 degrees to the cut surface. An exception was Minne2, which was embedded in triethylene glycol dimethacrylate because of it fragility and awkward shape for cutting; Once embedded, it was cut on a slow speed diamond saw producing a smooth finish that did not require polishing. AGG3 was not used in this study because it was felt that only two samples were needed for replication and AGG1-2 were the largest samples and would consequently have the thickest laminae.

To generate fluorescence in speleothems, it has been conventional to excite the calcite with either a) laser (White 1984, Baker et al. 1993), b) a UV-visible light photographic flashgun (Shopov et al. 1997) or c) a deuterium lamp (Shopov et al. 1994). In order to capture the fluorescence of the very fine banding in the Minneapolis samples, a variation of the laser technique used by Baker at al. (1993) was adopted. The Baradla Cave samples' laminae were examined under a microscope at 10x magnification and only very broad bands could be discerned. For efficient time management, it was decided that the growth rate and band thicknesses were such that the microscopic technique used for the Minneapolis samples was not required. The estimated maximum growth period of the speleothems is about 150 years and the speleothems measuring between 70 mm and 90 mm in length, grew at least four times faster than Minne1 or Minne2. Therefore, the photographic flash technique was used for the Aggtelek samples.

Laser Raman Spectrometer and Microscopic Technique

In earlier work, van Beynen et al. (1998) showed that speleothems display maximum fluorescence with excitation wavelengths at 255 nm and 340 nm. Unfortunately, a UV laser could not be used because of the glass optics of the microscope, so a longer wavelength had to be used. Fluorescence of the Minneapolis samples was measured using a Jobin-Yuon/ISA 1 meter Raman spectrometer, which uses an Ar laser. The laser was tuned to a wavelength of 472.7 nm. Although this is not the optimum wavelength to excite

a speleothem, it still produced sufficient fluorescence from the calcite to give meaningful results. The emission wavelength at which the intensities were measured was 526 nm. This appropriate detection wavelength was determined using a Perkin Elmer LS-5 fluorescence spectrophotometer, which showed that at the excitation wavelength of 472 nm, the peak emission intensity occurred at 526 nm. The Ar laser power of the Raman instrument was set at 1.5 mW and the integration time was 2.5 seconds. Using a specially built micrometric stage, the samples were moved 12.7 μm between each fluorescence measurement.

If the laser shone for more than a few seconds on a particular spot on the speleothem, the fluorescence would begin to decline presumably due to the photochemical breakdown of the fluorescing organic substances. This decay was discovered when the reproducibility of the fluorescence measurements was undertaken. To reduce the decay, minimal integration time and laser power were used while maintaining an acceptable signal-to-noise ratio. A very short integration time (<1 second) produces a weak signal since the number of counts is low and yields a relatively high amount of noise, but the photochemical breakdown effect is small. The optimum integration time and laser power, maximising signal-to-noise and yet keeping photochemical breakdown to a minimum, was 2.5 seconds and 1.5 mW respectively.

Photographic Flash Technique

The Aggtelek samples were excited using a Vivitar 285 HV zoom thyristor photographic flashgun and the fluorescence was captured on a Kodak TMAX ISO 3200 speed black and white film with an Olympus OM 4T camera equipped with a motordrive at 1/60 second and F 3.5. A series of four photographs were taken using the motordrive, the first taken simultaneously with the ignition of the flashgun and the subsequent ones within 1/60th of a second of each other. The first photograph is of the transmitted light of the flash through the speleothem and captures some of the fluorescence but also the density of the calcite and how it absorbs the transmitted light. The second photograph captures the

delayed fluorescence. Organic substances trapped within a solid crystal matrix will produced delayed fluororescence (Guilbault, 1973). The subsequent images capture the slow decay of the phosphorescence of the speleothem. The second image was used for the capture of fluorescence for it did not have the back lighting of the flash of the first image. It also had stronger intensity than the final two images. This image of the speleothem's variable fluorescence is then transferred on to CDROM and imported into IP-LAB Spectrum® for the retrieval.

Fluorescence vs Phosphorescence

To test that we are dealing with delayed fluorescence and not true phosphorescence when using the photographic technique, AGG2 was measured for fluorescence and phosphorescence on a Perkin Elmer LS5 spectrophometer. The phosphorescence capture time was set at 10 ms. Preliminary runs of both fluorescence and phosphorescence were undertaken to determine the peak intensity emission wavelength (411 nm). Figure 5.5 illustrates the very close similarity between the excitation and emission spectra of AGG2's fluorescence and phosphorescence with peak intensity excitation wavelengths at 345 nm and peak intensity emission wavelengths at 411 nm. The only difference is in the magnitude of the intensity, with the phosphorescence intensities being a little lower than their fluorescence counterparts. What is very surprising is that both have the same peak intensity wavelengths (Figure 5.5). Guilbault (1973) states that the peak emission wavelength should be longer for phosphorescence than for fluorescence. The similarity of the fluorescence and phosphorescence spectra (Figure 5.5) suggests that this is not phosphorescence, and in fact the phenomena being measured here may in fact be delayed fluorescence, caused by the organic substances being suspended in a solid matrix. Consequently, the fluorescent lifetimes of the organic substances are lengthened and therefore the emission is fluorescence and not phosphorescence.

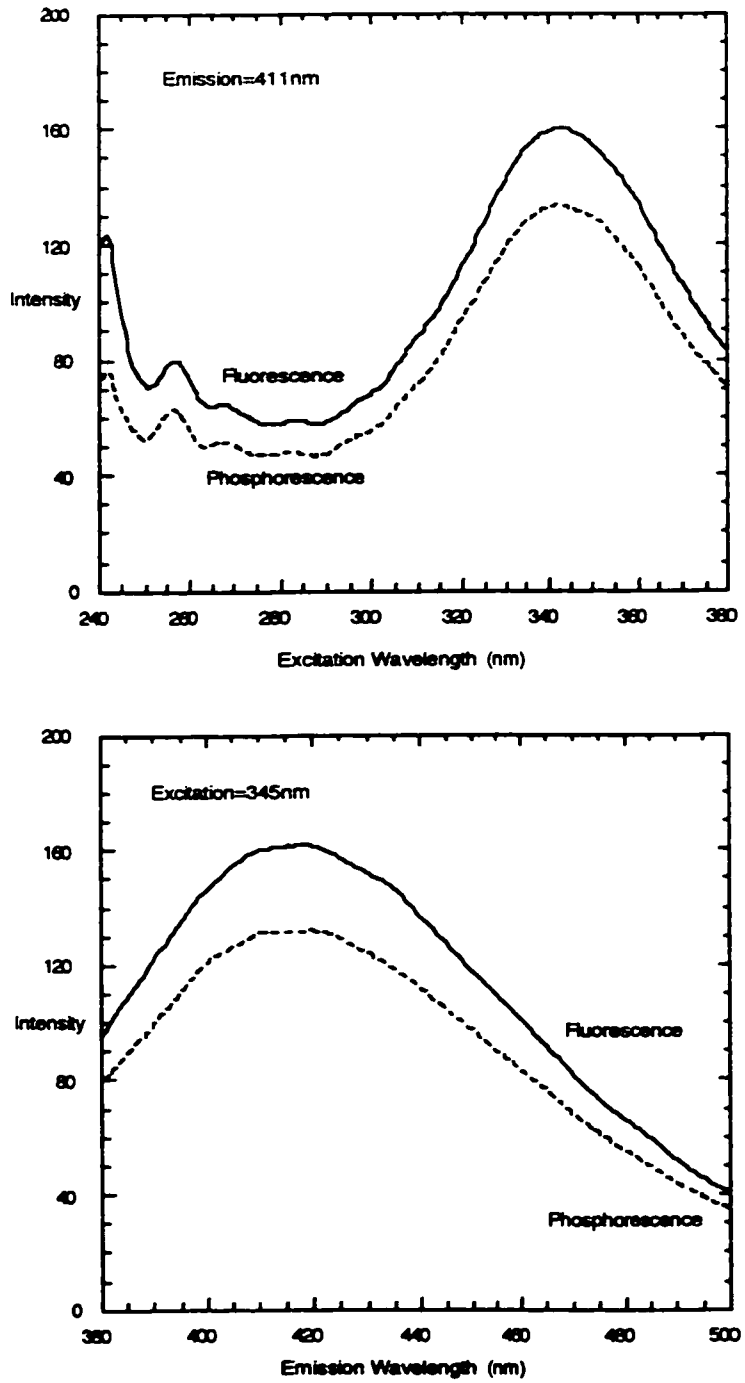


Figure 5.5. Comparison of Fluorescence and Phosphorescence Excitation and Emission Spectra for AGG2

Reproducibility of Fluorescence Signal

To determine the reproducibility of the two techniques, parallel transects approximately 2mm apart for AGG1 and 4mm apart for Minne1 apart were taken up their growth axes. The location of these transects is displayed in Figures 5.1 and 5.3. The first 4 mm of growth of Minne1 is shown in Figure 5.6. Unfortunately the laser power slowly declined over the night during capture and consequently Minne1a slowly declined in both fluorescence intensity and variability. However, over the first 4 mm the signal is still strong and has been detrended to remove the decline in it. The two signals for the Aggtelek sample are shown in Figure 5.7. Pearson correlations were undertaken to determine the reproducibility of the signals for each technique. Minne1's two fluorescence traces produced a positive correlation of 0.48 and AGG1 had a coefficient of 0.82; the significance of both correlations is 0.00001. These correlations confirm the close match of the AGG1 signals observed in Figure 5.7. Both traces possess 164 fluorescent peaks. Therefore the photographic technique does have strong reproducibility. The correlation was not as strong for the laser Raman technique, which could be due to the difficulties encountered in the capture of the Minne1a in this instance. However, the visual correlation between the two signals in the first 2 mm is close (Figure 5.6) and this was the period when no declining trend was seen in the raw data.

5.4 Results and Discussion

5.4.1 Annual bands in speleothems

The balance of current evidence (Baker et al. 1997, van Beynen et al. 1998) shows that where there is strong fluorescence banding producing light and dark couplets in a speleothem from a strongly seasonal setting, one couplet is likely to equal one climatic year. This thesis is tested in this study by counting back the couplets from the year of collection for each pair of samples from Minneapolis and Aggtelek.

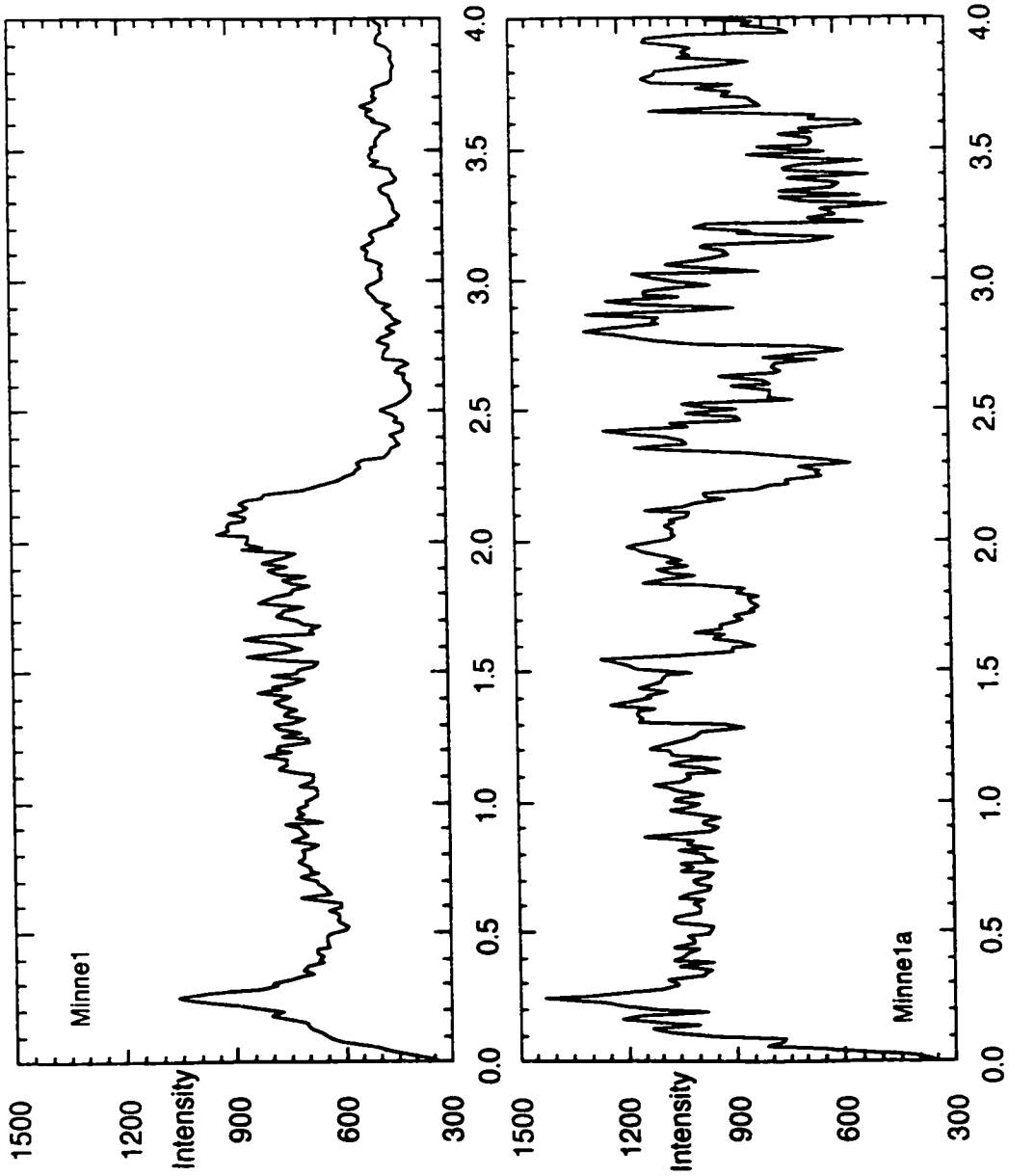


Figure 5.6. Reproducibility of the Laser -induced Fluorescence for Minne1

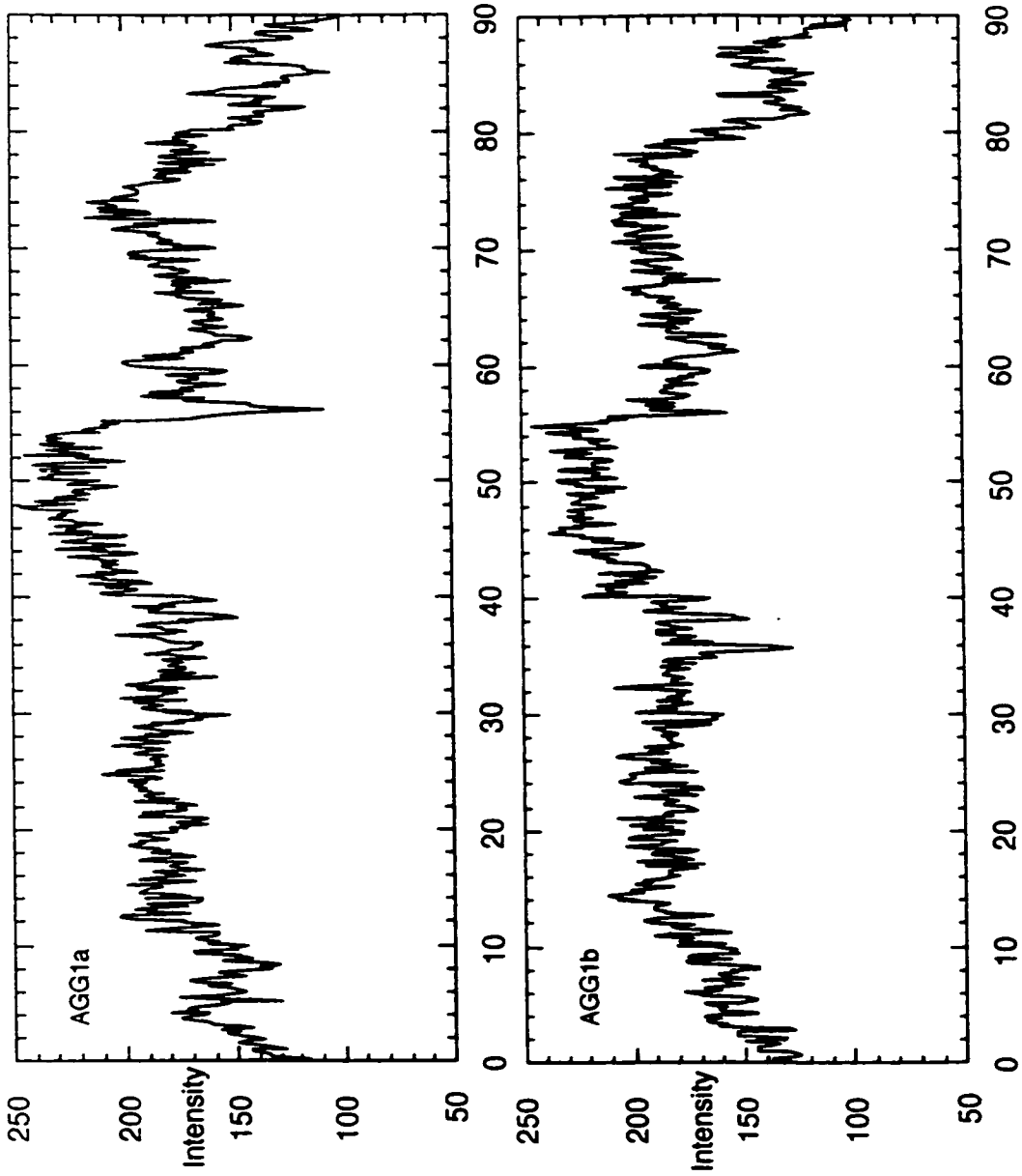


Figure 5.7. Reproducibility of the Photographic Flash Gun Induced Fluorescence for AGG1

a) Minneapolis Samples

By counting back the matching pairs of peaks and troughs of fluorescence intensities from the collection date (June 1996), the age of the speleothems and their laminae can be estimated (Figure 5. 8). As mentioned earlier, the speleothem could only have started growing in 1935, after the sandy tailings from the tunnelling were deposited in the cave and the loose sand consolidated and sealed by the earliest calcite being deposited within it. Using the counting back technique of the laminae fluorescence measured across Minne1, growth of the speleothem began in 1939-40 (Figure 5.8). This date leaves five years for the formation and sealing of the pit by the drip water, which seems entirely plausible.

Figure 5.8 compares the laser profiles of Minne1 and Minne2. Unfortunately the definition in the fluorescence signal for Minne2 is not as clear as it is in Minne1. However, a distinctive change in crystal structure and start of deposition of dark brown calcite can be used as a marker horizon in both samples. In Minne1 the dark banding is estimated to have started in 1974, hence this date was used in Minne2. Recognizable annual banding is only detectable in Minne2 after that date; before it, the fluorescence is featureless, lacking in the annual band structure. Overall the fluorescence signals for the two speleothems appear quite different. After 1974 sections of the fluorescence signal are quite similar, however, especially between 1976 and 1982, both in the shape of the yearly signal and its trend towards increasing values. Rapid growth in the mid 1980s appears in both samples and also the broad trough and peak of the late 80s to mid 90s. The intensity of fluorescence in Minne2 is also somewhat stronger than in Minne1.

An unusual feature of both signals is the large peak in fluorescence which occurs at the beginning of Minne1 and at the end of Minne2. Capture of the fluorescence signal using the argon laser and Raman spectrometer was begun at the start of growth for Minne1 and at the end for Minne2; i.e. both show a large peak at the start of the capture process. This

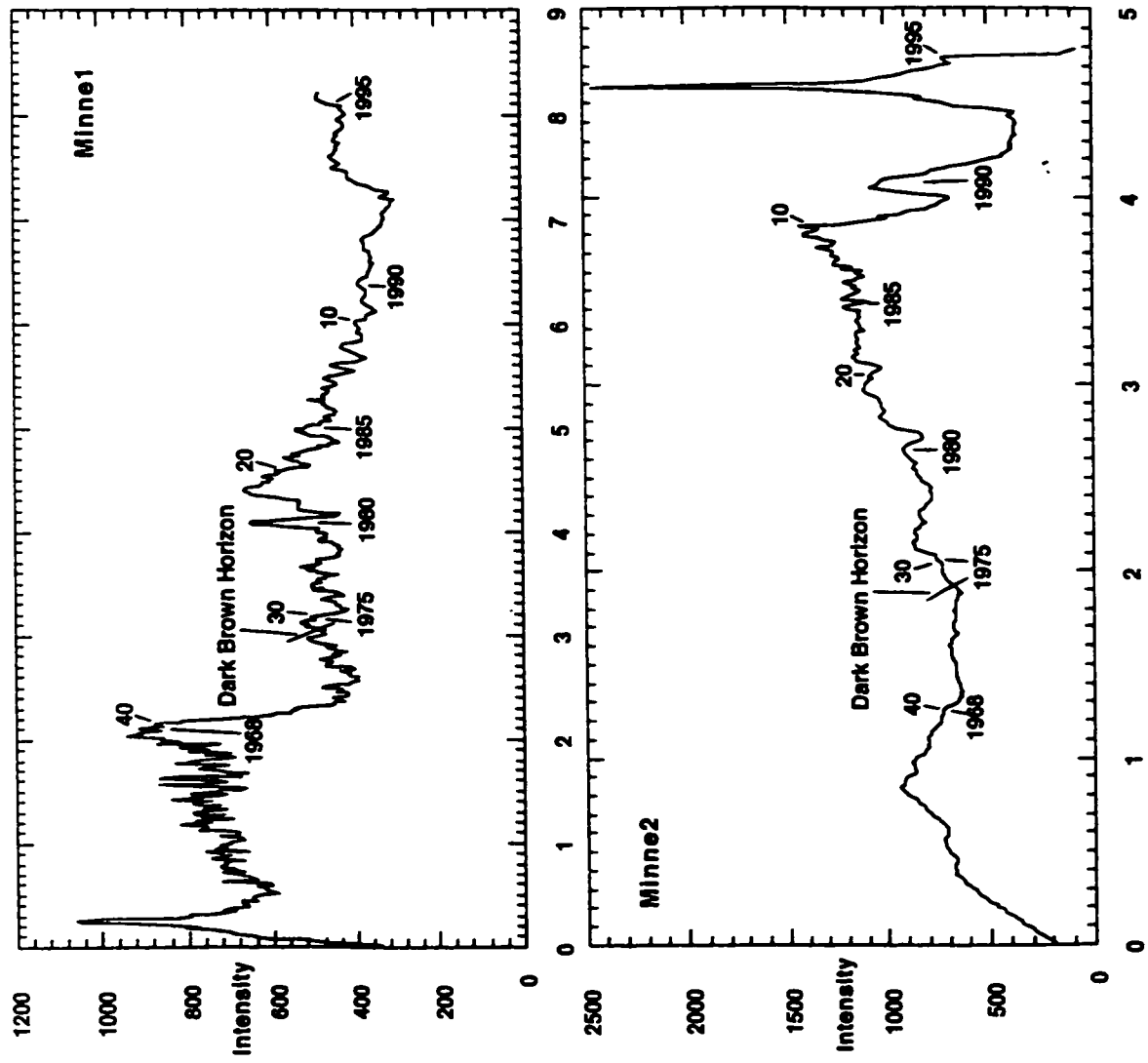


Figure 5.8. Production of Timeline for Minne1 and Minne2

suggests that the peak might be an artefact of the technique. Zones of darker calcite do occur in both speleothems where the large peaks of fluorescence were measured but they are not more prominent than other dark portions of the speleothems. If it were indeed related to technique, then the cause may be due to scattering of the laser light within the speleothem. This scattering will not only excite the organic compounds directly beneath the laser beam but also organic molecules in adjacent calcite thereby prematurely triggering the photochemical decay process. Subsequently, when this adjacent calcite is directly excited by the laser beam, it has lost some of its fluorescence potential, unlike the first portion of calcite which was not pre-exposed to the laser.

b) Aggtelek

The laminae in the Aggtelek samples, AGG1 and AGG2, were visualized using fluorescence from the sample, excited by a photographic flash gun, and photographed on high speed film (Figure 5.9). The sinusoid pattern clearly evident in these two traces may be annual bands. From the historical evidence, the age of these samples is estimated to be between 150 and 200 years. Assuming that the peaks in fluorescence are annual, we obtain an age of 165 years for the base of AGG1 and 156 years for AGG2. Some of the earliest growth of AGG2 was lost when it was collected, which probably explains the slight difference in speleothem age. Ages are assigned to each peak on the basis of this assumed annual cyclicity (Figure 5.9).

The most striking aspect of the fluorescence from both speleothems (Figure 5.9) is the similarity in trends of the fluorescence. The fitted age sequence shows that the major peaks and troughs occur in the same year for AGG1 and AGG2. The broad increase in fluorescence from the 1940s to the early seventies and the subsequent decline in the eighties and nineties are trends that match closely. Both also show the same gradual increase in fluorescence from the 1840s to the 1880s. The same peaks and troughs are also particularly reflected in the first half of the 1920s and early 1930s. It is therefore

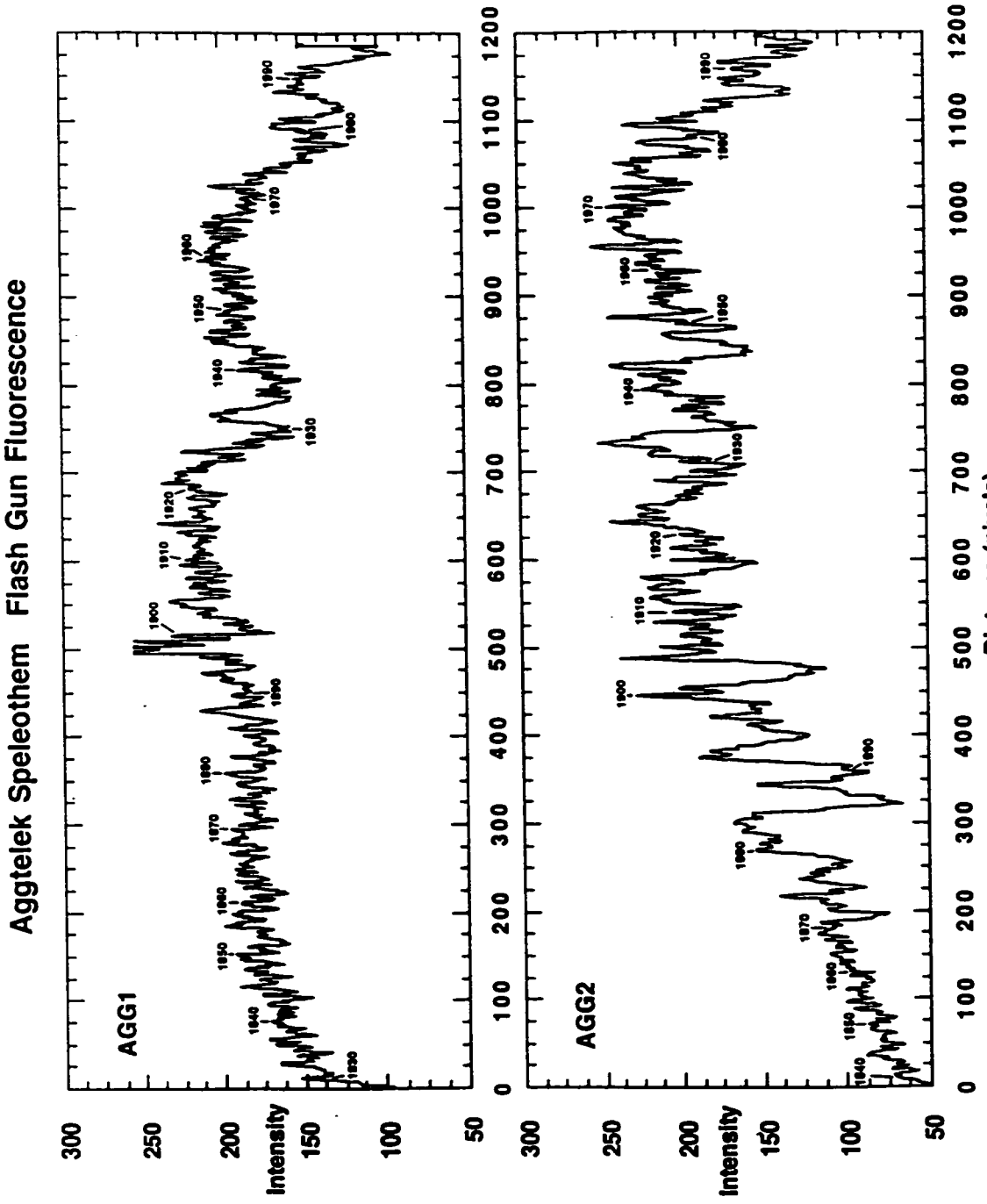


Figure 5.9. Production of Timeline for AGG1 and AGG2

reasonable to state that both the speleothems produce similar fluorescence patterns. Note that the measured intensity of fluorescence is also very similar in the two speleothems.

A criticism levelled at this technique is whether the "annual" variations in fluorescence are in fact real or just "noise" produced by the grain structure of the original photographic negative. When the negative is magnified, as in this case, the graininess of the image can become apparent. To investigate the problem, two parallel transects of the phosphorescence measured along the growth axis of AGG1. If the grain of the film was producing the signal then the two transects would not have shown the high correlation seen above (Figure 5.7). Therefore, the signal is indeed real and not an artefact of the film's grain.

5.4.2 Annual laminae thickness and annual peak fluorescence

Band widths for Minne1 and Minne2 were read directly from the micrometric stage of the Raman spectrometer. The thickness of an annual laminus for the Aggtelek samples was the number of pixels between fluorescence minima multiplied by a conversion factor to produce the actual width in millimetres.

a) Minneapolis Samples

Figure 5.10 shows the laminae widths and annual peak fluorescence for Minne1 and Minne2. Only the latter half of growth was used in Minne2 because as mentioned earlier, the period from 1940 to 1970 has very little variability in the fluorescence, making it impossible to delineate the annual bands. Both speleothems exhibit some variability in the thicknesses of the annual laminae. Minne1 ranges between 0.03 mm and almost 0.4 mm (a factor of 12). If the first 30 years of growth are averaged in Minne2, it ranges 0.04 - 0.25 mm (X6). The mean accumulation rate for Minne1 is 0.1322 mm, with a standard deviation of 0.1412 mm.

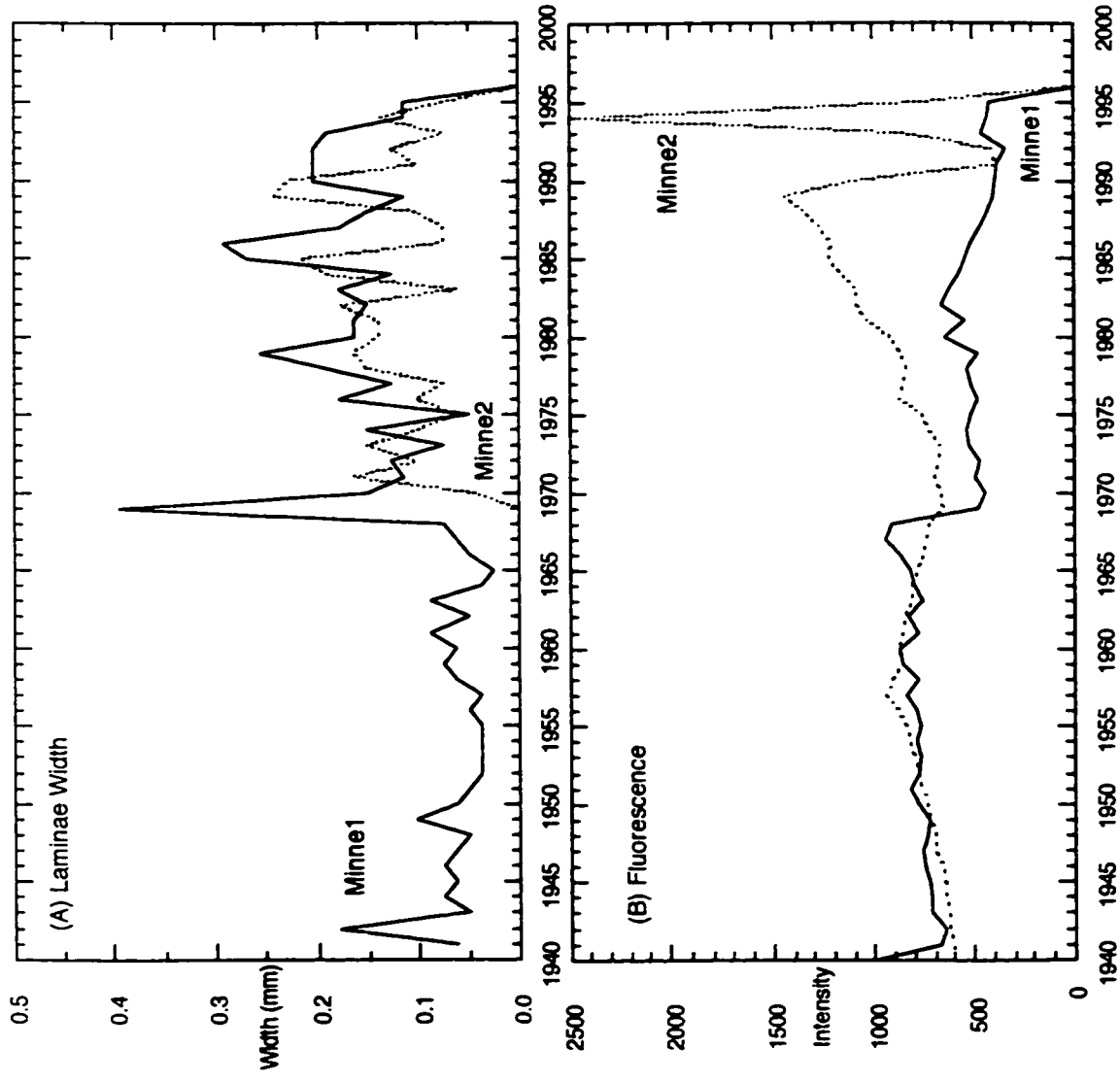


Figure 5.10. Minneapolis Samples Laser-induced Annual Peak Fluorescence and Laminae Widths

On viewing the photographic image of Minne1 (Figure 5.1), it is apparent that there were two distinct phases of growth. The first phase, approximately one quarter of the total accumulation, is of dense calcite with very fine banding structure. The subsequent growth is darker, less fluorescent, and has wider laminae with coarser crystal structure. This observation is supported when the growth as measured in annual bands is examined (Figure 5.10a). In the first 28 years of growth 2.04 mm of calcite was deposited; in the succeeding 28 years there was more than three times that amount - 6.25 mm. Minne2 also displays the same shift in growth rate, with 1.45 mm for the first half of growth and 3.3 mm for the second half. The reason for such a change in the growth rates probably lies in geometry of the deposits and their drip pits. As the pits grow, calcite is deposited on the inside of the "pot" and with time, the inner circumference becomes smaller. Such a decrease would produce thicker laminae because the year's quantity of precipitating calcite has less area to cover.

Peak annual fluorescence for both samples (Figure 5.10b) does not appear to be similar. Minne1 demonstrates a sharp drop in luminescence after 1968 which may be related to the increase in band width. When more calcite is deposited in a band and if the organic content of the drip waters remains constant, then organic concentrations in the calcite will drop, and consequently reduce fluorescence. However, Minne2's annual peak fluorescence is quite different from that of Minne1 with a steady rise in fluorescence from 1970 to 1988, although after this date both speleothems show the same trough and peak pattern, although Minne1's fluctuation is less extreme. The period between 1940 and 1965 does show some similarity between the two speleothems, however the variability in the signals is small and comparison is difficult.

b) AGG1 compared to AGG2

Figures 5.11a depicts the annual laminae widths for the period, 1880-1992 for both

samples. There appears to be a reasonable match between the laminae widths, with periods of low growth being common in each speleothem. For example, the mid 1880s, early 1910s, and the late 1970s are common episodes of high growth. Both speleothems also experienced low growth periods in the 1920s, 1940s, early 1960s, mid 1970s and early 1980s. AGG1 has a mean annual growth rate of 0.5493 mm (std. dev. = 0.2056); AGG2 yields 0.4692 mm (std. dev. = 0.1898). These findings indicate that, over comparatively long terms at least, neighbouring speleothems may grow at very similar rates in a cave.

Figure 5.11b illustrates the peak annual fluorescence for the Aggtelek samples. Although there are some periods when the two signals do not coincide, on more occasions than not, they exhibit a similar signal. The lack of match for certain years is probably an artefact of the counting assignment, e.g. the band assigned to 1940 AD in each sample could in fact be 1939 in one and 1942 in the other; it is doubted that greater error than this (~6%) could have occurred in counting back from the sampling year, 1996, to 1940 but, clearly, any such errors would be accumulative and thus greater further back in time. In addition, the slightly differing feedwater paths from the soil to the drip sites could cause differences of rate.

The simple correlation between the two raw data sets of Figure 5.11a is $r = 0.22$ (significance = 0.03). Attempts to improve the match by shifting the raw annual record of AGG2 in one-year steps up to a maximum of five years either side of an arbitrary starting point in the AGG1 record did not significantly improve this correlation. 2-year and 3-year running means (Figure 5.12) greatly improve the match, however; the correlation for the 3-year mean is $r = 0.45$ (significance = 0.0001). As suggested by the mean growth rates, the two specimens are responding to environmental controls in a very similar manner.

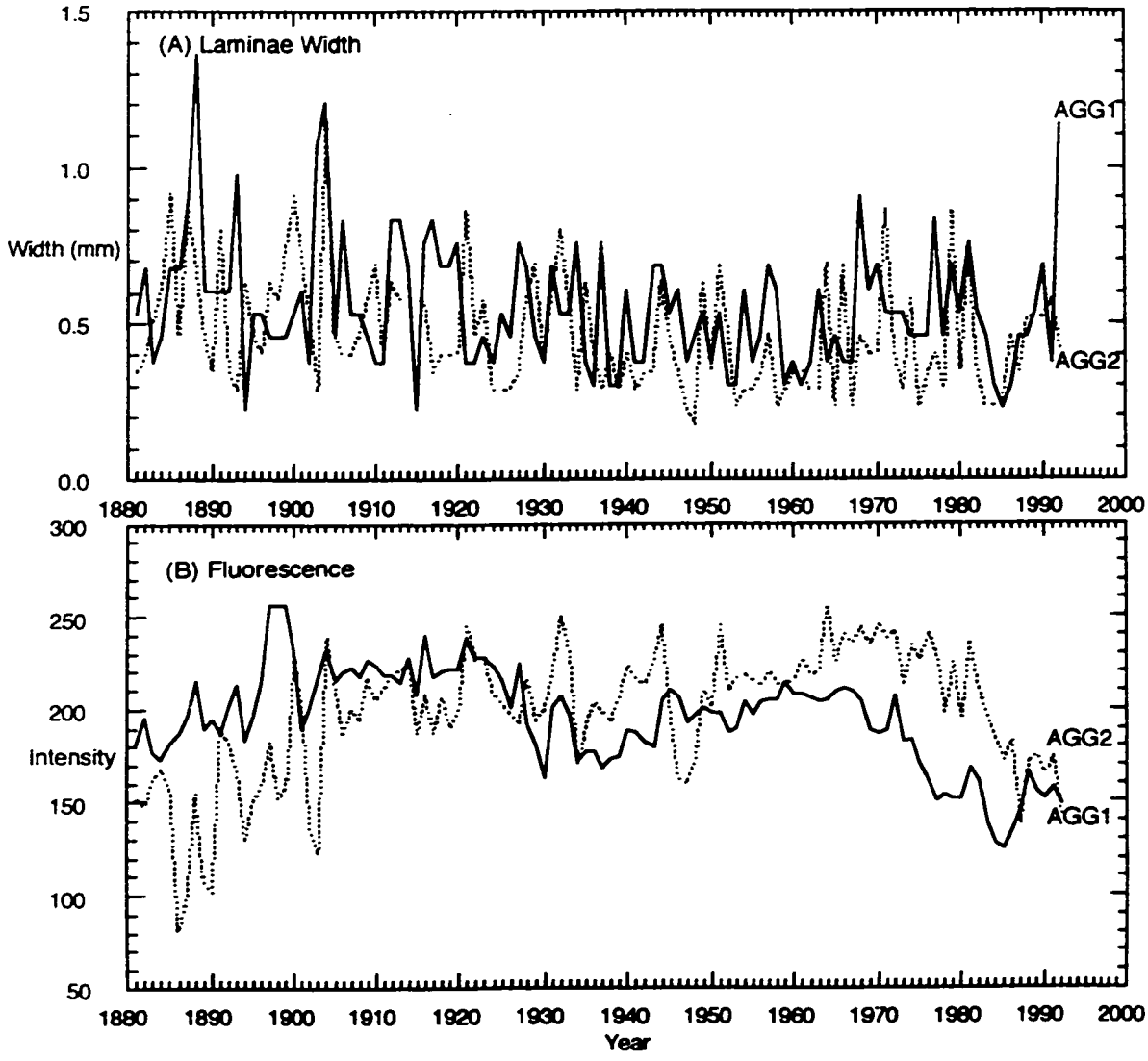


Figure 5.11. Aggtelek Speleothems Annual Laminae Widths and Annual Peak Fluorescence

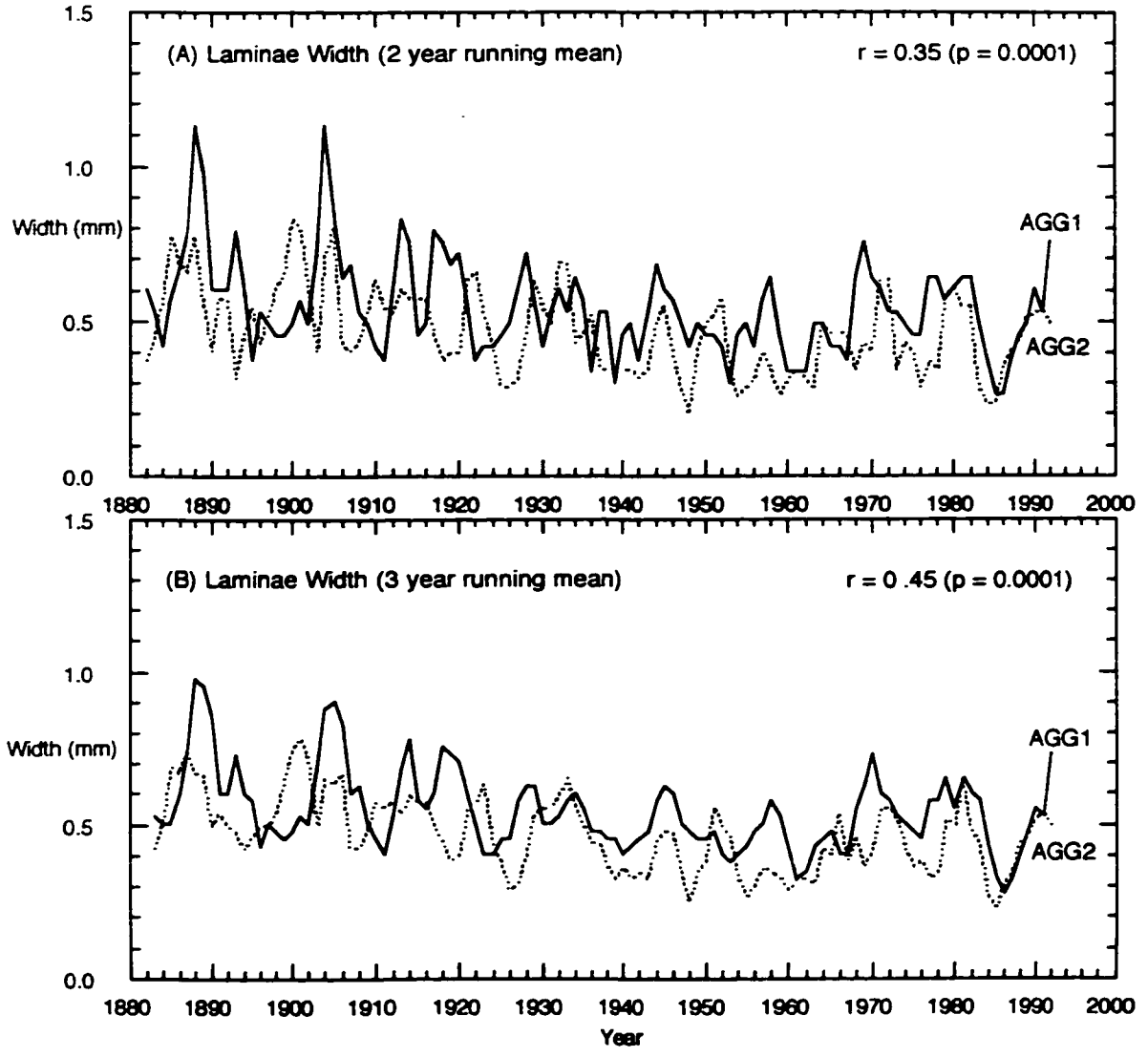


Figure 5.12. Two and Three Year Running Mean of Laminae Widths for the Aggtelek samples

5.4.3 Climatic Correlations with Fluorescence and Laminae Width

a) Minneapolis Samples

Maximum fluorescence intensity and laminae width of Minne1 and the post-1970 record of Minne2 were examined for correlation with annual total precipitation, mean annual temperature and annual total water surplus.The weather station used for the climate data was Minneapolis International Airport. The Thornthwaite water balance used to estimate the soil water surplus incorporated a soil storage value of 100 mm, as suggested by Borchert and Gustafson (1980).

Table 5.1 depicts the Pearson correlation coefficients and probabilities for each of the variables for the period 1940 to 1996 with significance being specified as probabilities ≤ 0.05. The only significant positive correlation is between Minne1 laminae width and precipitation. A positive relation between precipitation and Minne1 band width may be explained by augmented precipitation transporting more $CaCO_3$ in solution and thereby generating a thicker annual band. The weaker negative correlation between Minne1's fluorescence and precipitation is probably a consequence of the relationship just explained. More precipitation eventually leads to thicker bands, and as they increase in width, the concentration of organic substances in the calcite decreases and therefore so does the fluorescence, as seen in Table 5.1. Decreasing concentrations of organic substances in the calcite of the larger bands also explains the strong negative correlation between fluorescence and band width. The negative relationship between Minne1 fluorescence and the water surplus can be explained with the same argument. Water surplus is a measure of how wet the environment was for a particular year, and higher levels of precipitation would lead to greater water surpluses. No significant correlations were found between Minne2 and any of the climate data, nor between fluorescence and band width.

Table 5.1. Minneapolis speleothems fluorescence and band width related to climate.

	Minne1 Maximum Annual Intensity		Minne1 Annual Laminae Widths		Minne2 Maximum Annual Intensity		Minne2 Annual Laminae Widths	
	Correl.	Probab.	Correl.	Probab.	Correl.	Probab.	Correl.	Probab.
Minne1 Laminae Widths	**-0.68**	**0.0001**						
Minne2 Maximum Annual Intensity	0.06	0.77	-0.02	0.93				
Minne2 Laminae Widths	0.04	0.86	0.10	0.64	0.27	0.19		
Annual Total Precipitation	**-0.28**	**0.04**	**0.44**	**0.0001**	-0.001	0.99	-0.11	0.60
Surplus	**-0.29**	**0.03**	0.06	0.67	-0.03	0.90	-0.04	0.85
Annual Mean Temperature	-0.19	0.16	0.11	0.43	0.17	0.39	-0.14	0.49
January Mean Temperature	-0.06	0.66	0.03	0.82	-0.05	0.79	0.08	0.71
July Mean Temperature	-0.06	0.66	-0.04	0.79	0.17	0.42	-0.19	0.35

Table 5.2. Aggtelek speleothems fluorescence and band widths related to Josvafo climate, 1962-1987.

	AGG1 Maximum Annual Intensity		AGG1 Annual Laminae Widths		AGG2 Maximum Annual Intensity		AGG2 Annual Laminae Widths	
	Correl.	Probab.	Correl.	Probab.	Correl.	Probab.	Correl.	Probab.
AGG1 Laminae Widths	0.05	0.80						
AGG2 Maximum Annual Intensity	**0.72**	**0.0001**	0.02	0.93				
AGG2 Laminae Widths	0.13	0.47	0.29	0.16	0.23	0.21		
Precipitation	0.07	0.72	0.12	0.56	0.38	0.06	-0.01	0.97
Surplus	0.01	0.98	-0.02	0.93	0.04	0.83	-0.24	0.23
Annual Mean Temperature	0.12	0.56	0.19	0.37	**0.42**	**0.03**	0.24	0.26
January Mean Temperature	-0.15	0.47	0.02	0.92	-0.28	0.16	-0.15	0.48
July Mean Temperature	0.29	0.15	-0.07	0.73	0.08	0.70	-0.22	0.28

b) Aggtelek Samples

For the Aggtelek samples, only the flash gun technique was used for the capture of fluorescence and annual band widths. The closest weather station to the cave is at the village of Josvafo, ≈4 km from the sampling site, but it has only been established for just over 30 years (1962-1988). Longer climate records are available at the industrial city of Miskolc, (45 km distant) and at Budapest (160 km). In work in progress we are investigating the strength of correlation between the two latter and Josvafo to determine if valid meteorological time series can be extended further back than 30 years. This chapter considers only the Josvafo data.

Only two weak correlations have been obtained between the speleothem data and the Josvafo climate statistics (Table 5.2). The fluorescence record of AGG2 correlates positively with annual temperature and precipitation. Increased annual temperature may be a result of milder winters and hence with reduced freezing of the soil that, coupled with increased precipitation, causes more organic substances to be flushed from the soil into the cave, thereby increasing fluorescence. There were no significant correlations between water surplus and fluorescence or band width for the Aggtelek samples. Given that the AGG1 and AGG2 fluorescence are highly correlated for this period, it is surprising that AGG1 does not share the same relationship to the Josvafo data.

To determine the reliability of these correlations, scatter plots for the pairs of data sets showing significant Pearson correlations are shown in Figure 5.13. Figure 5.13a does confirm the significant negative relation between Minnel laminae width and fluorescence. However, Minnel fluorescence does not appear to be correlated with either the water surplus or precipitation (Figure 5.13b and c), in spite of statistically significant correlation coefficients. Figure 5.13d does show some semblance of the positive correlation between Minnel laminae width and precipitation. For the Aggtelek samples, AGG2 fluorescence does not show any real relation with Josvafo's annual temperature (Figure 5.14a).

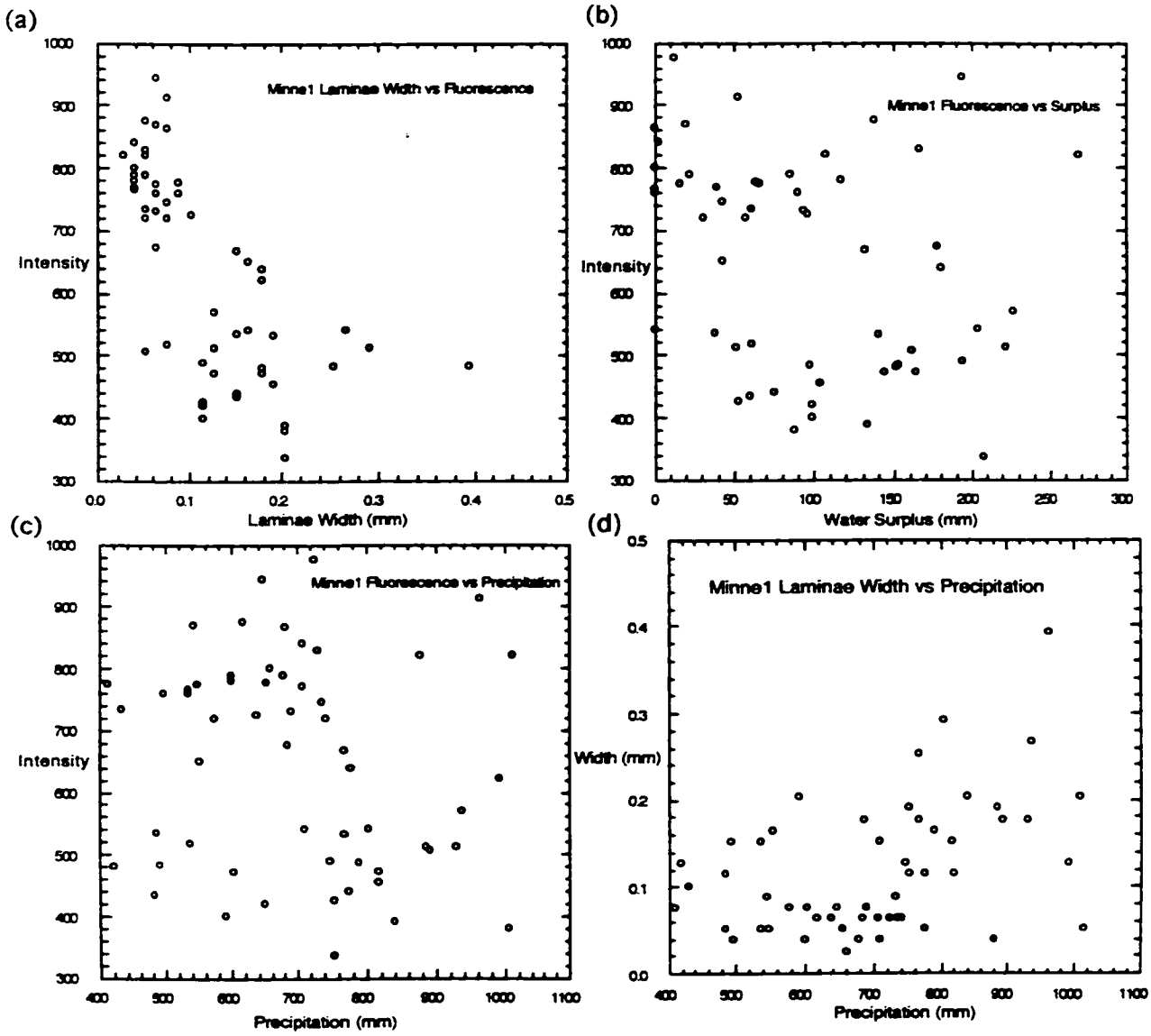


Figure 5.13. Scatter Plots of the Significant Correlations Between Climate Data, Fluorescence and Laminae Widths for the Minneapolis Samples

However the significant positive correlation between AGG2 fluorescence and Josvafo's precipitation may be real (Figure 5.14b).

Previous attempts to correlate properties of the annual laminae with standard climatic variables measured at nearby meteorological stations have also had very limited success and whether they could be correlated with climatic variables above the cave also had difficulty finding strong correlations. Genty and Quinif (1996) found that two speleothems from a canal tunnel in Belgium had a strong correlation between annual laminae width and water excess (r=0.84). They also discovered that dark calcite was deposited during one year with particularly high precipitation. Genty et al. (1997) used luminescence intensities to define annual band widths and correlated these with climate data for one stalagmite from the same tunnel as the samples used by Genty and Quinif (1996). They suggested that differences in the structure of the brightness of the banding could be correlated with seasonal variations in precipitation. However, no significant correlations were found between band width and annual precipitation, water surplus or temperature except for the year 1975, where band width matched with water surplus. They suggested that Ca ion loadings or some complex link between precipitation and cave drip rates may better explain the laminae variations than temperature or precipitation alone. We have obtained weak correlations between fluorescence and temperature and precipitation at Aggtelek, which the Belgian group did not, but cannot confirm their correlations between laminae thicknesses and what would appear to be the most significant external variable, water surplus. It is established that water balances and groundwater recharge in maturely karsted areas do not conform well to standard balance equations for soils and Darcian aquifers because, during heavy rains, for example, much water is drained directly into karst orifices and so is lost to the soil storage and evapotranspiration processes, (Ford and Williams 1989, p96 et seq). The effect is to diminish evaptranspiration losses over karst by amounts that vary with rainfall intensity, density of epikarst, etc, so creating a very complex relationship. There are intense investigations in progress at two field sites,

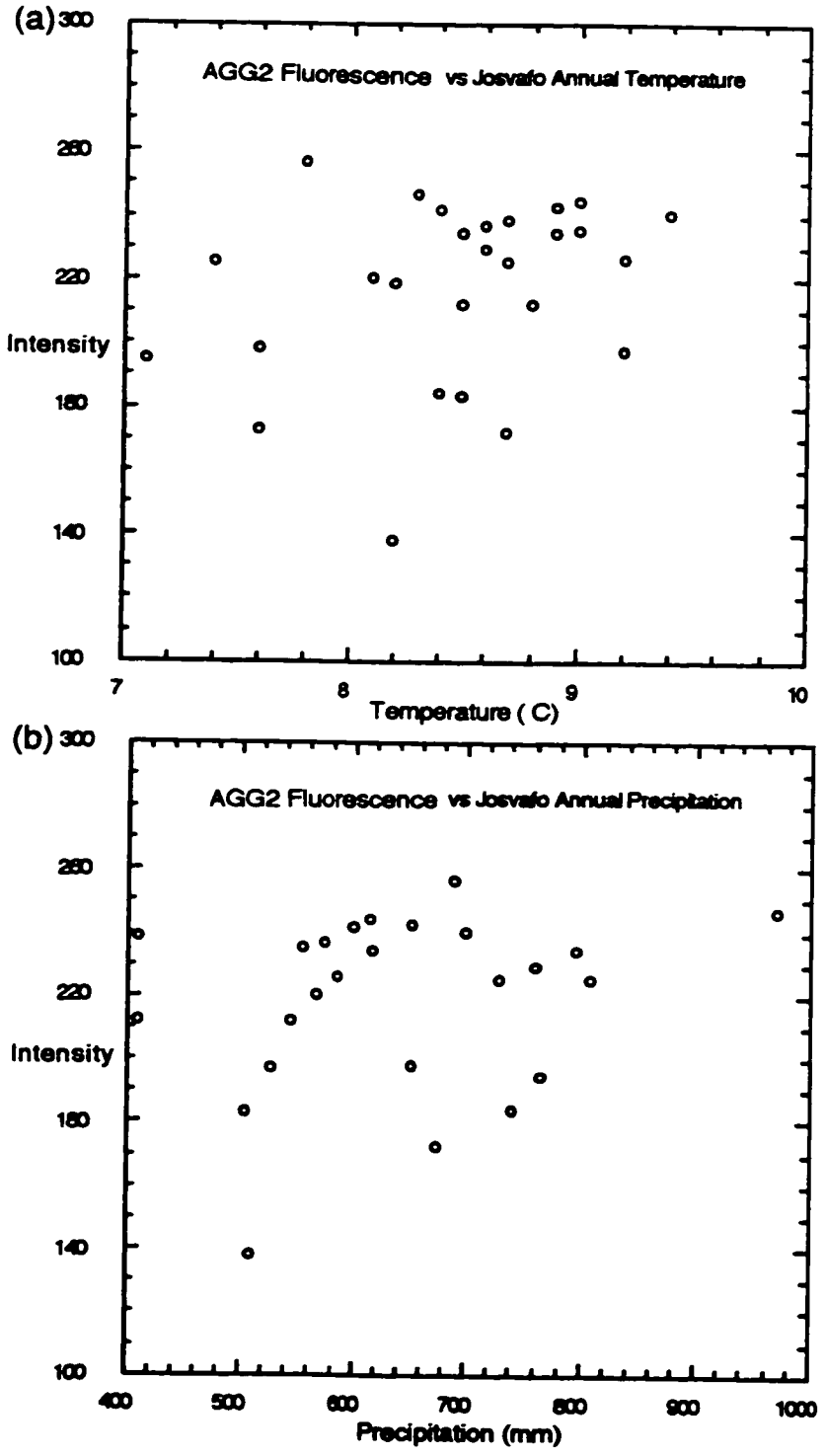


Figure 5.14. Scatter Plots of the Significant Correlations Between Climate Data, Fluorescence and Laminae Widths for the Aggtelek Samples

Vorosto (2 km from the Aggtelek sampling sites) and in the French Jura, which may clarify the problem in the near future.

The significant relationships between fluorescence and climate at both Minneapolis and Aggtelek pertain to augmented precipitation levels increasing fluorescence at Aggtelek and laminae width at Minneapolis. This can have important implications for paleoenvironmental interpretation of fluorescence in older Holocene and Quaternary speleothems, where conventional climate data do not exist. Increased fluorescence and laminae width may be indicative of a wetter periods. However, these results are tentative.

5.5 Conclusions

The aims of this study were to ascertain if annual bands could be clearly discerned in modern speleothems using fluorescence measurements and if so, whether any environmental relationships existed between the fluorescence, band widths and selected climate variables. Annual laminae as depicted by fluorescence were present both in Minneapolis and Aggtelek and the two speleothems from each cave yielded similar fluorescent signals. The reproducibility of the signal for AGG1 was tested and each run had the same number of annual peaks and very similar peaks and troughs in fluorescence. Minne1 did not have the same reproducibility, although inherent problems in its first run made comparison difficult. The thicknesses of the calcite bands as measured by the distance between the minima of fluorescence for each year could then be compared with fluorescence/fluorescence intensity and with climate data for each location. The relationship between band width and fluorescence for Minneapolis was negative, with increasing width reducing the fluorescence, which is to be expected due the lower organic concentrations in the calcite in wider laminae. Although the correlations were not strong for the period 1881-1992, AGG2 band width correlated positively with AGG1 fluorescence and band width. For the period 1963-1987, AGG1 and AGG2 fluorescence were more strongly correlated.

Correlations between climate data and fluorescence band width were attempted for each location, and for Minneapolis, there was a positive correlation between laminae widths and precipitation, but also a weak negative correlation for Minne1 fluorescence and water surplus. AGG2 gave the same result and also showed a positive correlation with precipitation and fluorescence while AGG1 did not. Such results suggests that increased precipitation may have generated wider laminae and enhanced fluorescence within speleothems. Correlations between band width and fluorescence was opposite at the two localities. This may relate to the proportions of non-fluorescent organic matter in each speleothem.

Two techniques were used in this research to induce fluorescence from the speleothems, the photographic flash gun and the argon laser, both techniques were used on the Minneapolis samples. Each technique has its advantages and disadvantages; the photographic flash gun is a simple and quick method but the resolution is not very detailed, whereas the laser technique provides great detail but is laborious and may cause decomposition of organic matter in the sample resulting in altered fluorescence readings.

Future research is needed to produce more solid conclusions about these techniques and their results. Firstly, a comparison between fluorescence and fluorescence of the Minneapolis samples is one such avenue that needs to be explored. If the same organic compounds produce both the fluorescence and fluorescence as is suggested in this paper, then the signals measured should be very similar. Secondly, multiple runs across a speleothem using the laser technique will allow a better insight into the reproducibility of the fluorescence signals obtained.

Chapter 6: Conclusions

6.1 Summary of results

The primary goal of this research was to ascertain the environmental significance of fluorescence of annual laminae in speleothems and to determine if it could be used as a proxy record for environmental change. To fulfil this goal, certain key questions needed to be answered. First, the mechanism of the production of the annual band structure required investigation through a detailed study of variations in the organic content of drip waters. Second, it is not known how conditions in the soil waters affect the drip waters beneath it and consequently the speleothem calcite precipitated from it. Third, it has not been conclusively proven whether organic substances or trace elements are the dominant cause of fluorescence in speleothems. Connected with this research is an examination into the characteristics of these organic substances and their relation to the colour hues and densities of the calcite. Finally, links between weather, climate and climate change and any variations in organic fluorescence in speleothems have yet to be conclusively demonstrated.

Marengo Cave in southern Indiana provided an ideal location to study the cave drip waters to determine what characteristics of the water could produce annual laminae. It was found that certain times of year yielded higher concentrations of organics substances than others. The spring seemed to have augmented levels of humic matter as seen in the DOC levels and fluorescence intensities. Spring waters were higher than the winter or summer because of the thaw allowed flushing from the soil of organic matter into the cave. It was also apparent that small organic molecules such as fulvic acids were the predominant substance reaching the cave. It appeared that there was a relationship between the fluorescence and temperature, whether it be with a lag or without, depending on the location within the cave. However, the sites in the cave which showed this relationship dried up for part of the summer and fall, and the sites which continued to flow did not

show the same strong relationship. Therefore, a second set of analyses of these continuously dripping sites was undertaken at a seasonal scale as it was thought that the differences may be more apparent than on a weekly basis.

Pertaining to the relation between surface and cave waters, the surface waters showed little variability in organic concentrations throughout the year, unlike the cave waters which demonstrated a bimodal peaks in organic yields in the Spring and Fall. Fluorescence and size fraction results suggested that the organic assemblages seemed to change as water percolated through the soil to the cave. Not only did concentrations display a 60-80% decrease upon reaching the cave, but there is also evidence of bonding occurring between the organic compounds and Ca^{2+} because certain size fractions decline while others increase in relative proportion with the water's passage to the cave. The maximum fluorescence of the excitation and emission wavelengths also become shorter. Changing surface conditions such as variable precipitation appears to affect both the soil and cave waters with the Fall's low levels increasing organic concentrations to an annual high in both the B-horizon water and cave drips. Finally, it appears that the larger hydrophobic compounds are preferentially removed by the soil and rock leaving the smaller hydrophilic compounds to reach the cave.

From the above results it was apparent that seasonality is important to production of annual bands. The seasonal fluxes of organic acids would produce light bands (Summer) and the darker band would extend from the fall to the spring. Calcite precipitated in the summer could appear white and porous because this period produced the highest Ca ion concentrations in the drip water but the lowest organic concentrations. This contrasts with the dark dense calcite for the rest of the year.

This finding was investigated in more detail in Chapter 4, but with bulk samples of

light and dark calcite instead of the microscopic laminae of the annual band. The fluorescence spectra measured in the cave waters were very similar to those in the speleothems, especially the fluorescence of the organic acids extracted from the solid calcite. The cave waters and these fulvic and humic acids all shared excitation maxima wavelengths of 255 nm and 340 nm and emission maxima wavelengths of between 410 - 440 nm. The dominant fluorophore in the calcite as well as the cave water were the fulvic acids, although the humic acids also contributed to a lesser extent. Trace elements were discounted as having any significant contribution to the fluorescence of speleothems used in this study. However, the most abundant organic substance by far was the particulate organic carbon (>0.7 μm). Its levels are so high (average of 204 ppm) that it is more likely to be a suppressor of fluorescence than an initiator due to self absorbance. In comparison, the fulvic and humic acids have respective average concentrations of 0.893 ppm and 0.064 ppm. These concentrations were thought to be the reason for the difference in colour between the light and dark calcite, but there is no significant difference between POC, FA and HA concentrations; nor did there appear to be any difference in the size fractions. Only the absorbance and fluorescence results showed differences, with solid dark calcite having greater absorbance and lower fluorescence than the light calcite. When dissolved and POC was removed, the dark calcite fluoresced more strongly than the light calcite.

Although FA is the dominant fluorophore and the speleothems should be excited at it's peak excitation wavelength to promote maximum emission from the speleothems, the reality of the situation is not so simple. The major problem lies in the hardware available to capture the fluorescence of the speleothem. If the photographic flash gun and high sensitivity light film is used, then the flash gun produces a broad spectrum of exciting light and will cover the peak excitation wavelength of FA. But if a laser is used for excitation, and is required to be focussed through the objective, the lens being glass will filter the UV laser and prevent any excitation light from reaching the sample. Consequently, a longer wavelength Argon laser has to be used, and once this is adopted, the optimal excitation

wavelength can no longer be used. However the laser can still be used at longer wavelengths which will excite the larger organics present but also the excite the smaller organics, although not at their optimal wavelength.

The findings from the above research were then taken and used in two locations with highly seasonal environments. The respective fluorescence signals of the Minneapolis and Aggtelek samples appear to contain annual laminae. The Minneapolis samples have 56 years of growth, compared to the 164 years for Aggtelek. These years fit well with the historically known limits for possible growth at these sites. The presence of these laminae are important because it allows the dating of the larger deviations in fluorescence by simply counting the number of bands. If the fluorescence is actually related to climate variables, then it can be used as their surrogate when climate data do not exist. The widths of the laminae may also be measured as delineated by the annual band and correlated with climate data for the modern speleothems.

Annual laminae peak fluorescence and their widths were correlated to climate data. The Minnepolis sample, Minne1, showed a positive relation between band width and precipitation. As precipitation increases, more water is available to wash the soil CO_2 into the phreatic zone of the karst system and enhance limestone dissolution. Increased levels of Ca ions in the drip water will therefore produce thicker laminae. AGG2's fluorescence correlated positively with precipitation. Increased annual precipitation may result in more flushing of the soil of organic substances into the cave increasing fluorescence. None of the relationships were particularly strong, probably because the climate data is too crude. However, it is hoped future work with more sophisticated climate variables may lead to stronger correlations.

6.2 Future research

To strengthen the work undertaken in this thesis, more research is needed:

1) Compare fluorescence and laminae widths for the modern speleothems with more sophisticated climate variables.

2) Compare the flash gun and laser induced fluorescence for the same speleothems

3) Older speleothems could be investigated to try to determine what factors are more conducive to the production of annual bands and what factors remove their presence. Other proxy climate records such as tree rings, palynological records and lacustrine sediments could be used to understand what is happening in the environment during the growth of the speleothems.

4) Compare band widths with tree ring widths for the same locations to see if any correlations exist. Tree rings are used as surrogates for both temperature and precipitation and comparison with laminae widths may shed some light on how speleothems are affected by these two climate variables.

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