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Fluorescence Excitation−Emission Matrix Characterization of Some Sewage-Impacted Rivers

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Fluorescence excitation−emission matrix (EEM) spectrophotometry was applied to 10 sample sites in six rivers in northeastern England, some of which were adversely impacted by sewage treatment works (STW) discharges, with the aim to investigate whether STW discharge has a significantly distinct fluorescence signature. Upstream, downstream, and STW discharge samples for two STWs demonstrated that treated sewage has a distinct fluorescence EEM, with high tryptophan and fulvic-like fluorescence intensities that are of approximately equal ratio. This signature could be seen in downstream samples. When all 10 sample locations were compared, two trend lines were apparent where STW impacted rivers plotted separately from the other sample locations. Fluorescence EEM signatures were compared to absorption at 254 nm and demonstrated to provide a better fingerprint of sewage-impacted water. It is suggested that fluorescence EEM spectrophotometry can provide a useful tool for the analysis of grab samples taken for both routine and investigative monitoring and has the potential for on-line monitoring of STW impacts on river systems.

Introduction

Fluorescent organic matter is a ubiquitous constituent of river waters, between 40 and 60% of natural organic matter is fluorescent (*1*). This fluorescent material principally comprises protein and organic acids derived from the decay of plant and animal matter from within the catchment. Fluorescence occurs when these molecules, having been previously excited by a high energy light source that raised the energy levels of the electrons within the molecule, release energy in the form of light. Recent advances in fluorescence spectrophotometry permit the collection of fluorescence data from waters at high optical resolution and the generation of excitation and emission data in the form of excitation−emission wavelengths (*2*). Figure 1 presents a fluorescence excitation−emission matrix (EEM) of a typical river water sample. Fluorescent organic matter exhibits discreet intensity peaks at known wavelengths: Figure 1 has labeled the fluorescence centers ascribed to tryptophan (A) and to organic acids (fulvic-like (B) and humic-like (C)) (*3*, *4*). Fluorescence intensities of these centers will predominantly depend on organic matter concentration, provided that other factors that affect fluorescence intensity (pH, metal ion interaction, etc.; *1*) remain relatively constant. Analysis of fluorescence EEM properties of river water and groundwater is becoming increasingly widespread (*5*−*7*).

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Several studies have investigated the fluorescence properties of sewage, either within the treatment process or in river systems, although none have applied the EEM technique. In a study of the Kurose River, synchronous scan fluorescence (SSF) was used to to differentiate natural organic matter from domestic wastes (*8*). Through the calibration of an intense fluorescence peak observed at 531 nm in sewage effluent, it was proposed that SSF could be used to detect different organic matter in rivers including a wastewater component. Fluorescence analysis has also been utilized within the sewage treatment process in an attempt to provide an on-line monitoring method superior to that of using absorption at 254 nm (*9*−*11*). High fluorescence intensity at 340 nm emission wavelength using fluorescence emission spectrometry has been demonstrated to correlate with high biological oxygen demand (BOD). Within the treatment process, untreated sewage was shown to have higher fluorescence intensity at 340 nm than treated sewage (*8*). In addition, it was demonstrated that fluorescence quenching by metal ions was not important due to the high concentration of organic matter in the wastewater. Lasers have also been utilized in an attempt to provide on-line process control in sewage plants through the analysis of fluorescence emission at 340 nm (*12*).

Here we use fluorescence EEM data for the first time to investigate the impact of sewage treatment works on some rivers in northeastern England. The relatively rapid data collection time (between 3 and 15 min depending on spectrometer model) and small sample size needed (<5 mL) mean that fluorescence EEM data have the potential to provide a quick analytical method for water quality monitoring to assess the impact of sewage treatment works on river systems. It is ideally suited to the analysis of grab water samples, such as those obtained in the U.K. by the Environment Agency, the regulatory body for water quality. Such water quality monitoring is becoming increasingly important in Europe in light of the implementation of the 1991 European Union Urban Waste Water Treatment Directive (*13*), whose quality objectives have to be met by the end of the year 2000 by sewage works with population equivalents of greater than 10 000. In addition, successful use of the EEM technique could be adapted in the future to on-line monitoring, provided that potential limitations such as fouling of the optical cell and spectrophotometer recalibration can be overcome.

Experimental Section

River water samples were collected at 7−14-day intervals from 10 sampling locations in northeastern England over the period May−July 2000. Six sample locations comprised those taken upstream, at the outfall, and downstream of two sewage treatment plants, East Tanfield STW on the River Team (National Grid reference NZ196553), and Hustledown STW on the Twizell Burn (National Grid Reference NZ214517). Both STWs provide a significant (typically >30%) proportion of the total river discharge. Hustledown STW has a consented dry weather flow of 2900 m^3/day, and East Tanfield has a consented dry weather flow of 5150 m^3/day. The average flow is usually 1.3−1.4 times the dry weather flow. Both are conventional percolating filter plants and produce effluent of reasonable quality; mean concentrations of BOD, suspended solids, and ammoniacal nitrogen have been 12.6, 20.5, and 1.8 mg/L at East Tanfield and 11.5, 27.1, and 1.1 mg/L at Hustledown over the last 3 yr (Environment Agency, unpublished data). The four other sampling locations were chosen from a variety of other local rivers. Two (Cong Burn

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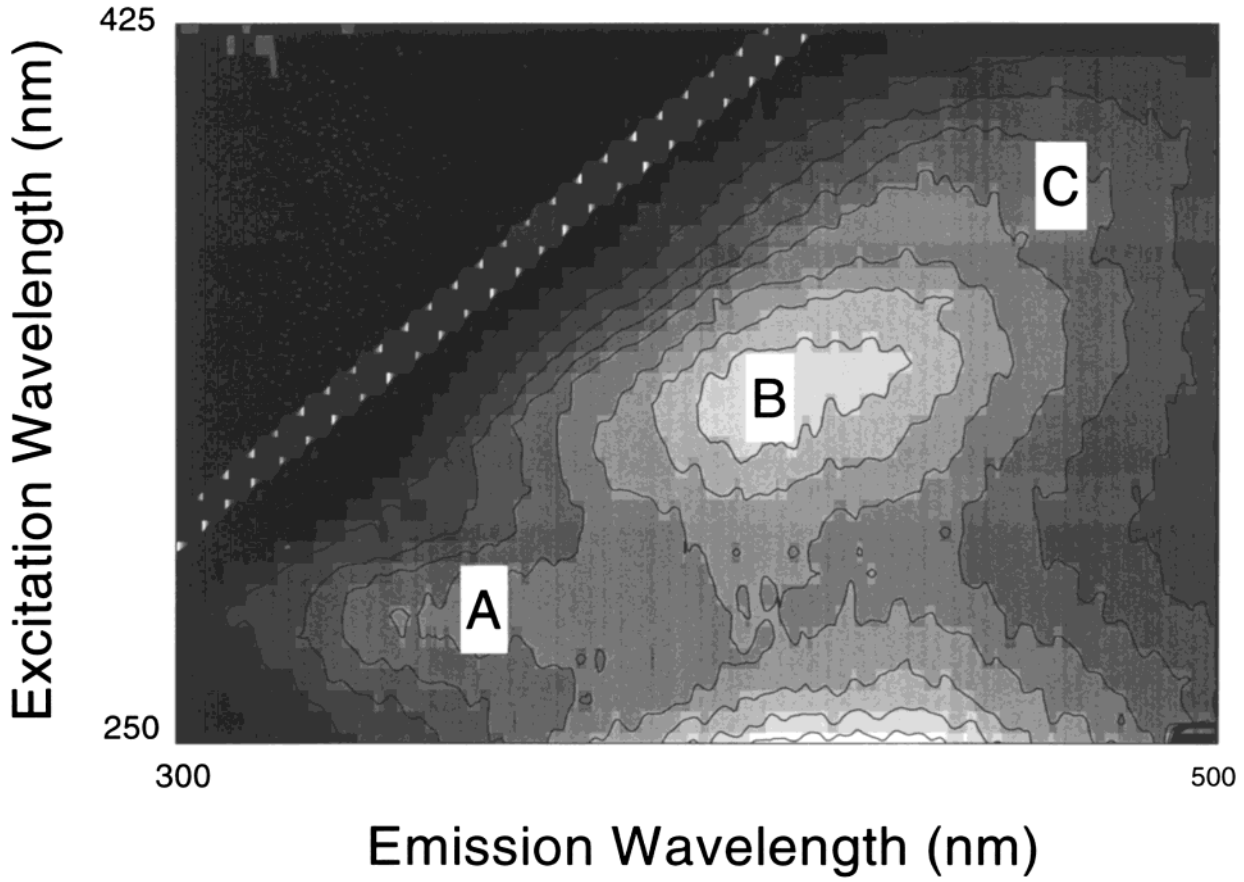

FIGURE 1. Typical river water fluorescence excitation−emission matrix (EEM). Excitation wavelengths vary from 250 to 425 nm, and emission wavelengths vary from 300 to 500 nm. Three fluorescence peaks are identified as A−C. The linear feature is the Rayleigh−Tyndall scatter when excitation wavelength equals emission wavelength. Tryptophan fluorescence (A) occurs at 275 nm excitation, 350 nm emission; fulvic-like fluorescence (B) at 320−340 nm excitation, 410−430 nm emission; and humic-like fluorescence (C) at 370−390 nm excitation, 460−480 nm emission.

and Lumbley Park Burn; National Grid References NZ 2775151 and NZ 285514) were known to experience poor water quality due to upstream STWs and combined sewage overflows (CSOs). In addition, the Twizell Burn is a tributary of the Cong Burn; therefore, sampling on this river at a distance of 10 km from the Hustledown STW would permit the investigation of dilution of the STW impact. Bogbins Burn (National Grid Reference NZ 205563) was chosen as it comprises a small agricultural catchment with no STW impact, and the River Wear (National Grid Reference NZ285507) was chosen as a high discharge end-member that has 40 upstream STWs with discharges less than 250 000 m^3/day. Water samples were grab samples taken at the same location on the river at each sampling trip. Samples were taken over a wide range of flow regimes, from immediately after an extreme June flood event through to summer baseflow.

Water samples were collected in 125-mL polypropylene bottles that had been precleaned in 10% HCl, Decon, and distilled water. Samples were refrigerated upon return from the field and analyzed within 24 h for their fluorescence properties, odor, pH, and conductivity. The samples were then analyzed for absorption at 254 nm, filtered to determine suspended solid concentrations, and titrated for chloride. Fluorescence measurements were undertaken 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, excitation wavelengths were incremented from 250 to 400 nm at 5-nm steps; for each excitation wavelength, the emission was detected from 300 to 500 nm at 0.5-nm steps. For each water sample, the tryptophan and fulvic-like fluorescence was measured as the maximum intensity at an excitation−emission wavelength pair. Analyses were performed at a constant laboratory temperature of 22 ± 2 °C, and blank water scans were run every 5−15 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 interlaboratory comparison. Raman emission at 395 nm averaged 18.8 ± 0.9 intensity units (n = 188), with no drift through the analytical period. Replicate analyses demonstrated that the wavelength of the fulvic-like fluorescence intensity maxima was reproduced within ±3 nm and protein fluorescence intensity maxima within ±5 nm; fluorescence intensities replicated within ±5% and ±15%, respectively. In addition, the stability of the Raman peak was assessed for a 10-min period at the start of each day of data collection, and analyses occurred only when the signal:noise ratio of the spectrometer was greater than 500:1. Absorption correction was not applied to the fluorescence spectra; absorption at 254 nm of 0.3−0.5 /cm decreased tryptophan fluorescence intensity by <10%. Absorption at 254 nm was undertaken using a WPA Lightwave UV−Vis spectrometer, chloride concentration was measured by silver nitrate titration, and suspended solids were measured by filtration using pre-ashed Whatman GF/C glass microfiber filter papers.

Additional background water quality data were obtained for 1996−1998 through the U.K. Environment Agency General Quality Assessment (GQA); the United Kingdom national method for classifying water quality in rivers. The Chemistry GQA describes quality in terms of three chemical measurements (BOD, dissolved oxygen (DO), and ammonia), which detect the most common types of organic pollution from

TABLE 1. Summary Fluorescence and Hydrochemical Results for 10 Sample Sites[a]

	fulvic-like fluorescence			protein luminescence			protein/ fulvic-like						general quality assessment (1996−1998)			
	excitation wavelength (nm)	emission wavelength (nm)	intensity (U)	excitation wavelength (nm)	emission wavelength (nm)	intensity (U)	fluorescence intensity	pH	conductivity (μs)	$A_{254\ nm}$ (cm^{-1})	Cl^- (mmol/L)	suspended solids (mg/L)	BOD (mg/L)	ammonia (mg of N/L)	DO (%)	grade
							Twizell Burn, Upstream of STW ($n = 6$)									
mean	330	418	140	278	363	82	0.62	7.6	906	0.17	4.1	24	1.93	0.23	91.31	good
SD	7	9	40	4	14	10	0.20	0.3	300	0.11	0.7	16	1.16	0.33	9.84	
							Hustledown STW ($n = 6$)									
mean	339	422	268	278	340	272	1.01	6.7	554	0.19	2.6	40				
SD	2	6	16	3	45	59	0.20	0.3	55	0.03	0.3	8				
							Twizell Burn, Downstream of STW ($n = 5$)									
mean	337	421	233	278	357	213	0.91	6.9	675	0.18	3.1	32	5.53	0.56	96.02	poor
SD	3	5	21	3	6	46	0.10	0.1	117	0.03	0.5	32	2.55	0.53	10.32	
							River Team, Upstream of STW ($n = 7$)									
mean	329	416	141	281	356	79	0.57	7.6	532	0.13	2.7	24	2	0.11	89.19	good
SD	7	4	28	5	11	25	0.20	0.2	67	0.05	0.5	16	1.59	0.14	10.84	
							Tanfield STW ($n = 7$)									
mean	339	420	257	279	353	258	0.99	7.1	518	0.21	2.6	40				
SD	2	4	36	2	6	71	0.20	0.1	46	0.03	0.2	8				
							River Team, Downstream of STW ($n = 8$)									
mean	336	420	216	279	360	192	0.88	7.4	555	0.18	2.6	8	2.99	0.59	83.86	fair
SD	6	4	26	2	8	50	0.10	0.1	93	0.02	0.2	8	3.91	1.53	22.05	
							Cong Burn ($n = 7$)									
mean	332	416	148	278	360	97	0.67	7.8	716	0.15	3.2	8	3.84	0.56	94.28	fair
SD	9	6	26	4	7	19	0.20	0.2	161	0.01	0.4	8	1.75	0.5	13.45	
							Lumbley Park Burn ($n = 7$)									
mean	329	414	179	276	370	168	0.93	7.8	1287	0.16	6.2	16	3.98	3.01	74.43	poor
SD	6	6	45	4	21	62	0.20	0.1	500	0.07	1.8	8	2.86	3.25	12.86	
							Bogbins Burn ($n = 6$)									
mean	328	416	115	278	358	82	0.75	8.1	527	0.15	2.6	nd				
SD	4	4	17	4	11	39	0.40	0.6	21	0.01	0.2	nd				
							River Wear ($n = 7$)									
mean	326	421	101	279	362	80	0.84	7.8	586	0.16	2.1	8	2.36	0.4	90.77	fairly good
SD	8	13	26	3	11	21	0.30	0.2	204	0.15	0.1	8	0.86	0.23	11.74	

[a] Fluorescence intensities are standardized to a Raman peak of 18.8 U at 395 nm emission. Fluorescence excitation and emission wavelengths are those of the intensity maxima. SD, standard deviation.

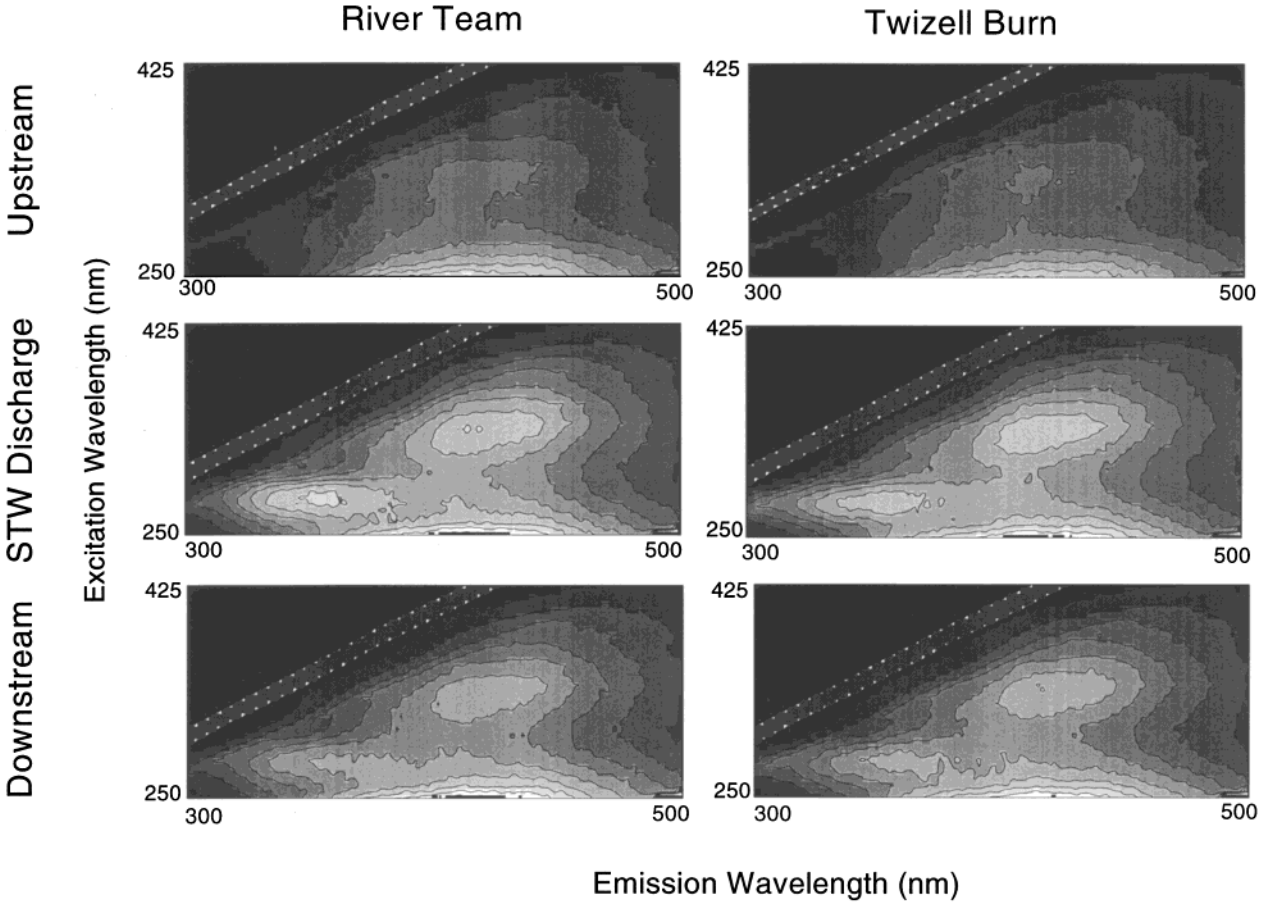

FIGURE 2. Typical fluorescence EEMs for the River Team (East Tanfield STW) and Twizell Burn (Hustledown STW) showing upstream, discharge, and downstream EEMs. Fluorescence intensity is scaled from 0 to 300 intensity units with contours every 30 units; contour labels are omitted for clarity. Note both increased intensity of peaks A and B at the discharge and downstream. Data are from samples taken on the July 4, 2000.

sewage treatment works, agriculture, and industry. Rivers are sampled at least 12 times a year, with a quality classification based on the results of 3 yr (a minimum of 36 samples).

Results and Discussion

Results of all water fluorescence and chemical analyses are presented in Table 1, together with GQA data for 1996−1998 where available. Fluorescence excitation and emission wavelengths for both the fulvic-like and tryptophan fluorescence centers are typical of those reported elsewhere, and the variability of fluorescence properties are typical of the temporal variability observed in natural waters (*3*−*6*). However, the intensity of fluorescence is particularly high at STW outfalls and at downstream sample locations. Other analyses confirm the impact of STWs in terms of odor and suspended solids. Conductivity measurements demonstrate that the rivers are not adversely affected by high ion concentrations.

Impact of Sewage Treatment Works on River Fluorescence Properties. Both the outfalls from the two STWs investigated plus the downstream samples exhibit statistically similar fluorescence properties. Fluorescence intensity of both fulvic-like and tryptophan fluorescence centers are significantly higher than the upstream samples for both rivers (significant at 99%, student's *t*-test). In addition, the ratio of tryptophan to fulvic-like fluorescence is distinctive (∼1.0) and significantly higher than the upstream ratio (significant at 95%, student's *t*-test). Figure 2 shows typical EEMs for upstream, outfall, and downstream samples.

Outfall from both STWs investigated is characterized by both high tryptophan and fulvic-like fluorescence intensity of approximately equal intensities. Downstream samples on both the River Team and the Twizell Burn were sampled at 1000 and 100 m from the outfalls, respectively. Fluorescence EEM properties are similar to that of the outfalls and demonstrate that the fluorescence signature is preserved even after dilution has occurred for these two rivers. Both STWs provide similar treated effluent; the ratio of tryptophan to fulvic-like fluorescence of ∼1.0 is significantly lower than that of untreated sewage observed elsewhere (2.7−3.1 in river waters; *8*), suggesting that further investigation of rivers where untreated sewage creates a pollution problem might also yield a distinctive fluorescence signature.

Fluorescence of STW-Impacted Rivers As Compared to Other Rivers. Figure 3 presents the tryptophan fluorescence intensity and the ratio of tryptophan/fulvic-like fluorescence for all 10 sample locations. STW outfall and samples downstream of the STWs are characterized by high tryptophan fluorescence intensity and high ratio as described earlier and can fit a linear trend line ($r = 0.94$; Figure 3). Outfall or downstream samples with lowest fluorescence intensity and ratio all relate to sampling immediately after the June 7, 2000, flood event and represent the effects of dilution under high flow conditions. The simplest explanation of the linear trend is that of initial high tryptophan and fulvic-like fluorescence intensity of STW-impacted waters that are subsequently diluted.

Upstream samples from the River Team and the Twizell Burn together with samples from the other four rivers plot

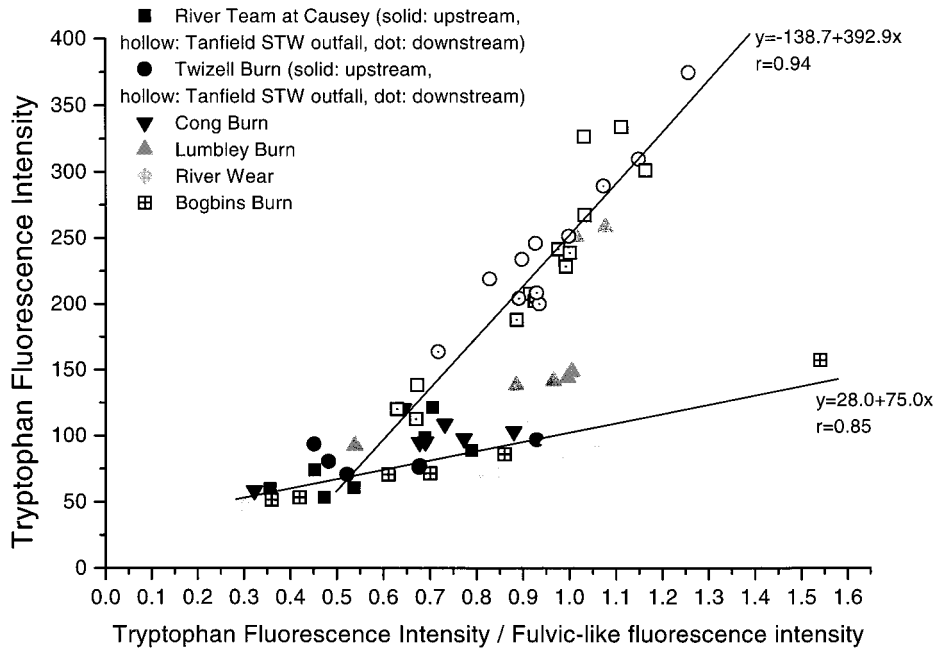

FIGURE 3. Graph of tryptophan fluorescence intensity against tryptophan/fulvic-like fluorescence intensity. The two trend lines are linear regressions fitted to (i) River Team and Twizell Burn STW impacted samples and (ii) all other samples except two Lumbley Park Burn outliers.

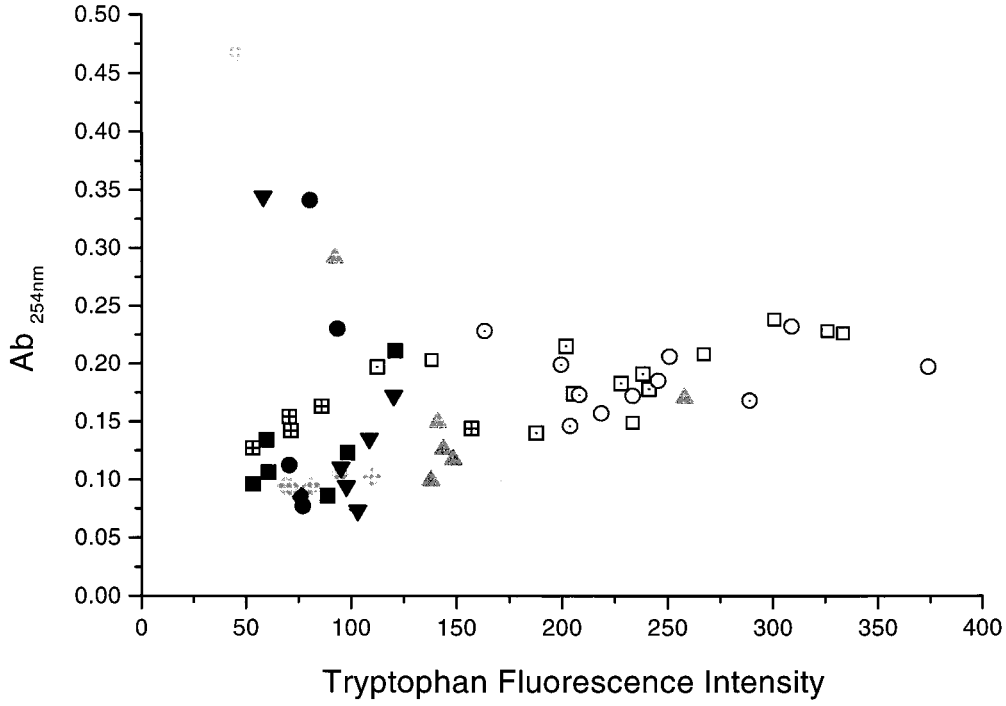

FIGURE 4. Graph of absorption at 254 nm against tryptophan fluorescence intensity. Symbols as for Figure 3.

on a trend line characterized by variable tryptophan/fulvic-like ratios and low tryptophan fluorescence intensity (r = 0.85 if two outlying samples are removed). Outfall and downstream samples from the River Team and the Twizell Burn together with the two outliers (two samples from Lumbley Park Burn) fall on a separate line than the STW-impacted trend. The two samples from Lumbley Park Burn that plot with the STW outfall samples may represent unconsented CSO or STW discharges; the river is known to be impacted by Sedgeletch STW, although no discharge data were available over our study period to prove this as the source. Samples from the Cong Burn, 10 km downstream from Hustledown STW, fall at the intersection of both trend lines. This reflects fluorescence signatures of waters that have upstream STW impacts but have subsequently undergone significant dilution effects or for which organic matter additions or transformations may have started to occur such that the fluorescence signature is lost. All other samples are characterized by variable tryptophan/fulvic-like fluorescence and low tryptophan fluorescence intensity. This trend line probably reflects a dilution line of river water that has experienced a combination of diluted STW impacts as well as other high protein sources such as farm yard wastes and industrial discharges. Further research into the fluorescence signatures at a catchment scale is necessary to elucidate this.

Comparison of Absorption and Fluorescence Properties. Absorption at 254 nm ($A_{254\ nm}$) has been suggested to be a useful indicator of sewage pollution (*9*, *10*). Figure 4 presents the relationship between $A_{254\ nm}$ and tryptophan fluorescence intensity for all water samples. $A_{254\ nm}$ ranges from 0.05 to 0.5, and samples of STW outfall or downstream samples exhibit intermediate values of $A_{254\ nm}$. This is in contrast to the fluorescence results; the protein fluorescence intensity is highest for STW outfall and downstream samples as described above. In general, it appears that there is a weak relationship between the two variables, with a group of outliers with high $A_{254\ nm}$. These are mostly from sampling after the June 7 flood and probably reflect the presence of high concentrations of humic material that may also absorb in the 254 nm region (Figures 1 and 2 show the presence of a fluorescence center at 250 nm excitation wavelength that is ascribed to humic substances; *3*). In general, Figure 3 demonstrates the improved differentiation of STW impacts on river systems using protein fluorescence rather than $A_{254\ nm}$.

Implications for the Characterization of STW Impacts. The results have demonstrated that by taking paired upstream−downstream samples around STWs, distinct fluorescence signature can be obtained. Sampling was manual, obtaining grab samples at regular intervals, a similar process as undertaken by the U.K. Environment Agency in order to obtain baseline water quality data. However, automatic sampling can be envisaged using an automatic line into the spectrophotometer, continuous EEM generation, and modem link. Such apparatus would require regular maintenance and calibration but could be used to investigate high-frequency variability in STW discharge. Previous research has suggested that untreated sewage should have significantly higher tryptophan/fulvic-like fluorescence, and although no untreated sewage was experienced in the study rivers, the technique should apply to such situations. Finally, for the European situation where compliance with Urban Waste Water Quality Directives is important, calibration of our fluorescence properties against key quality criteria (for example, BOD5 or COD) may help provide a rapid and precise measurement of water quality.

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