

# The freshwater dissolved organic matter fluorescence–total organic carbon relationship

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## Abstract:

The fluorescent properties of dissolved organic matter (DOM) enable comparisons of humic-like (H-L) and fulvic-like (F-L) fluorescence intensities with dissolved organic carbon (DOC) in aquatic systems. The fluorescence-DOC relationship differed in gradient, i.e. the fluorescence per gram of carbon, and in the strength of the correlation coefficient. We compare the fluorescence intensity of the F-L and H-L fractions and DOC of freshwater DOM in north Shropshire, England, featuring a river, wetland, spring, pond and sewage DOM sources. Correlations between fluorescence and DOC varied between sample sites. Wetland water samples for the F-L peak gave the best correlation,  $r = 0.756$ ; the lowest correlation was from final treated sewage effluent,  $r = 0.167$ . The relationship between fluorescence and DOC of commercially available International Humic Substances Society standards were also examined and they generally showed a lower fluorescence per gram of carbon for the F-L peak than the natural samples, whereas peat wetland DOM gave a greater fluorescence per gram of carbon than river DOM. Here, we propose the strength of the fluorescence–DOC correlation to be a useful tool when discriminating sources of DOM in fresh water. Copyright © 2007 John Wiley & Sons, Ltd.

KEY WORDS dissolved organic carbon; dissolved organic matter; fluorescence; wetlands; rivers

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## INTRODUCTION

Humic substances occur in every natural water sample that has ever been analysed for them (Malcolm, 1985). Dissolved humic substances can be defined as passing through a 0.45 µm membrane, and are made up of humic and fulvic substances, the latter being present in larger proportion and characterized as being soluble under all pH conditions (Aiken, 1985). Freshwater dissolved humic substances are derived from dead and decaying detritus, aquatic plants, animals and debris from overhanging vegetation, and tend to be of a younger state than that of soil humic substances (Hongve, 1999). River aquatic fulvic substances are derived from plant and tree residues, which contain more phenolic and lignin-derived organic compounds than those found in soil (Chen *et al.*, 2003). Historically, much work has gone into the determination and separation of these humic and fulvic substances, and yet still relatively little is understood about the molecular distribution and fluorescent characteristics of these molecules (Alberts and Takacs, 2004). Analysis of freeze-dried fulvic acid using <sup>13</sup>C nuclear magnetic resonance spectroscopy demonstrates that it contains a number of groups, such as aliphatic carbon atoms, carbohydrates, olefinic carbon atoms, aromatic carbons atom, carboxyl ester amide carbon atoms and ketonic and aldehydeic carbon atoms (Klapper *et al.*, 2002; Sierra *et al.*, 2005). They are responsible for water colour (Hongve, 1999) and

sometimes pH due to the carboxylic acid groups attached to them (Klapper *et al.*, 2002). The aromatic and carboxylic fractions provide the fluorescence of humic and fulvic substances (Senesi *et al.*, 1989); fulvic substances are more highly fluorescent than humic substances and humic fluorescence intensity is more pH dependent (Goslan *et al.*, 2004; Sierra *et al.*, 2005). Owing to the ambiguous characteristics of these substances, we feel that it is more appropriate to refer to them, hereafter, as humic-like (H-L) and fulvic-like (F-L) substances.

In freshwaters, it has been suggested that dissolved organic carbon (DOC) is usually dominated by the H-L and F-L fractions, making up 50–75% of the total (of which colloidal matter comprises up to 20% of the total DOC; Hope *et al.*, 1994). However, the proportion of H-L and F-L substances in the DOC is variable. For example, about 50% of uncoloured (USA) stream water consists mostly of humic substances and 90% of these humic substances found in dissolved organic matter (DOM) are the F-L fraction (Malcolm, 1985). In the Amazon basin, humic substances are attributed to as much as 60% of the aquatic total organic carbon (TOC; Patel-Sorrentino *et al.*, 2002). In stream/aquatic systems, DOC losses are possible due to biotic processes such as biofilm respiration and absorption onto algae and abiotic uptake onto mineral surfaces (Dawson *et al.*, 2001). Losses of CO<sub>2</sub> from volatile DOC fractions by degassing to the atmosphere, as well as variable DOM inputs throughout and between catchments, can also lead to this variability.

The presence of fluorescent DOM enables the comparison of H-L and F-L fluorescence intensity with DOC.

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There are many potential benefits of using fluorescence as a tool for determining and measuring DOC in aquatic systems. It is a relatively quick process, with a typical scan taking approximately 1 min; this is valuable, as labile DOM can degrade over time. The method is non-intrusive and thus does not interfere with the molecular structure (Klambitz *et al.*, 2000; Baker, 2002). However, given the variable fraction of fluorescent DOM as part of the total DOC pool, it is essential to investigate the fluorescence intensity–DOC relationship. Therefore, we compare the fluorescence intensity of the F-L fraction and DOC of freshwater DOM. The study site that this paper focuses on, Norton in Hales, in north Shropshire, England, is a site that features river, wetland, spring, pond and sewage DOM sources all within a small location (approximately 500 m), covering a wide range of variation in aquatic and soil-derived water samples.

#### SITE DESCRIPTION AND METHODOLOGY

Norton in Hales [UK National Grid Reference SJ 706 384], north Shropshire, UK, is situated along the River Tern, a tributary of the River Severn, 16 km from its source. The River Tern is a lowland catchment, primarily groundwater fed from red sandstone overlying the Permo-Triassic Sherwood sandstone. Its catchment boundary, thus, is not defined by its topography (described by Clay *et al.* (2004)). This amounts to a very stable base flow in the River Tern, showing minimal response to normal rainfall events. Local land use is generally under semi-intense mixed cereal/dairy/beef farming.

Figure 1 illustrates the diversity of the sample sites within a small area. In-stream water samples were collected (river sites A, C, E and H), as a transect down the river starting above the wetland (A) and finishing below the sewage treatment outflow pipes (H). Between these points, samples were also taken from the sewage

treatment works (STW) outlet itself (G), a groundwater-fed pond overflow, POND (B), a wetland discharge site (D) (not always connected to the main river) and a groundwater spring diverted through small concrete conduit (F). Additionally, three samples were taken from the wetland: surface water (I), 0–45 cm depth (J), and 50–100 cm depth (K). To obtain samples from the deeper peat, two perforated drain pipes, about 50 cm apart, were sunk (to 50 cm deep and 1 m deep, with the latter being perforated from 50 to 100 cm) and sealed at both ends, to reduce oxidation and contamination from other external sources. The sample was obtained by unscrewing a lid and dipping for the sample, throwing away the first few dips so that the sample was taken from the middle of the perforated column depth. The wells were then completely emptied before resealing. Thus, water in tubes was not allowed to stagnate, but perforation allowed ‘flow’ of the wetland and was representative of (actual) bog water. These samples (D, I, J and K) were grouped together to form BOG.

Water samples were collected fortnightly from Norton in Hales from March to October 2005. Plastic bottles (500 ml), pre acid washed (10% HCl, distilled-water rinsed), were used to ensure carbon- and fluorescence-free blanks (Bolton, 2004). Bottles were also pre-rinsed with sample before filling. On return to the laboratory (same day) the samples were then filtered through pre-rinsed Whatman GF/C filter papers under vacuum. Samples were kept in the refrigerator overnight between 4 and 6 °C they were then analysed for fluorescence and TOC the next day, with DOC analysis completed with 36 h of collection (on two occasions this was a day later due to machine downtime); this was critical, as the fluorescence properties of a sample can alter over time (Baker, 2002).

DOC was measured using a Shimadzu 5050 carbon analyser from filtered and unfiltered (not reported here) samples. DOC was obtained by measuring the total carbon and subtracting the measured inorganic carbon and carbonates (dissolved CO<sub>2</sub> and CO<sub>3</sub><sup>2-</sup>). As other studies

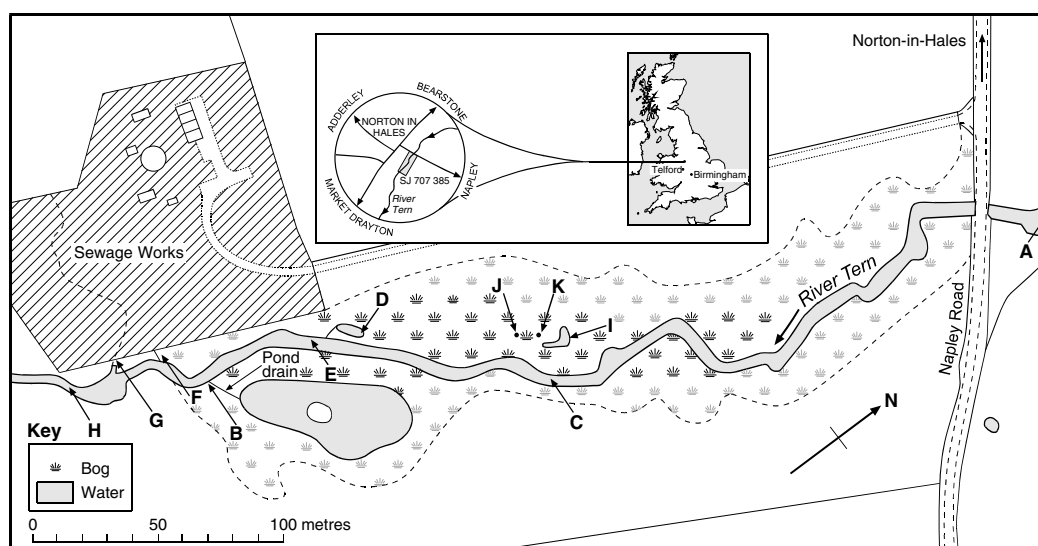


Figure 1. Location of sample sites

have demonstrated, some methodologies suggest purging with acid to remove the inorganic fraction (Clark *et al.*, 2002). This was not executed in this case because the total carbon and inorganic carbon concentrations are significantly different from each other, giving differencing errors of  $<1 \text{ mg l}^{-1}$  for DOC. Calibration was with certified organic and inorganic standards from Reagecon, and samples were analysed in triplicate and the mean taken from the best two (CV  $<2\%$ ). Glass vials were washed in 4% Decon 90<sup>®</sup> and 10% HCl and pre-rinsed with ultra-pure water and sample.

A fluorescence scan for the organic fraction of the sample was obtained from filtered samples within 48 h of sample collection. DOM fluorescence was measured in 4 ml capacity cuvettes using a Varian Cary Eclipse fluorescence spectrophotometer equipped with a multicell holder with Peltier temperature controller to enable the measurement of excitation–emission matrices (EEMs) at precisely controlled ( $\pm 0.1^\circ\text{C}$ ) temperatures at  $20^\circ\text{C}$ . Each EEM was generated by scanning excitation wavelengths from 200 to 400 nm at 5 nm steps and detecting the emitted fluorescence between 280 and 500 nm at 2 nm steps. Scan speed was  $9600 \text{ nm min}^{-1}$ , permitting collection of a complete EEM in  $\sim 60 \text{ s}$ . For all EEMs, two fluorescence peaks were identified that were always detectable. These were (1) fluorescence excited between 300 and 340 nm excitation and emitted between 400 and 460 nm, and (2) fluorescence excited between 220 and 250 nm excitation and emitted between 400 and 460 nm, both fluorophores attributed to F-L and H-L substances (Baker and Inverarity, 2004). For each peak, the excitation and emission wavelengths and the intensity of emitted fluorescence were recorded. To calibrate the fluorescence intensity, we also measured the strength of the Raman signal at excitation 348 nm (emitted between 395 and 400 nm) and all results are standardized to a mean Raman peak of 20 intensity units. Our results can be compared with a quinine sulphate standard: 32.5 intensity units are equivalent to one quinine sulphate unit ( $1 \mu\text{g l}^{-1}$  in  $0.1 \text{ M H}_2\text{SO}_4$ ). Samples were not diluted, with the exception of one intensely fluorescent BOG sample, for which a  $\times 2$  dilution, to bring to scale, was used.

To be able to put our sample sites in a wider context, baseline geochemical and nutrient data have also been collected (Table I). The pH was measured in the field using a Jenway hand-held meter. Determination of nutrients (nitrate, ammonium and phosphate) was undertaken at the University of Reading using a Chem-Lab continuous flow autoanalyser. Samples were then microwave digested (CEM Corporation) using the persulphate method as described by Johnes and Heathwaite, (1992) and then reanalysed to determine the organic fraction.

## RESULTS

Table I presents summary fluorescence and DOC results, together with secondary geochemical and nutrient data.

Data from sites A, C, E, and H are combined to form 'RIVER'; sites D, I, J, and K are combined to form 'BOG'. The treated final effluent (STW), pond site B (POND) and groundwater site F (SPRG) were analysed without grouping.

For all groups, the excitation and emission wavelengths and intensities of H-L and F-L fluorescence, as well as DOC concentrations, fall within the range reported from other rivers within the UK (Baker and Inverarity, 2004; Baker and Spencer, 2004; Worrall *et al.*, 2004), suggesting that DOM in the River Tern is typical of rivers in the UK. Intensities for the river F-L peak ranged between 113 and 424 a.u., with a mean of 172 a.u. and a standard deviation of 69 a.u. Intensities for the BOG F-L peak ranged between 77 and 951 a.u., with a mean of 409 a.u. and a standard deviation of 208 a.u.

EEM fluorescence intensity values of the F-L peaks (fluorophores) for all samples were compared with milligrams per litre DOC. The fluorescence per milligram per litre of DOC is presented in Table I, and the correlation coefficients between F-L fluorescence intensity and DOC in Figure 2, together with the fluorescence–DOC relationship of commercially available International Humic Substances Society (IHSS) standards: Nordic fulvic acid (referred to hereafter as Nordic FA), Suwanee River fulvic acid (SRFA), Suwanee River humic acid (SRHA) and Suwanee River natural organic matter (SRNOM). In general, the Norton in Hales samples showed a higher fluorescence per milligram of carbon for the fulvic peak than the IHSS standards, of which the Nordic FA showed the most fluorescence per gram of carbon of the IHSS standards, then SRFA, then SRNOM, then SRHA displaying the least intensity per unit carbon.

Correlations (product for normally distributed data; rank for non-normally distributed data) between fluorescence and DOC varied between sample sites. BOG gave the best correlation ( $r = 0.756$ ), then POND ( $r = 0.657$ ), SPRG ( $r = 0.631$ ) and RIVER ( $r = 0.409$ ), with the STW giving the lowest correlation ( $r = 0.14$ ). That BOG showed less scatter than SPRG, POND and RIVER could be explained by its early stage of decomposition and relative similarity in source and composition of the fluorescent molecules. The weak correlation observed in the sewage outlet could be attributed to other organic fluorescence chemicals, such as fluorescent whitening agents that are present in municipal sewage waste, and the low gradient due to the relative insignificance of the F-L fraction as a proportion of DOC compared with other fluorophores.

Results for the H-L fluorophores for all samples were also compared against DOC (Table I and Figure 3). H-L fluorophores generally had similar correlations with DOC than the F-L fluorescence. This is despite the fluorescence intensity for the H-L fluorophores being more dependent on pH (Patel-Sorrentino *et al.*, 2002; Sierra *et al.*, 2005), with fluorescence intensity increasing with increasing pH (Mobed *et al.*, 1996). The H-L peaks for the BOG samples were harder to locate, as they appear close or within the Rayleigh–Tyndall scatter line

Table I. Fluorescence, organic and inorganic carbon, nutrient and pH data for the five sample groups

	F-L		H-L		DOC (mg l <sup>-1</sup> )			Int./DOC	Organic (mg l <sup>-1</sup> )					C/N	pH		
	Ex. (nm)	Em. (nm)	Ex. (nm)	Em. (nm)	TC	IC	DOC		TON <sup>a</sup>	NO <sub>2</sub> -N	NH <sub>4</sub> -N	PO <sub>4</sub> -P	N			P	
<i>All data</i>																	
Min.	300	406	26	113	219	408	74	12.96	0.30	0.29	0.00	0.00	0.00	0.49	-0.01	6.31	
Max.	344	450	951	424	255	461	1558	122.30	86.73	41.62	28.92	0.32	5.65	4.89	0.71	8.15	
Mean	331	425	220	172	237	428	366	48.47	37.53	10.95	8.05	0.05	0.51	2.10	0.09	7.30	
SD	8	8	164	69	7	7	225	17.57	13.46	8.64	7.47	0.07	1.09	1.29	0.17	0.46	
No.	292	292	292	64	291	291	291	148	148	148	29	29	29	29	29	48	
<i>River</i>																	
Min.	315	417	113	424	235	414	217	23.83	18.01	2.74	7.60	0.02	0.00	0.13	0.79	-0.01	6.82
Max.	340	440	424	172	254	444	600	59.01	42.92	39.87	8.83	0.07	1.58	0.33	0.25	7.81	
Mean	330	427	172	122	239	428	309	42.94	34.04	8.89	7.64	0.04	0.41	0.18	0.07	7.54	
SD	7	5	69	5	5	6	88	6.23	5.51	7.54	2.33	0.02	0.65	0.09	0.10	0.27	
No.	64	64	64	64	64	64	64	64	64	64	12	12	12	12	12	15	
<i>BOG</i>																	
Min.	321	416	77	409	219	416	155	12.96	0.30	1.37	0.00	0.00	0.00	0.00	0.64	0.01	6.31
Max.	344	450	951	424	250	461	1558	122.3	86.73	41.62	5.59	0.06	5.65	0.29	2.61	0.09	7.77
Mean	334	427	409	172	231	429	645	63.16	46.56	16.60	1.17	0.02	0.87	0.08	1.28	0.03	7.00
SD	5	8	208	208	5	9	286	24.45	19.89	9.90	2.75	0.03	1.88	0.11	1.24	0.08	0.46
No.	49	49	49	49	48	48	49	49	49	49	9	9	9	9	9	20	
<i>STW</i>																	
Min.	320	406	177	424	240	420	284	22.42	15.91	4.79	14.87	0.18	0.17	2.21	3.72	0.25	7.01
Max.	340	426	311	409	250	436	407	46.97	35.33	17.95	28.92	0.32	0.74	4.67	4.89	0.71	7.50
Mean	333	419	249	172	245	426	340	38.99	26.44	12.55	17.30	0.19	0.34	2.88	3.09	0.37	7.30
SD	5	4	42	42	3	4	37	7.09	5.64	3.40	12.99	0.14	0.32	2.23	2.13	0.31	0.23
No.	16	16	16	16	16	16	16	16	16	16	3	3	3	3	3	5	
<i>B</i>																	
Min.	300	416	68	424	230	416	159	29.16	25.00	3.84	2.05	0.00	0.00	0.00	0.64	0.01	7.61
Max.	330	436	112	409	245	434	255	51.08	40.68	14.65	2.06	0.08	0.04	0.01	0.89	0.00	8.15
Mean	318	426	89	172	234	424	200	41.76	34.21	7.55	2.06	0.04	0.02	0.01	0.77	0.02	7.90
SD	10	5	14	14	4	5	34	5.44	4.14	3.18	1.19	0.04	0.02	0.00	0.45	0.01	0.20
No.	14	14	14	14	14	14	14	14	14	14	2	2	2	2	2	5	
<i>F</i>																	
Min.	315	412	40	424	235	412	77	35.30	29.75	0.29	12.79	0.02	0.02	0.14	2.06	-0.01	6.82
Max.	340	428	160	409	251	438	260	59.11	46.90	16.96	18.93	0.04	0.07	0.16	3.03	0.06	7.73
Mean	331	420	65	172	244	424	112	41.47	36.62	4.85	16.79	0.03	0.04	0.15	2.60	0.02	0.29
SD	6	5	28	28	5	8	47	6.20	5.14	4.31	8.86	0.02	0.03	0.07	1.36	0.03	0.38
No.	16	16	16	16	16	16	16	16	16	16	3	3	3	3	3	4	

<sup>a</sup>Total oxidized nitrogen (nitrate and nitrite).

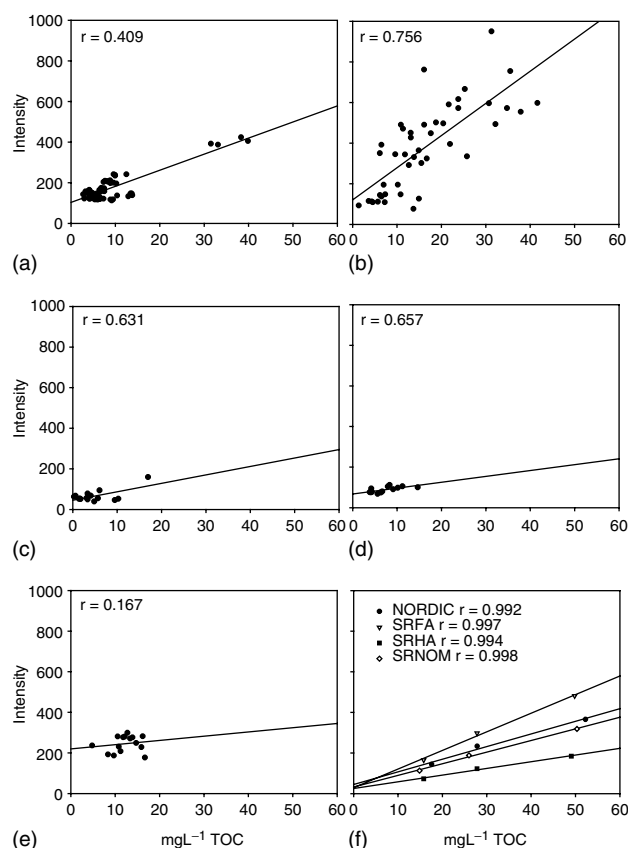


Figure 2. Comparisons of the fluorescence of F-L peak versus DOC for: (a) RIVER,  $y = 7.92x + 103.15$ ; (b) BOG,  $y = 15.87x + 121.03$ ; (c) SPRG,  $y = 4.15x + 45.8$ ; (d) POND,  $y = 2.89x + 67.77$ ; (e) STW,  $y = 2.09x + 219.82$ ; (f) IHSS samples

(Ex/Em 230/460 nm). As for the F-L fluorescence, the Norton in Hales samples showed a higher fluorescence per milligram of carbon for the H-L peak than the IHSS standards.

## DISCUSSION

Figures 2 and 3 demonstrate that H-L and F-L fluorophores have a correlation that varies with sample site. The strongest correlations are in the BOG and POND samples, where we hypothesize that natural H-L and F-L material dominates the DOM pool. In contrast, the weakest relationship occurs in the final treated effluent, a water source where H-L and F-L materials make up a relatively small component of DOM. These results match those of Baker (2002), investigating a small urban catchment. There, the F-L–DOC correlation for the whole catchment was  $r = 0.68$ , again with stronger correlations in the tributary containing greater proportions of natural DOM and poorer correlations in the subcatchments where anthropogenic influences were stronger (Table II). We suggest that the strength of the F-L–DOC correlation could be used as an indicator of anthropogenic influence on DOM. However, within-stream processing of DOM and mixing of DOM from different sources also has to be taken into consideration. For example, Clark *et al.* (2002) observed remarkable correlations (0.999) with DOM fluorescence

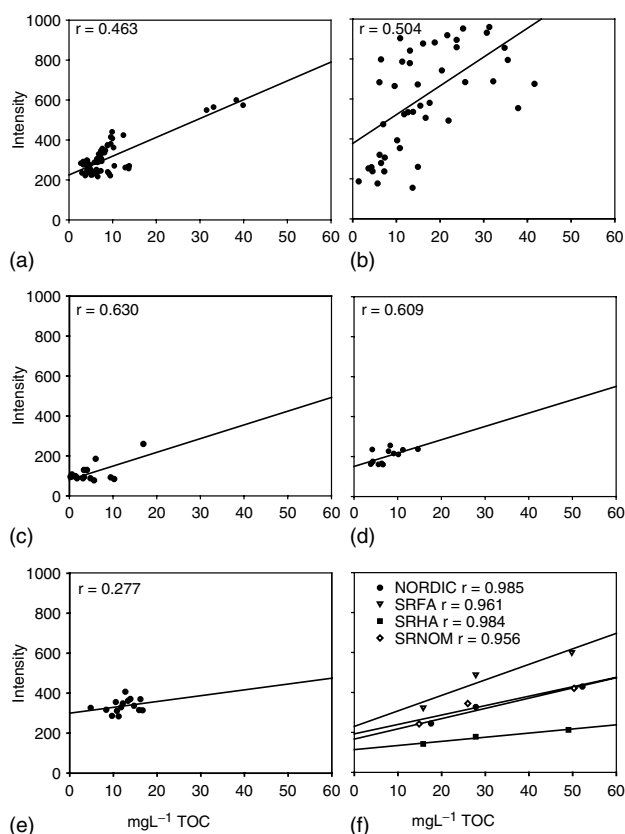


Figure 3. Comparison of the fluorescence of H-L peak versus DOC for: (a) RIVER,  $y = 9.42x + 224.62$ ; (b) BOG,  $y = 14.42x + 377.3$  (one data point is off scale, DOC = 16.5, Int. = 1558, not shown to facilitate equal scaling); (c) SPRG,  $y = 6.8621x - 80.69$ ; (d) POND,  $y = 6.66x + 150.88$ ; (e) STW,  $y = 2.919x + 299.69$ ; (f) IHSS samples

and DOC when they went from a fresh water to a saline water (Shark Head River to Florida Bay). Burdige *et al.* (2004) also reported good correlations from contrasting sites in the Chesapeake Bay, USA, with  $r = 0.92$ . In contrast, the scatter observed in the BOG, SPRG and RIVER TOC–fluorescence intensity relationships indicates the variability and instability of natural DOM when sampled closer to its source, as originally hypothesized in the river continuum concept (Vannote *et al.*, 1980). Dawson *et al.* (2001) showed changes in carbon fluxes in an upland stream ( $\text{CO}_2$  degassing) and that the source of the natural organic matter (NOM) may be unstable due to its state of decomposition and tributary contribution. By the time DOM reaches the estuary, within-stream processing and mixing of DOM from different sources leads to a final DOC–fluorescence relationship that is dominated by the stable, recalcitrant fluorescent fraction, leading to a stronger correlation.

The F-L fluorescence per gram of carbon of our samples (Table I and Figure 4) was shown to vary between sample sites. The BOG samples, with arguably the freshest DOM, have a greater fluorescence per gram of carbon than the river samples. F-L fluorescence per gram of carbon was also observed to be greater than the F-L IHSS standards, especially for the BOG samples site. For the H-L fluorescence per gram of carbon, this difference between our field samples and humic IHSS standards was

Table II. Comparison of the correlation coefficient between TOC and F-L fluorescence intensity with other studies

Study	Site ID	Description	Correlation coefficient	Comments
Ouseburn (Baker, 2002)				
Tributaries	4	Harey Burn	0.45	Impacted, sewerage failures
	11	Brunton tributary	0.78	Unimpacted, agricultural land cover
	17	Gosforth Park tributary	0.53	Impacted from occasional CSO discharges
	21	The Letch	0.75	Unimpacted, suburban and agriculture
	22a	Town Moor Tributary	0.78	Unimpacted, grassland and suburban
	6	Abbotswood Burn	-0.20	Impacted, cross connected sewers
Main stream	8	Airport tributary	-0.30	Impacted, airport deicer
	8	Woolsington	0.82	Unimpacted, agricultural land cover
	10	Brunton Bridge	-0.34	Downstream tributaries 6 & 8
	13	Red House Farm	-0.17	Downstream of tributary 11
	16	Great N Road	-0.29	Downstream of tributary 17
River Tern (this study)	22	Castle Dean gauge	0.42	Downstream of tributaries 21 & 22
		RIVER	0.87	
		BOG	0.76	
		SPRNG	0.63	
		POND	0.66	
	STW FTE	0.17		
Clark <i>et al.</i> (2002)		Florida Bay	0.99	Estuary
Burdige <i>et al.</i> (2004)		Chesapeake Bay	0.92	Estuary

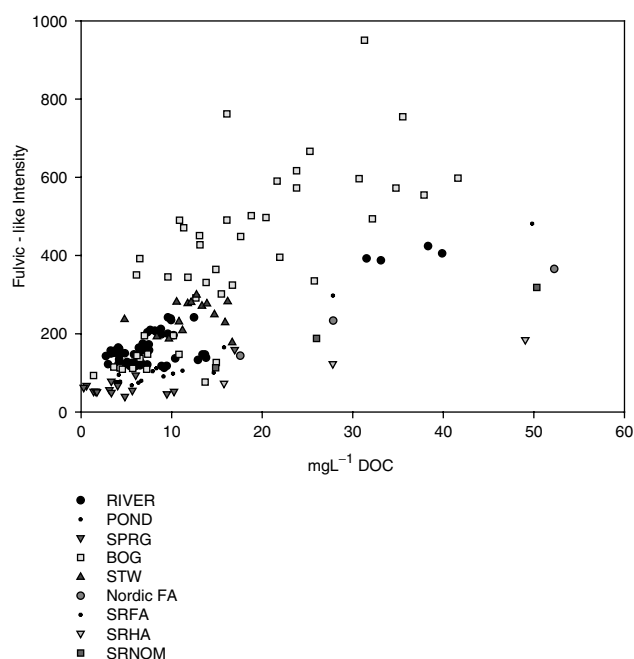


Figure 4. DOC versus F-L fluorescence intensity for all samples, showing the different fluorescence intensities per gram of carbon for different carbon sources

even greater. These results suggest that a fluorescent fraction is lost in the IHSS standard preparation procedure, especially the humic (SRHA) standard. This is in agreement with previous investigations that have suggested that no ideal system is available for isolating pure hypothetical humic substances from a water sample, and that certain changes in the structural composition of the DOM take place during each isolation procedure based on a chemically assisted sorption-desorption technique (Peuravuori *et al.*, 2005). Studies that use IHSS standards (e.g. Mobed *et al.*, 1996; Klapper *et al.*, 2002; Chen *et al.*, 2003; Alberts and Tackacs, 2004; Sierra *et al.*, 2005), and

in particular humic standards to quantify carbon fluxes from DOM fluorescence properties, could be considerably overestimating carbon quantities.

## CONCLUSIONS

We demonstrate different fluorescence intensity-DOC relationships for H-L and F-L fluorescence centres, depending on the source of DOM. These relationships differ in both gradient, i.e. the fluorescence per gram of carbon, and in the strength of the correlation coefficient. Strongest correlations are observed at sites where natural DOM dominates the DOC pool, whereas greater fluorescence per gram of carbon is observed in our peat wetlands than with within-river DOM. IHSS standards, in particular humic extracts, have significantly lower fluorescence per gram of carbon than our field samples. Assuming that all catchments have sufficient variability in both DOC and fluorescence, we propose the strength of the fluorescence-TOC correlation to be another useful tool when discriminating sources of NOM in freshwaters. Even when there is little enough variability of DOC and fluorescence, our results show significantly different fluorescence per gram of carbon between sources. Analyses of both fluorescence and DOC potentially fill a gap in the available data required to identify the mechanistic process, and magnitudes of, aquatic carbon fluxes to the estuary (Eatherall *et al.*, 1998).

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