Fluorescence Excitation−Emission Matrix Characterization of River Waters Impacted by a Tissue Mill Effluent

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Fluorescence excitation−emission matrix (EEM) spectrophotometry was applied to five neighboring rivers, including one that is impacted by wastewater from a large tissue mill, to determine if fluorescence spectrophotometry could be used to differentiate between the river waters. River water samples from both the tissue mill effluent and the impacted river, the Park Burn, exhibited significantly higher fluorescence intensity than the other sites. This fluorescence was dominated by tryptophan fluorescence and a fluorescence center possibly due to the presence of fluorescent whitening agents. In contrast, the three other rivers exhibited lower fluorescence intensities typical of river systems with tryptophan (sewage), humic-like (peat derived color), and fulvic-like (natural organic matter) sources. It is suggested that fluorescence EEM spectrophotometry has the potential to provide a useful tool for pollution detection, monitoring, and control of paper industry impacts on river systems.

Introduction

Wastewater originating from the paper and pulp industries has high organic and suspended solids loads; treatment requirements are therefore high in order that direct discharges meet national discharge standards. For example, untreated effluent biochemical oxygen demand (BOD_5) of 1600−3100 mg/L and suspended solids (SS) of 200−220 mg/L was observed for a pulp mill whose effluent was derived from chip washings, press filtrate, and white water purge (*1*). Wastewater properties of a Finnish paper mill before secondary treatment were an SS of 3800 mg/L and a chemical oxygen demand (COD) of 1120 mg/L (*2*), and at a hardboard production plant that utilized clean wastepaper, wastewaters had a COD of 2000−9000 mg/L and an SS of 800−3500 mg/L that were significantly reduced by treatment (*3*).

BOD_5 and COD have been used as standard methods of measuring paper and pulp wastewater quality as opposed to spectrophotometric (fluorescence, absorbance) methods, despite the latter being applied in other areas of water quality monitoring and control (*4*−*7*). Two studies that have used spectrophotometery include the use of UV aborbance to estimate BOD_5 from two different pulp and paper mill effluents (*8*) where it was demonstrated that absorbance between 200 and 350 nm correlated with BOD_5, but that the correlation was site specific. A separate study investigated paper pulps from wheat straw, fiber sorghum, and sweet sorghum stalks and demonstrated that fluorescence emission spectra could be used to give information of pulp source and pulping method used (*9*). In this study, it was claimed that fluorescence had not been widely used given the heterogeneity and chemical complexity of the lignin/cellulose-based compounds, an unsurprising result given that about 250 chemicals have been identified in pulp mill effluents, including acids, phenolic compounds, and sugars (*10*).

Further research is warranted into the use of fluorescence properties to characterize wastewater effluent from pulp and paper mills. Recent advances in fluorescence spectrophotometry permit the rapid (∼1 min) collection of fluorescence data from small samples (5 mL) of river waters and wastewaters at a higher optical resolution than previously possible and the generation of excitation and emission data in the form of excitation emission matrixes (EEMs). Analysis of fluorescence EEM properties of river water and groundwater is becoming increasingly widespread (*11*−*14*) and can detect fluorescence centers attributed to aromatic proteins and humic- and fulvic-like substances at concentrations down to the ppb level. Recent technological advances including autosamplers, flow cells, and fiber-optic probes have all led to the possibility of using fluorescence EEMs as a continuous monitoring tool. Therefore, further research is needed to investigate whether fluorescence has a use either in the process control of wastewater from the paper and pulp industries or in the monitoring of pollutants from these industries when discharged into river systems.

Experimental Section

Here we use fluorescence EEM data to investigate the fluorescence properties of wastewater from a tissue mill in Northumberland, NE England, together with comparative data from nearby rivers. The process operated at our research site consists of the manufacture of 80 000 ton of tissue products/yr from the recycling of fiber. De-inking of the resultant secondary fiber pulp is carried on at the mill; the recycled fiber process plant is designed to repulp approximately 400 ton of waste paper/day and can produce up to 90 000 air-dried ton of de-inked secondary fiber pulp/yr. Of particular relevance to this study is the recycling process: waste paper is mixed with recycled water and warmed with steam; mechanical agitation is used to disintegrate the paper into individual fibers and to separate the inks, ash, and other contrary material. The pulp is screened to remove plastic, wire, staples, etc. followed by washing and fine mesh filtration, which retains the paper fibers but allows the inks and fine fibers to pass through. Aqueous effluent passes into the site effluent treatment plant, some water is recycled from there, and the rest is discharged into the Park Burn. Discharge consent is for waters with SS <30 mg/L (48 h average), discharge of <20 000 m^3/day, pH of 5−9, ammoniacal nitrogen of <5 mg/L, and BOD_5 <15 mg/L (monthly average) (*15*).

Water samples were taken at 7−14-day intervals over the period December 2000−February 2001, when sampling was halted due to countrywide access ban due to foot and mouth disease. Water samples were taken from sampling locations on five rivers for both fluorescence and comparative water quality parameters (Figure 1). One sample site was the pulp mill treatment plant effluent, which was sampled at 15-min intervals on each sampling trip for a period of up to 2 h; this discharged directly into the Park Burn and comprised >90%

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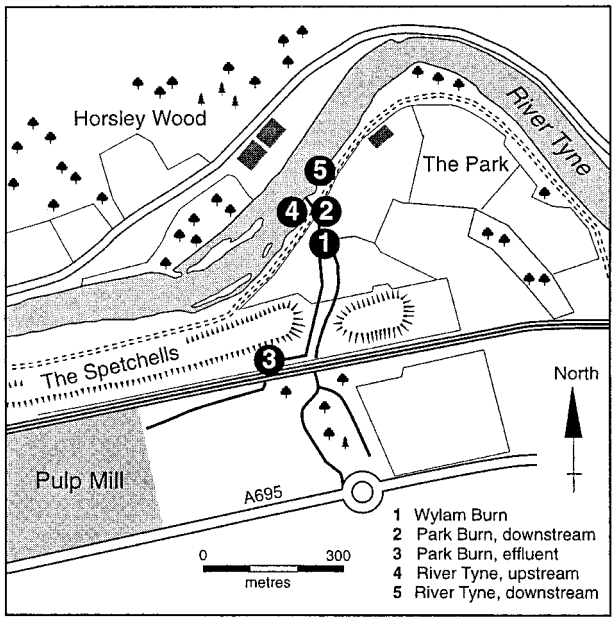

FIGURE 1. Sample sites and location of the tissue mill, NE England.

of the flow of this stream. Grab samples of river waters were also taken at four other locations within 500 m of the mill to compare the fluorescence properties of the river waters. These sample sites comprised (i) a tributary of the Park Burn, the Wylam Burn, which joined the Park Burn downstream of the effluent input; (ii) the Park Burn, which was sampled 50 m downstream of this tributary and before the Park Burn joins the River Tyne; (iii) the River Tyne, 50 m upstream of the Park Burn−Tyne confluence and close to the intake of river water into the tissue mill (the River Tyne has an upstream catchment of ~2200 km^2 and a mean discharge of 33 $m^3 s^{-1}$); and (iv) 50 m downstream of the Park Burn−Tyne confluence to investigate if any impact of the wastewater could be seen.

Water samples were collected in 125-mL polypropylene bottles that had been precleaned in 10% HCl, Decon, and distilled water. Temperature and dissolved oxygen were measured in the field using a YSI Dissolved Oxygen Probe. Samples were refrigerated in the dark on return from the field, filtered using Whatman GF/C glass microfiber filter papers, and analyzed within 24 h for their fluorescence properties, absorbance, pH, electrical conductivity, total carbon (organic and inorganic), ammoniacal nitrogen, and chloride. Chloride was measured by silver nitrate titration, ammoniacal nitrogen was measured by a Hanna Instruments colorimeter, and total carbon analyses (made in triplicate) were made using a Shimadzu 5000 TOC analyzer. Fluorescence measurements were made using a Perkin-Elmer LS-50B luminescence spectrophotometer. The spectrophotometer 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 420 nm at 5-nm steps; for each excitation wavelength, the emission was detected from 280 to 500 nm at 0.5-nm steps. Typical EEMs observed in this study are presented in Figure 1. For each water sample, the fluorescence peaks were 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 10−20 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 comparisons; Raman emission at 395 nm averaged 21.0 ± 0.7 intensity units with no drift over the analytical period. Absorption measurements were also made on all samples using a WPA Lightwave UV−Vis spectrophotometer

TABLE 1. Summary Fluorescence and Water Quality Results[a]

		T (°C)	pH	conductivity (μS)	Cl^- (mg/L)	total C (mg/L)	inorg C (mg/L)	total org C (mg/L)	diss O (%)	ammon N (mg/L)	absorption 254 nm/cm	absorption 340 nm/cm	absorption 410 nm/cm	fluorescence peak T ex (nm)	fluorescence peak T em (nm)	fluorescence peak T int (U)	fluorescence peak F ex (nm)	fluorescence peak F em (nm)	fluorescence peak F int (U)	fluorescence peak H ex (nm)	fluorescence peak H em (nm)	fluorescence peak H int (U)
mill discharge	mean	21.6	7.38	972	216.6	91.4	61.1	32.1	61.3	0.25	0.362	0.091	0.014	277.3	351.1	481	343.5	432.7	4728	np	np	
(*n* = 16)	SD	2.2	0.15	132	28.4	22.4	9.6	18.9	5.1	0.06	0.074	0.025	0.015	2.6	1.9	72	5.8	2.9	1941			
Wylam Burn	mean	6.4	7.53	1434	468.6	45.5	35.4	10.2	78.8	0.26	0.172	0.072	0.046	276.0	351.4	65	336.0	422.9	131	381.0	528.0	66
(*n* = 6)	SD	0.9	0.14	406	234.3	9.4	11.3	2.8	8.2	0.15	0.113	0.075	0.068	2.2	2.0	25	6.5	13.7	29	5.5	131.4	21
Park Burn at River Tyne	mean	19.8	7.36	972	191.7	83.8	59.5	24.3	59.4	0.24	0.331	0.084	0.014	277.5	352.0	436	343.0	430.8	3459	np	np	
(*n* = 6)	SD	2.6	0.19	158	56.8	16.6	5.1	12.3	5.9	0.04	0.092	0.034	0.017	2.9	1.5	78	6.7	3.5	1314			
River Tyne upstream	mean	2.9	7.50	208	117.2	24.5	12.1	12.4	100.8	nd	0.440	0.170	0.065	277.9	351.1	28	334.3	434.6	138	387.1	471.5	87
(*n* = 6)	SD	1.2	0.10	61	17.8	3.9	5.8	2.3	4.5		0.146	0.060	0.021	2.7	1.1	13	8.4	11.2	15	3.9	4.1	12
River Tyne downstream	mean	3.7	7.56	241	110.1	26.7	14.8	11.9	101.6	nd	0.447	0.172	0.068	280.0	351.4	31	343.8	436.0	193	386.3	471.5	93
(*n* = 6)	SD	0.4	0.14	102	14.2	6.6	8.0	1.4	0.8		0.079	0.036	0.016	4.1	2.4	8	7.5	7.3	83	4.8	5.2	5

[a] np, peak not present; nd, not determined due to interference between natural water color and colorimetry test.

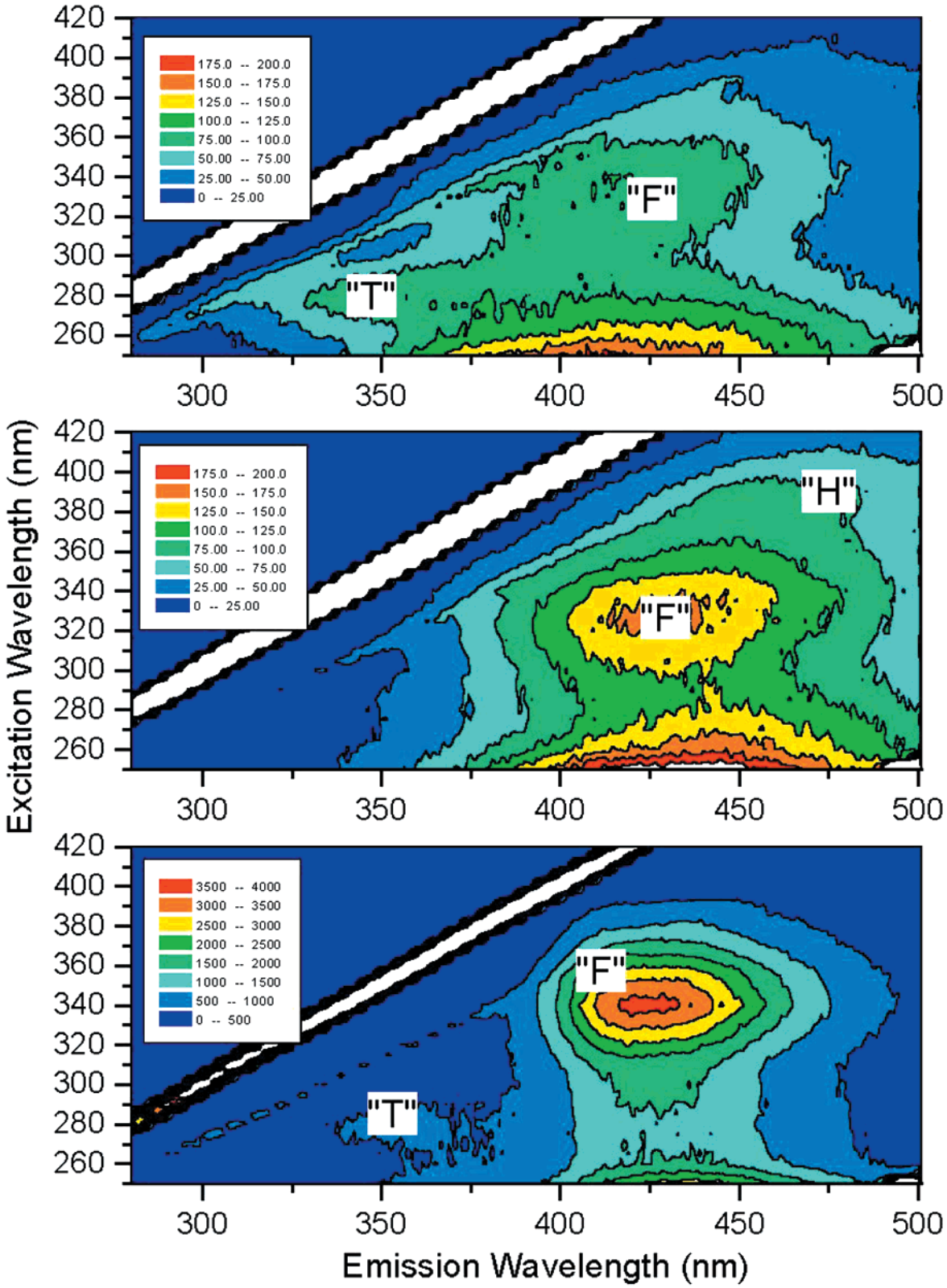

FIGURE 2. Typical fluorescence EEMs observed in this study: (a, top) Wylam Burn, (b, middle) River Tyne, (c, bottom) Park Burn. Note that the fluorescence intensity scale is different for the Park Burn. Peaks T, P, and H are as referred to in the text. The linear features are Rayleigh Tyndall and Raman scattering of water, respectively.

in order to check if inner-filtering (internal absorption and re-emission) of fluorescence was likely due to the high organic carbon concentrations present samples. Absorption correction was not applied to the fluorescence spectra (*16*); absorption at 254 nm of 0.1−0.5/cm and at 340 nm of 0.01−0.24/cm (highest values of each were for the River Tyne samples) decreased tryptophan and fulvic-like fluorescence intensities respectively by 0−14% and had no effect on fluorescence wavelengths. For further fluorescence technical details, see ref *12*.

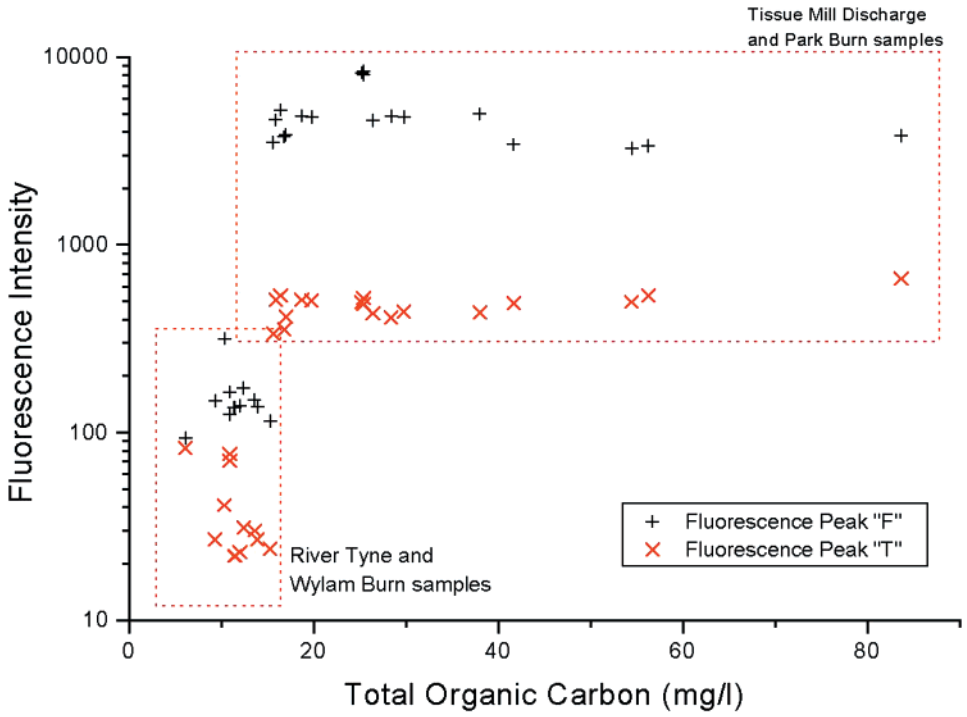

FIGURE 3. Comparison of total organic carbon and fluorescence intensity for all samples.

Results and Discussion

Results of all fluorescence and water quality analyses are presented in Table 1, and typical fluorescence EEMs are presented in Figure 2. Water quality and geochemical data demonstrate significant differences between the sample sites. Most notably, the Park Burn and the tissue mill effluent samples are similar in all characteristics, with the Park Burn being thermally impacted and having the lowest dissolved oxygen due to the impact of the tissue mill effluent. The Wylam Burn has a relatively high conductivity and chloride and intermediate dissolved oxygen concentrations. The River Tyne has low conductivity and high dissolved oxygen. On the basis of the U.K. Environment Agency water quality classification, Park Burn, Wylam Burn, and River Tyne would be classified for dissolved oxygen as fair, fairly good, and very good, respectively.

Fluorescence results are also tabulated in Table 1 and are best visualized in Figure 2, which contrasts typical EEMs observed on Wylam Burn, Park Burn, and River Tyne. Figure 1a, from the Wylam Burn, exhibits two fluorescence centers, one attributed to fulvic-like fluorescence (F) and a peak T attributed to the protein tryptophan (*17*). Figure 1b, from the River Tyne upstream sample site, has the peak F and a second peak attributed to humic-like substances (H) (*11*). Figure 1c, from the Park Burn, exhibits an order of magnitude higher fluorescence with peaks at both fluorescence centers T and F, although for the latter a double-peak shape can be observed. For the River Tyne and the Wylam Burn, the differences in fluorescence properties agree with the known sources of organic matter in the rivers and previous research (*11*, *12*). The tryptophan peak is often attributable to sewage or farm waste pollution (*7*, *13*), and the Wylam Burn is impacted by emergency sewage pumping station overflows; sewage litter was observed during the course of this study. In contrast, the River Tyne has a discernible brown color due to its peaty headwaters in the Pennine mountain range of north central England, which accounts for the presence of the H fluorescence center (*11*). For the tissue mill effluent and the impacted Park Burn, the fluorescence EEM results are different from those reported previously in river systems with an order of magnitude higher fluorescence intensity of both peaks T and F. In addition, the latter peak has a more symmetrical shape than observed in the other samples and has a distinctive double-peak structure and a subpeak at excitation 300 nm/emission 425 nm, suggesting that it is dominated by a different fluorophore.

Comparison of Fluorescence Center Intensities and Total Organic Carbon. Figure 3 plots the intensity of the fluorescence centers at the peaks F and T against total organic carbon (TOC) for all samples. Samples from the Park Burn and the effluent can be seen to have a higher TOC than the other rivers. A positive correlation between TOC and tryptophan fluorescence intensity is observed ($r = 0.62$, $n = 18$) for these sites, suggesting that fluorescent protein is a significant contributor to the TOC. This is probably due to the lignins and sugars produced by the pulping and recycling process, which are likely to be rich in aromatic proteins (*10*). In contrast, no correlation is observed between TOC and the fluorescence intensity at peak F for the Park Burn and the effluent, despite its order of magnitude greater fluorescence intensity than the tryptophan fluorescence intensity. The correlation between tryptophan fluorescence intensity T and TOC is not observed at the other three river sample sites, suggesting that here it is a less important component.

Figure 4 presents the fluorescence intensities of peaks T and F plotted against each other for all samples, showing the sampling dates. Both the tissue mill effluent and Park Burn demonstrate no correlation between the two fluorescence centers. Significant variability is observed in fluorescence properties at 15-min sampling intervals, although samples cluster on particular dates. This suggests that short-term variations in the tissue mill recycling process are important in determining the fluorescence properties, a not unexpected result given the discharge rate of the effluent, the variability in pulp used for recycling at the plant, and the recycling of water within the manufacturing process.

Fluorescence and Pulp Mill Discharges. Fluorescence intensity of peak F of the effluent shows some agreement with the "brightness" (measured as the reflectivity of the pulp at a fixed wavelength) of the pulp to be used in the

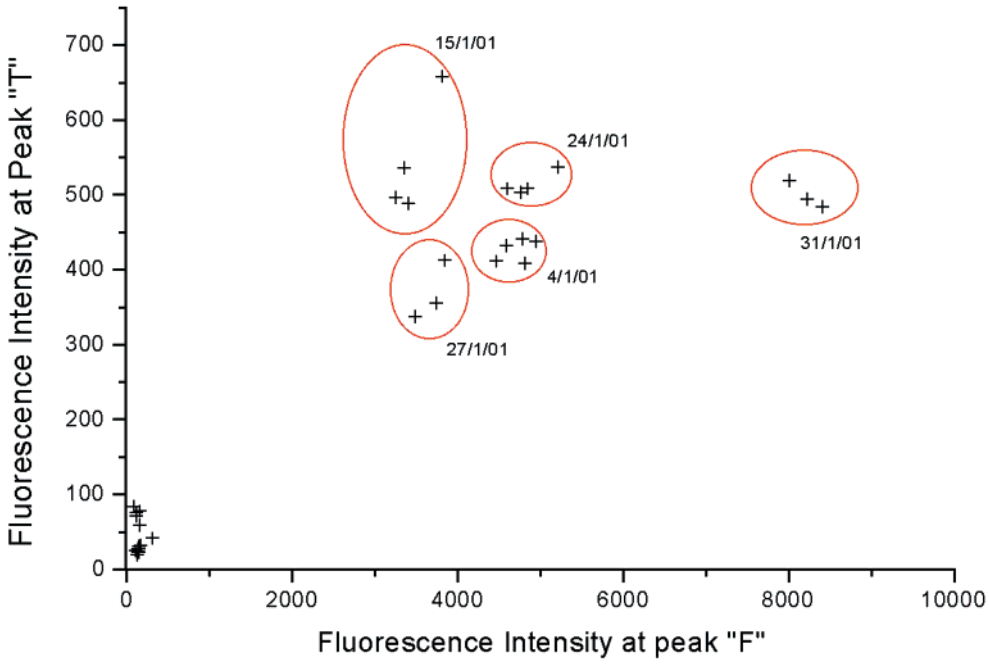

FIGURE 4. Plot of fluorescence intensity at peak T (tryptophan) against fluorescence intensity of and peak F. Sampling dates are also shown.

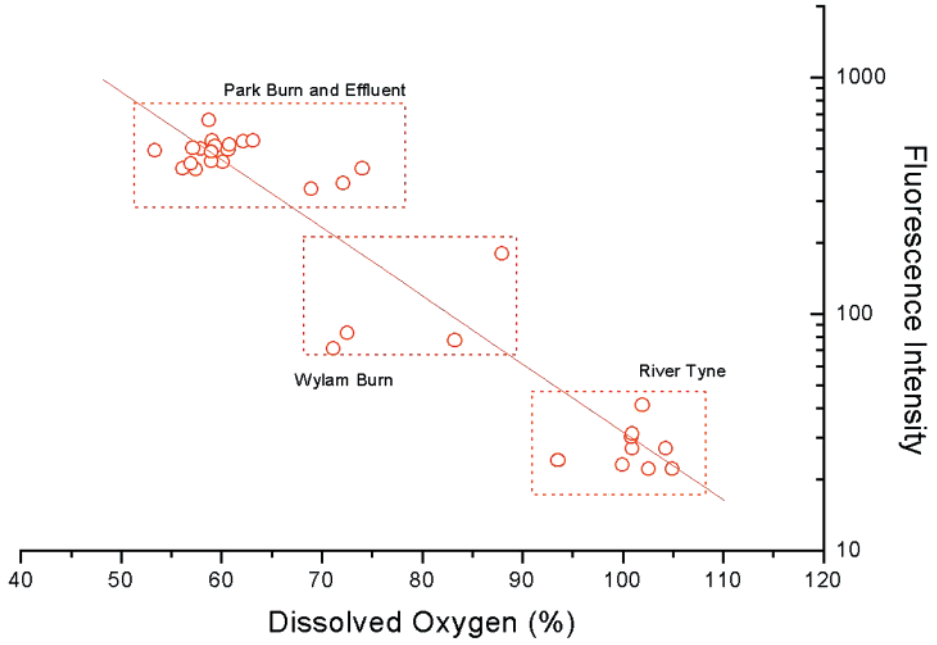

FIGURE 5. Tryptophan fluorescence intensity vs dissolved oxygen for all samples.

recycling process in the previous 24 or 48 h (Jim Smith, Plant Manager, personal communication). Given this observation, together with the relatively high fluorescence intensity and quantum efficiency of peak F and its distinctive double-peak shape, we suggest that this peak be due to the leaching of optical brighteners or fluorescent whitening agents (FWAs) from the pulp during recycling. FWAs such as the stilbene type such as distyrl biphynl (DSBP) and diaminostilbene types (DAS1 and DAS2) are commonly used in papers. FWAs are not readily biodegradable, but due to their ability of absorb sunlight, they photochemically degrade in natural waters (*18*). Photochemical degradation rate varies between FWA type with oxygen concentration and exposure to sunlight. In laboratory experiments over a 24-h period, we observe a ~40% decrease in fluorescence intensity of peak F in the effluent and the Park Burn samples when they are exposed to sunlight, again suggesting the presence of FWAs in our water samples. In contrast, samples from the upstream River Tyne and the Wylam Burn showed no change in fluorescence intensity. Detail chemical analyses such as those perfomed on Swiss river waters (*18*−*21*) would confirm the existence of these species.

Our results have several potential applications. For pollution detection and monitoring, grab water samples analyzed for fluorescence may be used to trace the dispersion of effluent, especially when combined with TOC measurements. Further research is undoubtedly still required. This includes the investigation of the use of fluorescence as a tracer of effluent dispersion in the River Tyne. Table 1 clearly shows that downstream water samples on the River Tyne have

a higher fluorescence intensity of both peaks T and F than upstream due to the impact of the Park Burn. Additionally a comparison of tryptophan fluorescence intensity with a wider range of dissolved oxygen and also with BOD_5 is required in order to investigate its use as a proxy for this water quality parameter. Figure 5 demonstrates the correlation between tryptophan fluorescence intensity and dissolved oxygen for our five sample sites ($r = -0.95$) and suggests that there is considerable promise for further research. Finally, given the known variability of effluent quality and characteristics between pulp and paper mill sites, the results observed here should be extended to other sites in order to compare the utility of fluorescence EEM analyses with other paper and pulp industrial complexes (*22*). These are the focus of current research.

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