Fluorescence of Dissolved Organic Matter as a Natural Tracer of Ground Water

by Andy Baker[1] and John Lamont-Black[2]

Abstract

The fluorescence properties of dissolved organic matter (DOM) in ground water in the Permian limestone of northeast England is determined from six monitoring boreholes, a private water supply well and from a natural resurgence in a flooded collapse doline in the environs of Darlington, County Durham, northeast England. Measurements of both protein and "fulvic-like" fluorescence was undertaken from January to December 1999. The wavelengths of fulvic-like fluorescence excitation and emission and of protein fluorescence emission were all determined to be sensitive fingerprints of organic matter fluxes through the ground water, with water within the till and within both gypsum and limestone strata deep inside the Magnesian Limestone being differentiated by these parameters. Previous research has suggested that proteins in waters are "young" in age, hence our seasonal variations suggest that we are sampling recently formed DOM. The rapid response of all deep borehole samples suggests relatively rapid ground water flow, probably through karstic cave systems developed in the gypsum and solution widened features in the dolomitic limestone. Our results suggest that use of both protein and fulvic-like fluorescence wavelength variations provides a DOM signature that can be used as a natural tracer.

Introduction

Dissolved organic matter (DOM) is a ubiquitous constituent of natural water, derived from the decay of plant and animal matter from within the catchment. In most river systems, most DOM is thought to be soil derived, with typical concentrations in the British Isles ranging from 1 to 15 mg/L (Tipping et al. 1997). Of the biodegradable fraction, research has suggested that this predominantly comprises humic substances, with smaller amounts of carbohydrates and amino acids; with most carbohydrates and amino acids being humic bound (Volk et al. 1997). Between 40% and 60% of DOM is fluorescent (Senesi 1993); this fluorescent material principally comprises protein and organic acids. The presence of fluorescent DOM in river and ground water systems has long been recognized. It may generate problems when artificial fluorescent dyes are used to trace water within, for example, cave systems (Smart et al. 1976). Figure 1 presents fluorescence excitation-emission matrices (EEMs) of typical ground water and riverine organic matter. DOM fluoresces with the same wavelengths as photine (340 to 350 nm excitation, 430 to 450 nm emission; Smart and Laidlaw 1977), one commonly used artificial tracer, and thus if present in significant quantities would interfere with any tracing experiments. Conversely, given its fluorescence properties, DOM could be used as a natural tracer in place of artificial dyes if its characteristics change with either space or time.

Recent advances permit the rapid generation of fluorescence EEMs that permit both fluorescence intensity and wavelength to be measured. Fluorescent DOM exhibits discreet intensity peaks at known wavelengths: Figure 1 has labeled the fluorescence centers ascribed to organic acids ("fulvic-like" [A] and "humic-like" [B]) and protein [C]; Coble 1996; Baker and Genty 1999; Baker 2001). Variations in the relative wavelengths of these fluorescence centers both temporally and spatially might provide information on DOM source within a catchment and, hence, provide natural tracers. Fluorescence intensities will predominantly depend on DOM concentration, providing other factors that affect fluorescence intensity (pH, metal-ion interactions) remain relatively constant. For example, increased fulvic-like fluorescence intensity has been observed with increasing pH, with an intensity increase of ~50% with a pH

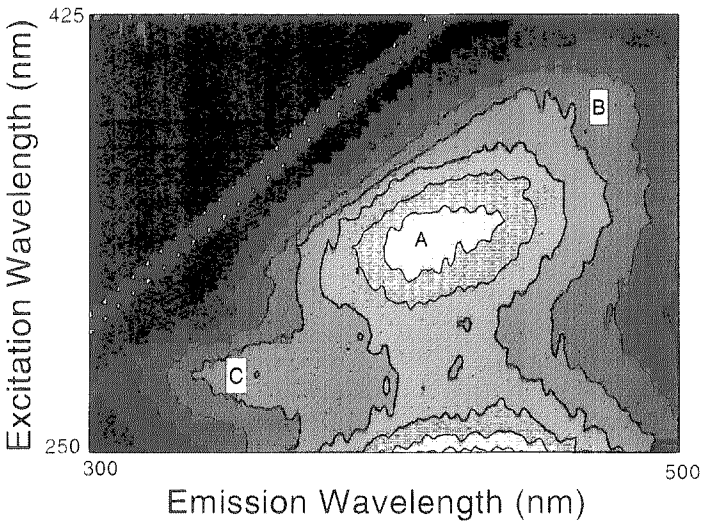

Figure 1. Fluorescence excitation-emission matrix (EEM) of a typical water sample, with fluorescence centers ascribed to "fulvic" and "humic" fluorescence (A and B) and protein fluorescence (C). Peak B is typically quenched in carbonate ground water; the diagonal lines are caused by Rayleigh-Tyndall and Raman properties of water.

[1]Department of Geography, University of Newcastle-upon-Tyne, Newcastle, NE1 7RU, United Kingdom; 0191 222 5344; fax: 0191 222 5421; andy.baker@ncl.ac.uk

[2]Department of Civil Engineering, University of Newcastle-upon-Tyne, Newcastle, NE1 7RU, United Kingdom; 0191 222 6420; fax: 0191 222 6613; john.lamont-black@ncl.ac.uk

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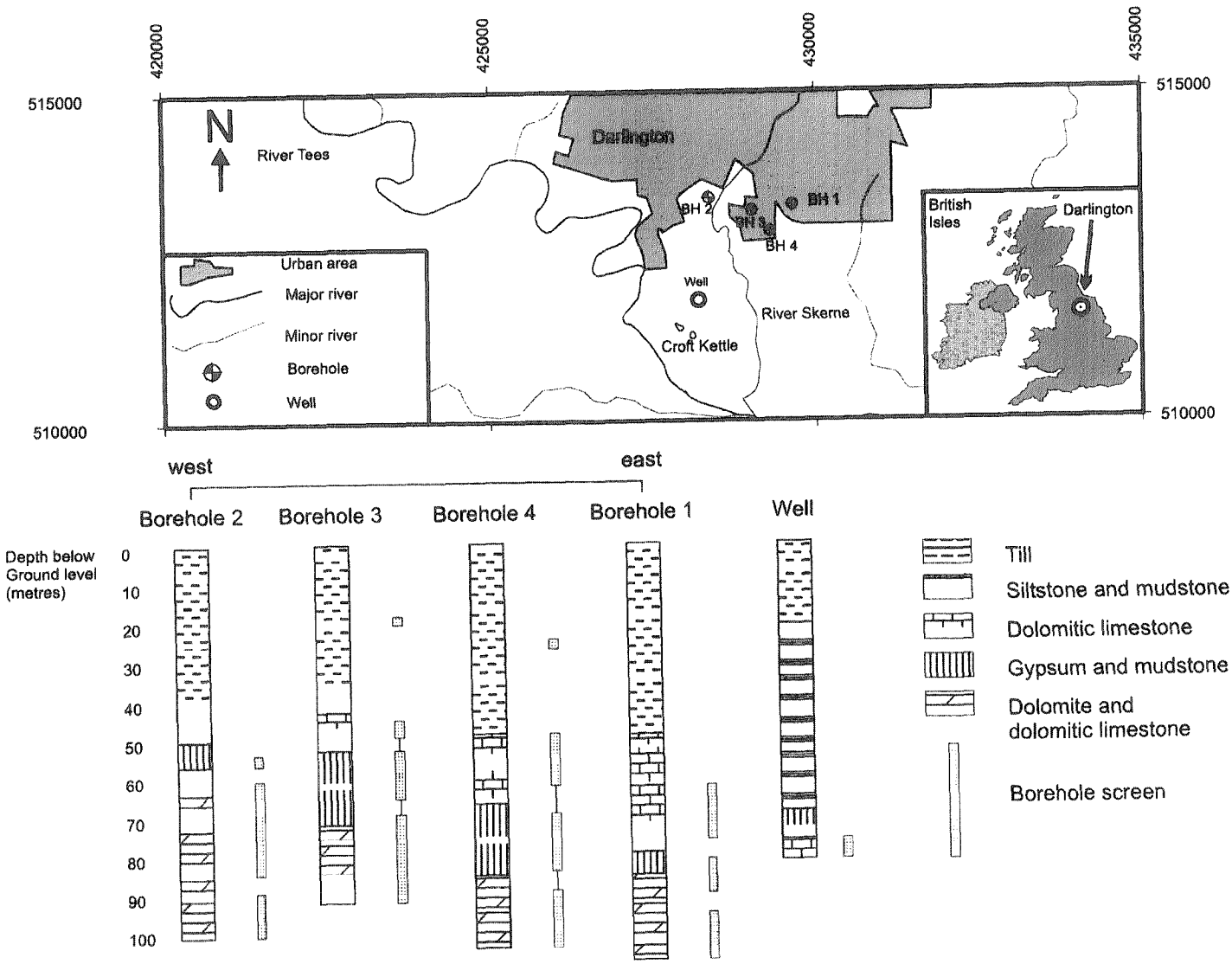

Figure 2. Location of the four boreholes and the well at Croft Kettle in Darlington, northeast England. Grid references are United Kingdom national grid; the map area is 20 × 10 km. Borehole screens are at: BH1, 49–55 m, 63–77 m, 82–91 m, 96–108 m; BH2, 45–50 m, 54–57 m, 60–85 m, 90–101.5 m; BH3 18.7–21 m, 46.1–50 m, 53.2–66.1 m, 70–93 m; BH4, 25–27 m, 49–62.9 m, 70–85 m, 90–105 m. For BH3 and BH4, borehole screens in the solid geology sections are connected via a continuous piezometer tube that is slotted in the screen sections.

change from 4.0 to 9.0 (Miano et al. 1988). Metal-ion interactions with humic and fulvic acids have been demonstrated to quench fulvic-like fluorescence by ~20% to 50% with metal-ion concentrations of just 1 mmol/L (da Silva et al. 1998). However, in carbonate aquifers, dissolved metal-ion concentrations (Ca, Mg) are much greater than the DOM. Therefore, it can be assumed that all metal binding sites on the organic molecules are occupied and that changes in metal-ion concentration will not induce variations in what is a fully quenched fluorescence intensity. Similarly, owing to its buffering, and near neutrality, pH induced fluorescence variations are relatively invariant. Laboratory studies suggest that fulvic-like fluorescence intensity will vary by < 10% in the pH range 7.0 to 8.4 (Miano et al. 1988).

Previous research has investigated fluorescence variations in one specialized ground water case, that of cave waters (Baker and Genty 1999; Baker et al. 1999). These investigations focused only on the organic acid fluorescence center (peak B on Figure 1). Results demonstrated fluorescence intensity peaks of an order of magnitude higher intensity than that observed at other times of the hydrological year during times of hydrologically effective precipitation, when soil derived DOM, was flushed into the aquifer. In addition, the first flush of the hydrological year (in autumn) comprised fluorescent DOM that had a lower emission wavelength than the annual mean, and a second (late winter) organic matter flush comprised fluorescent DOM that had a lower excitation wavelength than the annual mean (Baker and Genty 1999; their Figure 6). These fluorescence wavelength differences were interpreted as derived from soil DOM of different ages and therefore different extent of decomposition. However, as with fluorescence intensity, pH and metal-ion interactions may also affect fluorescence wavelength variations. For example, Mobed et al. (1996) observed an ~10 nm red shift in fluorescence wavelength of the humic-like and blue shift of the fulvic-like centers for a shift from pH 6.0 to 10.0. Erich and Trusty (1997) observed up to 20 nm decrease in excitation and emission wavelengths of the fulvic-like fluorescence (peak A) in soil water after liming, which increased soil pH from 4.8 to 6.3 to 7.2 to 7.8 and dissolved calcium from 0.03 to 2.2 to 0.2 to 15.3 mmol/kg organic matter. Again, in carbonate aquifers where dissolved metal-ion concentrations are much greater than the DOM, and where pH is buffered and typically near neutral and relatively invariant, fluorescence wavelength variations may be indicative of DOM of different structure and composition.

Table 1

Summary Fluorescence Properties (Mean and 1 Standard Deviation) for BH1-4 and Kettles Sample Sites for the Sampling Period January to December 1999

Borehole I.D. Depth (m) and Strata		Number of Samples	Fulvic-Like Fluorescence Center λex (nm)	λem (nm)	I_{fa} (units)	Protein Fluorescence Center λex (nm)	λem (nm)	I_p (units)
BH1 63.0-77.0	OL	17	320.6 ± 12.1	409.0 ± 4.0	32.1 ± 17.7	277.5 ± 3.2	348.3 ± 10.0	26.4 ± 15.1
BH1 82.0-91.0	G	17	316.2 ± 12.4	405.5 ± 6.3	21.0 ± 5.3	277.5 ± 6.1	346.9 ± 10.1	25.1 ±1 5.0
BH1 96.0-108.0	UL	17	322.5 ± 11.8	406.8 ± 5.9	23.3 ± 4.1	278.4 ± 4.4	347.3 ± 9.5	27.8 ± 21.3
BH2 45.0-50.0	G	17	322.2 ± 13.9	408.09 ± 5.6	84.9 ± 32.4	277.0 ± 4.1	346.6 ± 8.7	63.4 ± 40.0
BH2 60.0-85.0	G	18	313.0 ± 16.1	405.3 ± 4.3	27.7 ± 8.5	277.2 ± 4.9	345.8 ± 11.8	30.6 ± 39.1
BH2 90.0-101.5	UL	18	316.5 ± 11.0	407.8 ± 5.4	36.5 ± 8.6	277.94 ± 6.4	349.1 ± 9.8	28.4 ± 22.0
BH3 18.7-21.0	T	13	323.8 ± 10.7	408.7 ± 3.1	105.5 ± 12.7	277.0 ± 4.2	344.67 ± 8.9	49.5 ± 11.5
BH3 46.1-93.0	OL, G, UL	10	318.5 ± 14.9	405.9 ± 6.1	27.8 ± 6.6	274.5 ± 5.0	337.5 ± 11.6	33.1 ± 30.5
BH4 25.0-27.0	T	10	3245.0 ± 10.0	407.2 ± 2.6	115.7 ± 20.0	278.1 ± 4.6	338.5 ± 5.1	44.4 ± 9.6
BH4 49.0-105.0	OL, G, UL	9	317.8 ± 14.6	406.3 ± 3.5	36.5 ± 14.8	276.7 ± 6.1	350.1 ± 8.6	39.1 ± 42.7
Croft Kettle	OL	15	313.0 ± 12.7	406.8 ± 4.5	16.2 ± 8.5	275.9 ± 7.8	341.3 ± 7.5	25.9 ± 13.7
Well 78.0-82.0	OL	14	313.8 ± 18.5	407.8 ± 4.5	10.6 ± 3.7	276.9 ± 7.2	350.5 ± 12.7	12.8 ± 8.9

For precise sampling depths see Figure 2. T, OL, G, and GL refer to till, overlying dolomitic limestone, gypsum, and underlying dolomites and dolomitic limestone, respectively.

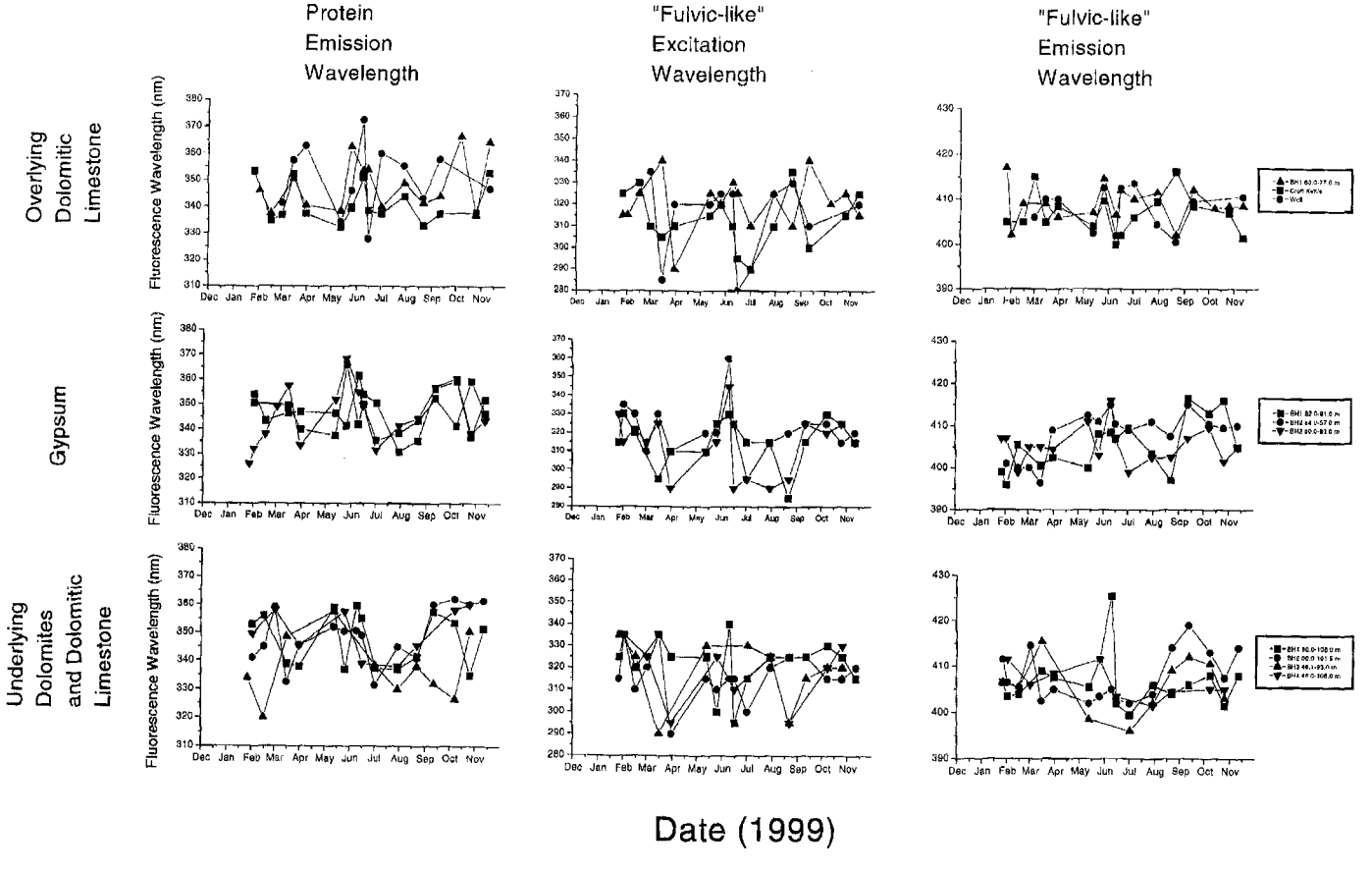

Figure 3. Protein fluorescence emission wavelength, and fulvic-like excitation and emission wavelength variations the overlying dolomitic limestone, gypsum, and underlying dolomites and dolomitic limestone for the period January to December 1999.

Aside from the work of Baker and Genty (1999), to the authors' knowledge there has been little detailed timeseries investigations of DOM fluorescence wavelength variations or on the investigation of fluorescence centers. Mopper and Schultz (1993) identified both protein-type and humic-type fluorescence in marine waters, and suggested that they could be used to study the nature and distribution of "recent biochemical" vs "old humic" substances, respectively. Mayer et al. (1999) investigated dissolved protein fluores-

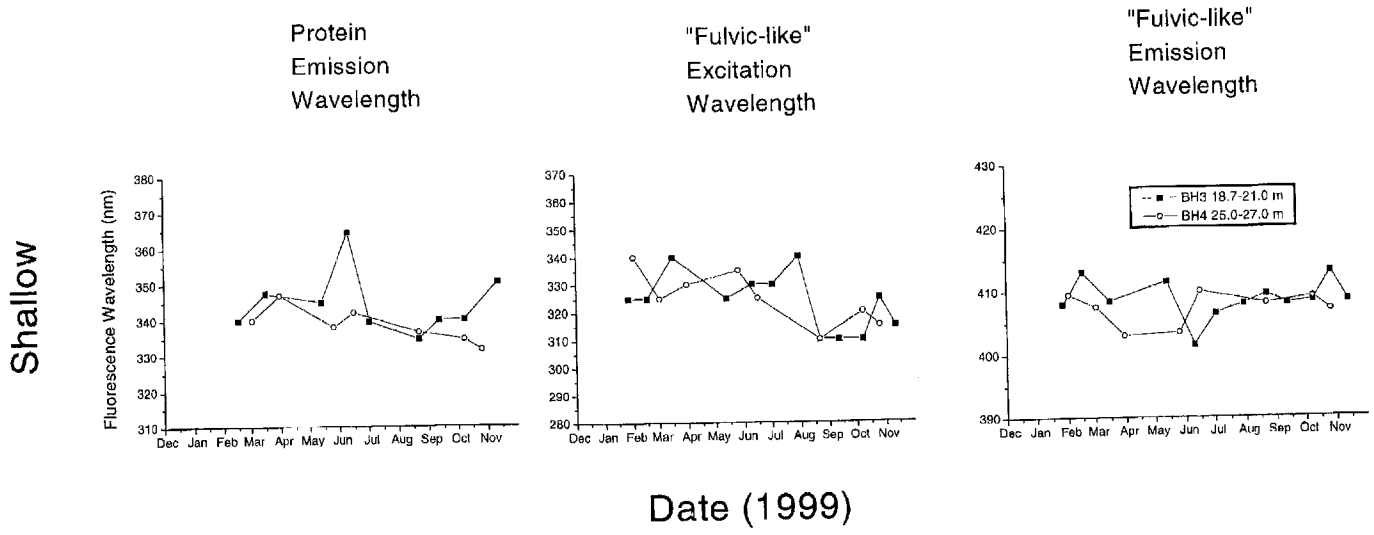

Figure 4. Protein fluorescence emission wavelength, and fulvic-like excitation and emission wavelength variations for shallow samples for the period January to December 1999.

cence in two river estuaries and found that protein fluorescence reflected the short lifetimes of proteins in sea water. Here we present EEM results from ground water sampled over the period 1999–2000 from Darlington, northeast England, to investigate use of DOM fluorescence wavelength variations to trace water through the Permian strata in the region.

Site Description and Methodology

The geology of the area comprises Permian dolomites and dolomitic limestones (informally known as the "Magnesian Limestone") with interbedded gypsum, siltstones, and mudstones overlain by 40 to 50 m of glacial till with glacio-lacustrine clays toward the top (Cooper 1998). Cavities filled with silty and sandy deposits were recorded in boreholes drilled through the gypsum strata and probably represent caves and cave sediments. High concentrations of dissolved calcium and sulphate measured in water from boreholes and springs indicate that gypsum dissolution is an active process in the area.

Water samples were retrieved from four monitoring boreholes, a private water supply well, and a natural resurgence in a flooded collapse doline (Croft Kettle) in the environs of Darlington, County Durham, northeast England (Figure 2). The monitoring boreholes comprised nested piezometer tubes with the tips at depths ranging from 21.0 to 101.0 m; details of screen lengths are shown in Figure 2. Results are presented in the form analyses relating to separate stratigraphic levels comprising (in descending order): glacial till; overlying dolomitic limestone; gypsum, siltstone, and mudstone; underlying dolomite and dolomitic limestone.

Water samples were collected every two to three weeks from January through December 1999. Samples were collected in plastic bottles before being transferred to 30 mL dark-glass bottles and refrigerated immediately upon return from the field. The fluorescence spectra of the water samples were determined using a Perkin-Elmer LS-50B luminescence spectrometer. The spectrometer used a xenon excitation source, and slits were set to 5 nm for both excitation and emission. To obtain fluorescence EEMs with appropriate optical resolution, excitation wavelengths were incremented from 250 to 425 nm at 5 nm steps, the step size chosen as a compromise between data resolution and data collection time. For each excitation wavelength, the emission was measured from 300 to 500 nm at 0.5 nm steps. For each water sample, the fluorescence intensity was measured at the maximum on the excitation-emission matrix. Analyses were performed at a constant laboratory temperature of 22 ± 2°C, and blank water scans were run every five to 15 analyses using a sealed distilled water cell. The Raman peak of water at 348 nm was used as a test for machine stability and to permit inter-laboratory comparison. Raman emission at 395 nm averaged 20.8 ± 2.2 intensity units (n = 306), with no drift through the analytical period. Duplicate samples demonstrated that the wavelength of peak organic acid fluorescence was reproduced within ± 3 nm and protein fluorescence ± 5 nm; fluorescence intensities replicated within ± 5% and ± 15%, respectively. In addition, the stability of the Raman peak was assessed for a 10-minute period at the start of each day and data collection occurred only when the signal-noise ratio of the spectrometer was greater than 500:1.

Results

Fluorescence data for all sample sites in the form of mean and standard deviation over the whole sampling period are presented in Table 1. No humic-like fluorescence (peak C in Figure 1) is observed in our samples, and therefore these data are not tabulated. From the table it can be seen that both protein and the fulvic-like organic acid fluorescence are observed in all samples (peaks A and B of Figure 1). The lack of the humic-like peak is identical to that observed in carbonate ground water elsewhere (Baker and Genty 1999 and unpublished data). For protein excitation and emission wavelengths and fulvic-like excitation and emission wavelengths, there is no statistical difference between any of the samples (t-test, 95%) for the period January through December 1999, although this hides significant intra-annual variations in fluorescence excitation and emission wavelengths of both the fulvic-like and protein fluorescence. Within the January through December 1999 sampling period, protein emission wavelengths vary by up to 30 nm (1 s.d. variability at individual depths is from 5 to 13 nm); fulvic-like excitation wavelengths range up to 55 nm (1 s.d. variability from 10 to 19 nm) and fulvic-like emission wavelengths range the least at up to 20 nm (1 s.d. variability from 3 to 6 nm). The seasonal range of

the fulvic-like excitation wavelength of up to 55 nm is significantly greater than that reported as typical of pH and metal-ion interactions (~10 nm; da Silva et al. 1998; Mobed et al. 1996), suggesting changes in the DOM itself are dominating this variability.

Figures 3 and 4 present a time-series of the protein fluorescence emission wavelength variations, and fulvic-like fluorescence excitation and emission wavelength variations. Protein excitation wavelength time-series are not shown as they were observed to be invariant through the sampling period. Figure 4 demonstrates that for two of the hydrostratigraphic levels—those within the gypsum and deep within the underlying limestone—show similar time- series records. For these depths, fluorescence wavelength properties are variable through time, with similar irregularity in terms of fluorescence wavelength throughout the data, although with no particular pattern. In contrast, samples from the overlying dolomitic limestone, which include the private well and Croft Kettle samples, show seasonal variations in fluorescence wavelength similar in magnitude and to those in the gypsum and underlying dolomite and dolomitic limestone but with different timing (Figure 4). Finally, Figure 3 demonstrates that the shallow samples show long-term trends in fluorescence wavelength without the seasonal variability observed at all other hydrostratigraphic levels as shown in Figure 4.

Interpretation

Figures 3 and 4 demonstrate that water sampled from shallow depths within the till, within the overlying dolomitic limestone, the gypsum, the underlying dolomite and dolomitic limestone and at the kettles sites are differentiable by their fluorescence wavelength time-series, and therefore has potential as a natural tracer. We do not consider fluorescence intensity variations, as we know that from borehole data and have interpreted from piezometric data that the gypsum, dolomitic limestones, and dolomites contain solution cavities and widened fissures and therefore fluorescence intensities will be affected by both transport and dilution effects, depending on the size and nature of these karst features. Indeed, fluorescence intensity data is much noisier than that of fluorescence wavelength (Table 1). Therefore, in this case study we consider that fluorescence intensity variations will be of relatively limited use as a tracing tool.

Shallow water samples are identified by relatively constant fluorescence wavelengths, which show no intra-annual trends similar to the deeper sites (Figure 3). The difference in time-series fluorescence characteristics between the shallow borehole samples in the till and samples from deeper levels suggests that lateral flows may dominate over vertical flows, particularly between the till and the solid rocks. Measurements of piezometric levels seem to confirm this hypothesis. Ground water levels in the till are quite stable with intra-annual variations of about 100 to 200 mm. The same data from the solid rocks shows 800 to 1000 mm variation in levels, thus indicating poor vertical interconnection between the till and the solid geology.

The gypsum-bearing formation and the underlying dolomitic limestones demonstrate more variable fluorescence wavelength properties. Variability through time is observed with similar irregularity in terms of fluorescence wavelength throughout the data, although there is no particular pattern. Given that the sites are spatially separated by several kilometers, this variability may reflect our sampling of water with different sources within the same sampling run. Similarly, the relatively noisy signal could be generated by higher frequency variations in water fluorescence properties than our sampling frequency of two to three weeks. Previous research has suggested that proteins in water are "young" in age (Mayer et al. 1999), given the relatively rapid breakdown of protein in the natural environment. However, the multiple peaks observed at different times of the year within 1999 are not just in late summer/autumn, when the flushing from the soil zone of recently formed DOM might be expected. This suggests that an anthropogenic (for example, sewage or spreading of farm wastes) rather than a natural source may be generating at least some of the observed trace. The sharp nature of the increase in fluorescence wavelength that occurs at several sites in June might be indicative of such a point-source pollutant, compared to the broader fluorescence peaks observed at other times of the year that possibly indicate a more diffuse source. The rapid response of all deep borehole samples suggests relatively rapid ground water flow, probably through karstic systems developed in the gypsum (which lies at the base of the gypsum-bearing formation) and solution widened fissures in the dolomitic limestone.

Results from the overlying limestone, which includes the private well and Croft Kettle sites, also show some intra-annual variability, but has a different timing to that in the underlying strata (Figure 4). This unit is separated from the underlying dolomite and dolomitic limestone and gypsum by a band of mudstone, which varies in thickness from 0 to 7 m. This mudstone forms the upper portion of the gypsum-bearing formation and is thinnest in Borehole 1. Therefore, a likely interpretation is that the upper part of the gypsum-bearing formation is behaving as an aquitard, which might explain the observed differences in fluorescence signatures across the gypsum-bearing formation. The difference between the two dolomitic limestone formations is most clearly expressed by data from the Croft Kettle and Well sites. This difference appears less distinct in Borehole 1, which may be owing to the thinner mudstone at this location.

Conclusion

Our results suggest that that use of both protein and fulvic-like fluorescence wavelength variations provides a DOM fingerprint that can be used as a natural tracer. Seasonal variations in fluorescence excitation and emission wavelengths of protein and fulvic-like fluorescence centers are observed and are greater than those ascribed to change in pH and metal-ion interactions. In this study, we can discriminate temporal variations in DOM properties, but there is no reason that the fingerprint would not work in large catchments with spatial variations in DOM. Further studies might include the simultaneous measurement of both fluorescence and absorption properties of DOM to further improve their optical characterization, the categorization of the fluorescence of anthropogenic fluorescent DOM (Galapate et al. 1999; Baker 2001), as well as the use of autosamplers to provide continuous monitoring. It is anticipated that the use of DOM fingerprinting as previously described will be of additional benefit in determining flow patterns in karst aquifers. Work is presently under way combining DOM fluoresence with data on water quality data, water level, and structural geology. The results of this work will be the subject of a future publication.

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