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Can fluorescence spectrometry be used as a surrogate for the Biochemical Oxygen Demand (BOD) test in water quality assessment? An example from South West England

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ABSTRACT

The fluorescence intensities of tryptophan-like, tyrosine-like and humic-like materials were determined using excitation-emission-matrices (EEMs) for a wide range of samples including natural surface waters, sewage and industrial effluents and waters that have experienced known pollution events from the South West of England (n=469). Fluorescence intensities reported in arbitrary fluorescence units (AFU) were correlated with standard five day Biochemical Oxygen Demand (BOD_5) values which were used as an indicator of the amount of biodegradable organic material present. Tryptophan-like fluorescence, which has been found to relate to the activity of the biological community, showed the strongest correlation with BOD_5. Fluorescence analysis of the tryptophan-like peak (excitation/emission wavelength region 275/340 nm) is found to provide an accurate indication of the presence, and relative proportions of bioavailable organic material present (natural or anthropogenic). It therefore provides an insight relating to its oxygen depleting potential. Thus fluorescence spectroscopy is recommended as a portable or laboratory tool for the determination of the presence of biodegradable organic matter with intrinsic oxidising potential in natural waters. The novel application of Geographically Weighted Regression (GWR) to the data illustrates that strong local relationships exist between the two parameters and that site specific character may be a strong factor in the strength of the tryptophan-like fluorescence/BOD_5 relationship.

1. Introduction

Fluorescence spectroscopy is commonly used in the study of dissolved organic matter (DOM) in natural waters including marine waters (Coble, 1996), rivers (Patel-Sorrentino et al., 2002), groundwaters (Baker and Genty, 1999) and lakes (Cammack et al., 2004). It is a rapid, reagentless technique that requires little sample preparation. An Excitation–Emis-

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sion–Matrix (EEM) can be created by simultaneously scanning excitation and emission wavelengths through a set path-length of aqueous sample. Each fluorophore, distinct fluorescent spectra from a single fluorescent compound or overlapping spectra from a range of fluorescent moieties, appears on the EEM as a peak or series of peaks associated with specific excitation and emission wavelengths. The intensity of the peak can be used as a measure of the concentration of the fluorophore to ppm or ppb levels, depending upon the fluorophore.

The most common fluorophores in natural surface waters are humic-like derived from the breakdown of plant material (peaks C and A, (Coble, 1996)). In this study these fluorophores exhibit fluorescence at excitation/emission wavelengths λ_{ex} 304–347 nm λ_{em} 405–461 nm (Peak C) and λ_{ex} 217–261 nm λ_{em} 395–449 nm (Peak A). In addition to humic-like material tryptophan-like and tyrosine-like material as "free" molecules or bound in amino acids and proteins (commonly referred to as peaks T and B respectively, (Coble, 1996)) also exhibit fluorescence at distinctive wavelengths in natural waters. Tryptophan-like fluorescence (peak T_1) occurs in this study at $\lambda_{ex/em}$ 275–296/330–378 nm while tyrosine-like fluorescence (peak B) was not commonly seen and is not addressed in this work. Peak *T* also has a shorter wavelength excitation/emission pair (named T_2) with excitation at between λ_{ex} 216–247 nm and emission at between λ_{em} 329–378 nm. Tryptophan-like fluorescence may be exhibited by natural waters where tryptophan is present as 'free' molecules or else bound in proteins, peptides or humic structures. Peaks *T* and *B* are related to microbial activity (Parlanti et al., 2000) and may be transported into a system (allochthonous) or be created by microbial activity within a system (autochthonous). For an example EEM showing fluorophores common in natural waters see Fig. 1.

Previous studies have identified different specific wavelengths of excitation and emission in the study of fluorophore T_1 and its relationship with BOD. These are presented in Table 1. The variation in wavelengths is likely to be due to the physical characteristics of individual samples such as pH, metal ions, sample concentration (Vodacek and Philpot, 1987). These factors have not been analysed on a sample by sample basis for this (or any previous) study. For this reason the wavelengths identified in each individual body of work are presented with no correction for the contributory factors. Table 1 also illustrates the sample types and number of samples used in studies of the BOD/tryptophan-like fluorescence relationship.

Surface waters are commonly rich in humic-like material which may be allochthonous or autochthonous, and may be new or old, and more or less bioavailable, with character being influenced by source (Newson et al., 2001; Katsuyama and Nobuhito, 2002) and season (Newson et al., 2001). Surface waters become more influenced by anthropogenic (human) factors with increasing urbanisation, and demonstrate a different organic character dependent upon processes and inputs along the reach (Westerhoff and Anning, 2000). The fluorescent signature of the water changes with increasing human impact from humic-rich (peaks *A* and *C*) to protein rich with *A*, *C*, *T* and *B* peaks (Galapate et al., 1998; Baker and Spencer, 2004). The *T* and *B* peaks are related to bacterial activity and may represent the presence of a bioavailable, labile organic substrate or the product of microbial or algal activity (Cammack et al., 2004; Nguyen et al., 2005; Elliott et al., 2006; Urban-Rich et al., 2006).

Waste waters including sewage effluents (Reynolds and Ahmad, 1997; Reynolds, 2002; Chen et al., 2003), farm wastes (Baker, 2002) and landfill leachates (Baker and Curry, 2004) have been found to be rich in microbial derived *T* and *B* fluorescence and these peaks have been used as tracers of waste waters in natural waters (Baker and Inverarity, 2004; Baker et al., 2004). Reynolds and Ahmad, (1997) determined that the sewage treatment process reduced peak *T* intensity to a much greater extent than the humic-like *A* and *C* peaks. This suggests that the *T* peak in untreated sewage, derived from

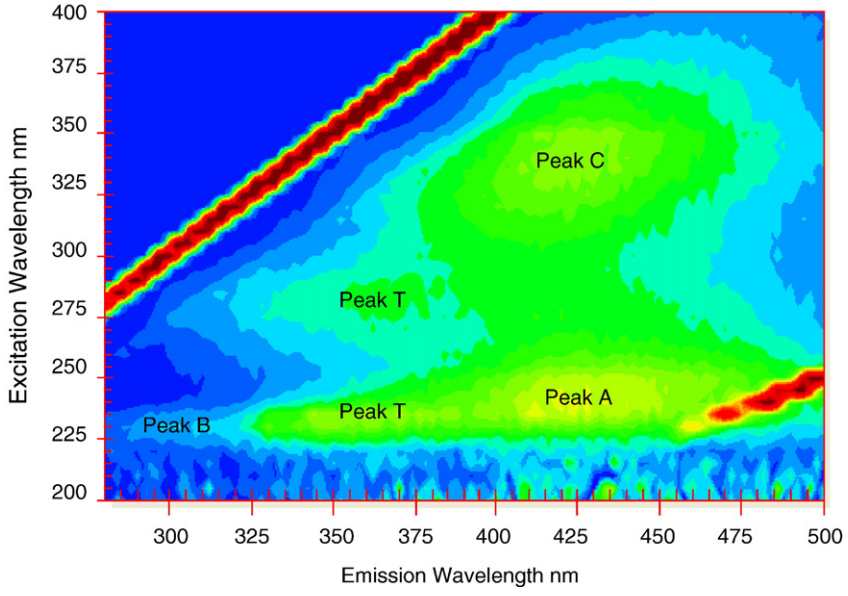

Fig. 1 – Example EEM illustrating positions of T_1, T_2, *C* and *A* peaks.

Table 1 – Summary of previous work relating fluorescence to BOD_5

Analytical parameter	Optical parameter for correlation	Correlation coefficient	Number of samples	Comments	Water type	Author
Biochemical Oxygen Demand	Fluorescence intensity at excitation 250 and 350 nm, emission 430 nm	–	c.200 samples from different sites	No relationship found. Wrong wavelength pairs examined.	Rivers and sewage and industrial effluents, U.K	(Comber et al., 1996)
Biochemical Oxygen Demand	Tryptophan (280/340 nm) (T_1)	0.94–0.97	129 samples, 3 sites	Relationship is site specific.	24 h composites of raw, settled and treated waste from 3 sewage works.	(Reynolds and Ahmad, 1997)
Biochemical Oxygen Demand	Tryptophan (248/340 nm) (T_2)	0.97	25 samples, 1 site	Up to 350 mgl^{-1} BOD. Relationship expected to be site specific.	Single sewage works.	(Ahmad and Reynolds, 1999)
Biochemical Oxygen Demand	Tryptophan (280/350 nm) (T_1)	0.96–0.99	101 samples, 2 sources		Single sewage works and a synthetic sewage	(Reynolds, 2002)
Biochemical Oxygen Demand	Tryptophan (220–230 nm/340–370 nm) (T_2)	0.94–0.98	40 samples, 3 sites	Relationship site specific.	3 Landfill leachates, U.K	(Baker and Curry, 2004)
Biochemical Oxygen Demand	Tryptophan (220/350 nm) (T_2)	0.85	434 samples, 62 sites	Samples not paired.	River Tyne catchment, N.E. England, U.K	(Baker and Inverarity, 2004)
Biochemical Oxygen Demand	Tryptophan-like fluorescence (280/350 nm) (T_1)	0.906	294 samples, from 267 sites of which 141 effluents, 124 surface waters and 2 pollution incidents	Paired samples.	River, sewage effluent and industrial effluents S.W. England, U.K	This work

anthropogenic activity, represents fresher, less degraded material with a high potential for oxidation, and that the fluorescence intensity of this peak may be used as a surrogate for the Biochemical Oxygen Demand (BOD) test.

The BOD test is a measure of the oxygen depleting potential of an organic/inorganic load in natural waters. It is a laboratory based biodegradation test that relies upon the presence of a viable microbial community that may be naturally present in the sample or artificially introduced by addition of a seed, commonly a known volume of sewage effluent of known BOD. It is carried out using standard operating procedure (HMSO, 1988) and usually is run as 5–21 day test, 5 days being the most common period (BOD_5). The concentration of biodegradable material present is calculated from the decrease in dissolved oxygen concentration over the 5 day period as labile organic material is oxidised by the microbial community. The BOD_5 test has a number of inherent difficulties associated with reliance upon the bacterial community and its sensitivity to environmental conditions. However the biggest inconvenience of the test is the 5 day test period which delays analysis of potential pollution events. For this reason, alternative techniques such as fluorescence spectroscopy, which delivers an indication of polluting load and the proportion of biodegradable material in ~1 min, as a laboratory or field test would be of great value to environmental regulators and those who discharge material to the environment.

Fluorescence spectroscopy has also been used for a number of years in the study and identification of microbial communities, either fingerprinting and characterising particular species (Seaver et al., 1998; Smith et al., 2004; Elliott et al., 2006), species identification (Gray et al., 1998; Leblanc and Dufour, 2002) or in process monitoring (Farabegoli et al., 2003; Saadi et al., 2006). These papers, and that of Cammack et al. (2004), illustrate that fluorescence in particular spectral regions is associated with microbial activity. For this reason it is considered that analysis of the fluorescence intensity of the tryptophan-like region can be used to indicate the size and activity of the microbial community. As the BOD test is a microbial based assessment of polluting load, there should be a correlation between "microbial" tryptophan-like fluorescence and absolute BOD measurement.

This paper will assess the potential of fluorescence spectroscopy for use in water quality assessment by studying the relationship between fluorescence intensities and the BOD_5 values for a range of surface waters and various effluents from South West England.

2. Materials and methods

2.1. *Sample collection*

Surface waters, sewage and industrial effluents and samples of pollution incidents were collected from South West England (area ~1700 km^2) for water quality analysis by the Environment Agency, Starcross, Exeter, UK. The samples were transported to the Environment Agency laboratory and stored under refrigerated conditions (c. 4 °C). BOD_5 analysis was performed within 24 h of collection. On the day BOD_5 analysis commenced, sub samples (50 ml) were decanted and sent to the University of Birmingham for fluorescence analysis. In this

paper the analysis of BOD_5 and fluorescence on portions of the same original sample will be referred to as "paired". The sub sampling was undertaken on 13 occasions over 12 months (March 2005–February 2006) typically with 25 effluent and surface water samples each occasion. Samples were transported to the University in HDPE bottles, previously washed in 10% HCL and rinsed with distilled water, and were stored in the dark under refrigerated conditions throughout transport and until analysis to reduce possible changes in sample character through microbial or photo degradation. Fluorescence analysis was performed within 24 h of sample receipt at Birmingham University, which is within 48 h of the start of BOD_5 analysis. Samples were not filtered prior to fluorescence analysis in order that fluorescence data could be directly related to BOD_5 results for which samples are not filtered. To reduce the influence of scattering by particles fluorescence analysis was performed on settled samples.

2.2. *Biochemical Oxygen Demand (BOD_5)*

BOD_5 was analysed by the Environment Agency using the method described in the HMSO (1988). Dilution waters were seeded with a known quantity of treated sewage effluent with a known BOD_5. For all samples, seeding was conducted after a sub sample had been removed for fluorescence analysis.

2.3. *Fluorescence spectroscopy*

An EEM was generated for each sample using a Varian Cary Eclipse fluorescence spectrometer at 20 °C. Excitation and emission were scanned simultaneously at wavelengths from 200–400 nm and 280–500 nm respectively at 5 nm intervals, with a 5 nm slit width at 9600 nm/min scan rate. The position (excitation and emission wavelength) and maximum fluorescence intensity in fluorescence units (AFU) of each peak was recorded. No post-manufacturer instrument corrections were applied as the same instrument was used for all analyses making results comparable and such corrections are only usually applied to make data comparable between instruments. The Raman line of water at excitation wavelength 348 nm was used as a standard to monitor instrumental drift and results are normalised to an intensity of 20 U.

Samples were also analysed for fluorescence using an SMF-2 portable fluorimeter (Safe Training Systems, U.K). The SMF-2 uses a xenon flash light lamp. Excitation is targeted at 260 nm through the use of an interference filter with 85% transmission in the 260 nm region and zero transmission at 350 nm. A cut off filter is used to selectively advance the emitted light at wavelengths which have been identified as most appropriate for the application. The intensity of the fluorescence is measured by 250 detectors following a stationary grating. The SMF-2 is proposed as a field measurement device for identifying anthropogenic pollution.

2.4. *Total Organic Carbon (TOC)*

For comparative reasons total organic carbon was determined for all samples. Undiluted samples were analysed for both total carbon and inorganic carbon and the total organic carbon then calculated. For this a Shimadzu TOC-Vcpn analyser was used throughout. Total carbon was analysed by combustion of the sample at 680 °C with a platinized alumina catalyst and the resulting CO_2 production measured. Total inorganic carbon was analysed by phosphoric acid digestion combined with CO_2 determination. From these tests the total organic carbon was calculated using total carbon — total inorganic carbon. The instrument was calibrated using total carbon and inorganic carbon 1 molar standards and for each analysis the mean of 3 measurements was used. TOC data is briefly presented in this paper, as a common analytical technique, for comparison with BOD only.

2.5. *Sample dilution*

Samples with high fluorescence intensities (>c. 400 IU) upon initial analysis, or which were visibly turbid, were diluted with laboratory distilled water (upon which fluorescence analysis had been previously carried out) until fluorescence intensities were measurable. Fig. 2 shows that the fluorescence intensities of tryptophan-like fluorescence at excitation and emission wavelengths 275/340 nm demonstrate a quasi-linear relationship with BOD_5 upon dilution. This demonstrates that no step change in response occurred with increasing dilution, suggesting that minimal inner filtering (reabsorption of emitted energy by surrounding molecules in concentrated solutions) was observed even at high concentrations. However, to standardize the approach to inner filtering the data presented is from samples with minimal or no dilution and any errors as a result of this phenomenon are incorporated within the error of the relationships observed.

3. Results

469 samples (246 "surface waters" and 223 "effluents", comprising sewage effluents, trade effluents and samples from pollution events of unknown origin) were analysed for fluorescence and BOD_5.

Peaks *T* and *B* have two emission peaks. Here peak *T* will be referred to as T_1 or T_2 (T_1 $\lambda_{ex/em}$ 280/350 nm, T_2 $\lambda_{ex/em}$ 215–220/340 nm) and peak *B* results are excluded from this study as it was rarely observed in these samples. Table 2 illustrates the general fluorescence character of i) all samples ii) surface water samples and iii) effluent samples. Surface waters contained humic-like material with peak *A* ($\lambda_{ex/em}$ 260/380–460 nm) being more intense than peak *C* ($\lambda_{ex/em}$ 380/420–480 nm). Peaks T_1 and T_2 were commonly present and varied in fluorescence intensities. T_2 fluorescence intensities were always greater than T_1 (average ratio 2.94). Throughout this study only 2 surface water samples required dilution. Sewage effluents contained peaks T_1 and T_2 at greater intensities than surface waters with T_2 intensity exceeding that of T_1 (average ratio 1.87). In some samples peak *A* was obscured by the fluorescence of peak T_2 only becoming measurable as a peak after dilution. In total 15 sewage samples required dilution prior to fluorescence analysis.

The fluorescence intensities (AFU) of each peak were correlated with BOD_5 (mgL^{-1}). Samples with oxygen depletion less than 1 mg L^{-1} over 5 days are quoted by the Environment Agency as a "<" BOD_5 value and have no true numerical value

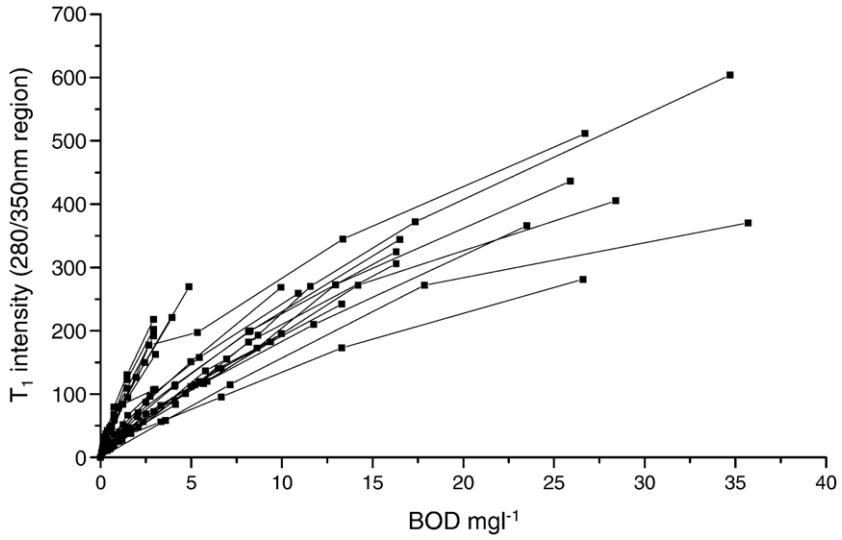

Fig. 2 – T_1 fluorescence intensity plotted against BOD_5 as a dilution series where more than 4 dilution stages undertaken.

with which to correlate fluorescence intensity. In essence such samples exhibit a zero or near zero biochemical oxygen demand as determined by the standard five day BOD test. For clarity correlation between the fluorescence intensities and BOD_5 data for all samples has been performed excluding the BOD_5 values of "<". By excluding these values the number of presented samples is reduced to 294 (135 surface waters and 159 effluents and pollution incidents). Site identification data, dilution factor, fluorescence intensity, TOC and BOD values are shown for these sites in Supplementary Table 1.

Table 3 illustrates the relationships with BOD_5 and TOC for all peak fluorescence intensities for all samples and surface water and effluent subsets (excluding "<" values). Note all correlation coefficients "r" are non-parametric (Spearmann's Rho) as the BOD_5, TOC and fluorescence data do not conform to a normal distribution and so a ranked correlation is the most appropriate method of statistical analysis. Correlation coefficients (rho "r") quoted are linear, however in Figs. 3–5 the data is represented on log/log graphs as this more clearly illustrates the distribution of data.

For the whole dataset, peaks T_1 and T_2 always show a stronger correlation with BOD_5 ($r=0.906$ and 0.848 respectively) than the humic-like C and A peaks ($r=0.771$ and 0.720 respectively) and the relationship between BOD_5 and T_1 is always strongest. BOD_5 correlates less well with TOC ($r=0.782$) than with T_1 and T_2 fluorescence intensities.

TOC shows a strong correlation with peaks C and A ($r=0.870$ and 0.808 respectively); the strongest correlation is with T_1 ($r=0.876$), and the correlation with T_2 is the least strong ($r=0.802$). This suggests that fluorescence may be a good indicator of TOC, however T_1 or T_2 fluorescence is a better indicator of BOD_5 than TOC. The TOC and T_1 relationship is not

Table 2 – Total dataset sample fluorescence data (i), surface water samples fluorescence data (ii) and effluent sample fluorescence data (iii)

Fluorophore	Coble (1)	Name in this study	Excitation (nm)				Emission (nm)				Intensity (IU)			
			Min	Max	Mean	S.D	Min	Max	Mean	S.D	Min	Max	Mean	S.D
i) All samples														
Tryptophan-like	T	T_1	275	296	282	3	330	378	353	8	6	2424	137	178
Tryptophan-like	T	T_2	216	247	233	4	329	378	356	9	15	7327	281	474
Humic-like	C	C	304	347	334	6	405	461	423	7	14	1632	194	168
Humic-like	A	A	217	261	238	7	395	449	424	9	47	4966	313	318
ii) Surface water Only														
Tryptophan-like	T	T_1	275	288	281	2	339	373	351	5	6	557	41	55
Tryptophan-like	T	T_2	216	239	231	4	329	377	356	9	15	1547	109	135
Humic-like	C	C	304	347	332	6	408	461	424	8	14	760	93	79
Humic-like	A	A	222	261	236	5	401	449	425	9	47	2189	192	176
iii) Effluent only														
Tryptophan-like	T	T_1	276	296	283	3	330	378	356	10	15	2424	244	204
Tryptophan-like	T	T_2	221	247	235	3	331	378	356	9	51	7327	472	620
Humic-like	C	C	318	347	336	5	405	445	422	5	40	1632	306	169
Humic-like	A	A	217	253	241	7	395	443	423	9	71	4966	446	380

Table 3 – The relationship between all fluorophores, TOC and BOD_5 excluding "<" values

	Tryptophan-like (T_1) 280/350 nm	Tryptophan-like (T_2) 225–237/340–381 nm	Humic-like (C) 300–370/400–500 nm	Humic-like (A) 237–260/400–500 nm	Total organic carbon (TOC) mgL^{-1}
Correlation with BOD_5 mgL^{-1}					
All samples	0.906	0.848	0.771	0.720	0.782
Surface water	0.612	0.532	0.315	0.316	0.188
Effluent	0.714	0.472	0.341	0.331	0.516
Correlation with TOC mgL^{-1}					
All samples	0.876	0.802	0.870	0.808	–
Surface water	0.457	0.401	0.507	0.534	–
Effluent	0.768	0.502	0.749	0.618	–

improved by dividing the data into surface water and effluent subsets.

Fig. 3 shows a log/log plot of the correlation between BOD_5 and T_1 (again excluding "<" values). Table 3 illustrates that the relationship between BOD_5 and T_1 is also not strengthened by dividing the data into surface water and effluent subsets. Figs. 4 and 5 demonstrate the relationships between BOD_5 and T_1 for surface waters and effluents respectively (excluding "<" values). The effluent subset correlation is influenced by a number of samples which demonstrate the presence of organic material with high fluorescence intensity and low biodegradability (BOD_5). These samples may be better investigated using the Chemical Oxygen Demand (COD) test, although this is not addressed by this work.

Samples were analysed for T_1 fluorescence using an SMF-2 portable spectrofluorimeter manufactured by Safe Training Systems Ltd. Fluorescence intensity measured by the SMF-2 correlates well with the T_1 fluorescence intensity measured using the Cary spectrophotometer ($r=0.974$) for the total dataset similar to the relationship found by Baker et al. (2004) of $r=0.91$. The relationship between the two instruments for surface water samples is $r=0.868$ and for effluents is $r=0.932$. When correlated with BOD_5 values the SMF-2 displays a good correlation for the total dataset ($r=0.850$), while the correlation with sample subsets are less good with surface waters ($r=0.397$) and effluents ($r=0.573$) respectively. This is in line with results from the Cary Eclipse.

4. Discussion

4.1. *Reasons for a T_1/BOD_5 relationship*

Microbial activity, measured by oxygen depletion in the BOD_5 test, is thought to relate to fluorescence peak *T*, either because peak *T* is present in a bioavailable substrate, or because peak *T* is produced by microbial action whilst it is using a bioavailable organic fraction. Cammack et al. (2004) proposed that observation of the *T* peaks is actually an observation of the balance of decline in substrate and increase in community. Elliott et al. (2006) show that the *T* peaks intensities increase with colony forming units for a riverine microbial community. Interestingly, strong correlations have also been found between peak *T* fluorescence and COD (Lee and Ahn, 2004). In contrast, humic-

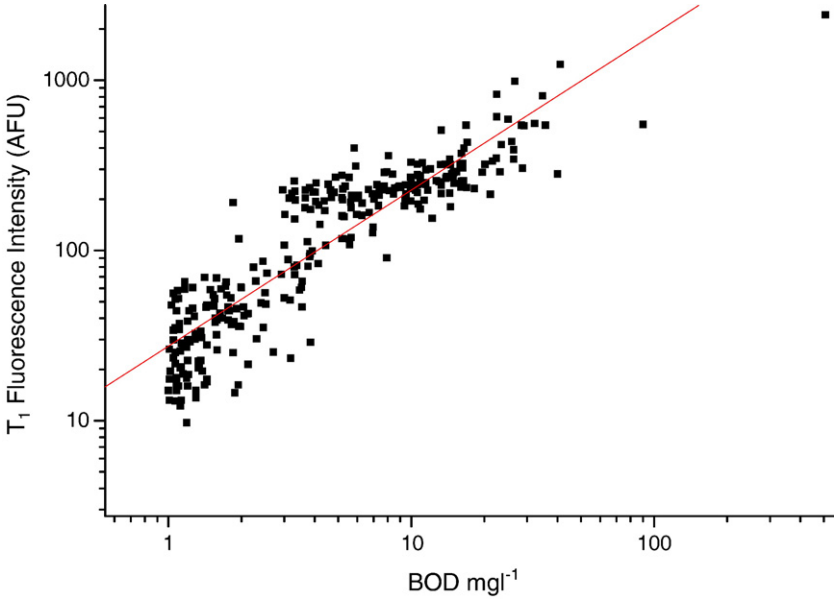

Fig. 3 – Relationship between Cary Eclipse T_1 fluorescence intensity and BOD_5 for all samples excluding "<" values, shown on log/log scale for clarity. Linear regression (Y = 1.44 + 0.92 * X).

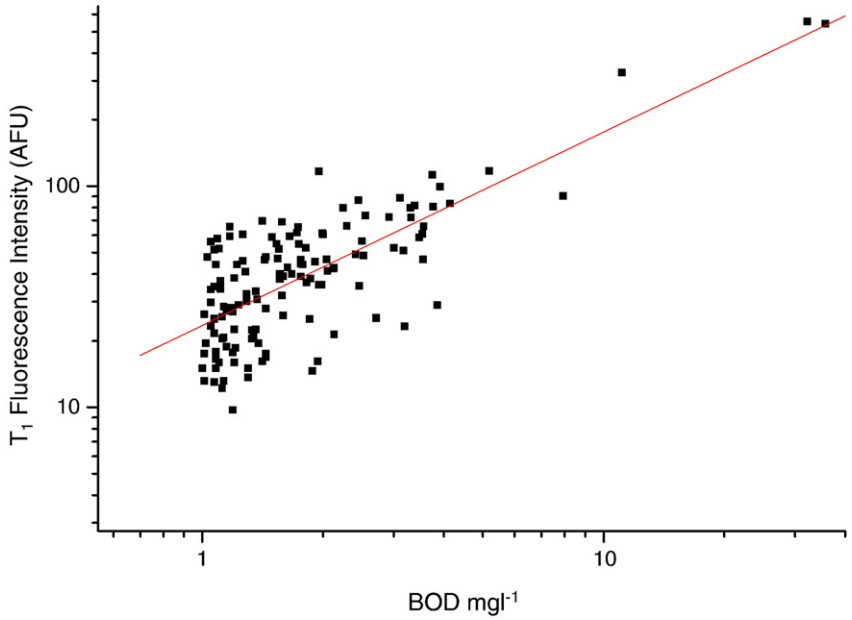

Fig. 4 – Relationship between Cary Eclipse T_1 fluorescence intensity and BOD_5 for surface waters excluding "<" values shown on log/log scale for clarity. Linear regression (Y = 1.37 + 0.87 * X).

like material relates poorly to the BOD_5 test, indicating that it is not readily available to the bacterial community and therefore is more stable and not easily degraded.

4.2. *Geographical variability in the T_1/BOD_5 relationship*

To demonstrate a strong correlation between T_1 fluorescence and BOD_5 for a large dataset of such geographically diverse samples it is necessary to include both the high and low end members of the dataset. In subdividing the data into sample types the relationship, although statistically significant, begins to decrease as site specific factors, microbial community and BOD_5 method error (quoted as 20%) begin to exert an influence. However, there are no sites from which sufficient replications were analysed to directly determine the effect of site influenced variability. An improvement in the relationship after splitting the dataset into water types would suggest heterogeneity of sample character from site to site. However, as this work represents a natural system, from a range of geographical locations and seasons, there is enormous

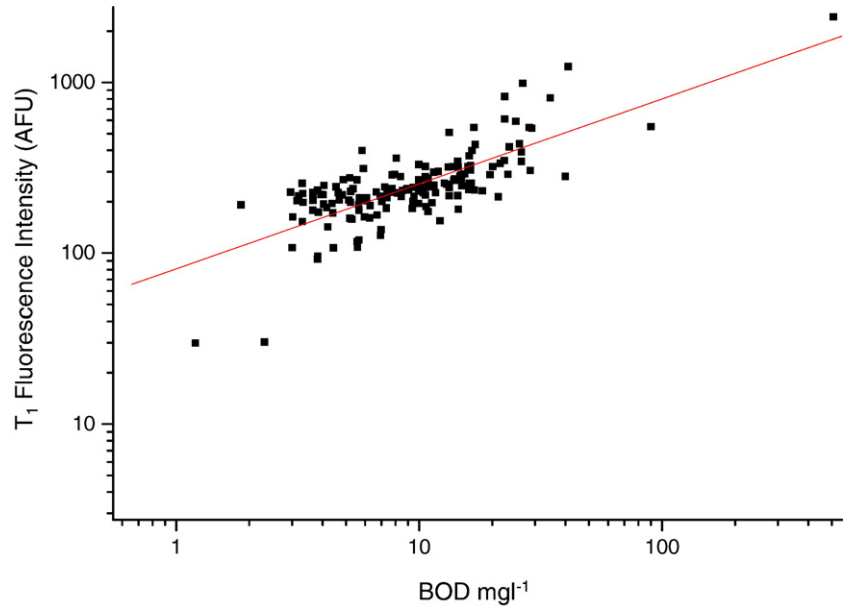

Fig. 5 – Relationship between Cary Eclipse T_1 fluorescence intensity and BOD_5 for effluents excluding "<" values shown on log/log scale for clarity. Linear regression (Y = 1.91 + 0.5 * X).

character variability in both the surface water and effluent datasets. Surface waters are subject to geological and climatic variation, agricultural and anthropogenic inputs whilst wastewater effluents are subject to variations in character determined by treatment specific impacts.

Relationships were further investigated using Geographically Weighted Regression (GWR) (Brunsdon et al., 1996). Preliminary assessment of the GWR between surface water T_1 and BOD_5 indicates that strong relationships (R^2) exist between geographically proximate samples. GWR relates the relationship between the parameters in question for a group of sample sites that occur within a specified region area, in this case 14 km^2 which has been calculated to be the optimum bandwidth for this dataset. The GWR method determines the relationship between all sites within 14 km of each other site, in all directions by weighting local regression models using a moving window filter. The model for this relationship is $BOD = a(x,y) + b(x,y)^* T_1 + \text{error}$ where (x,y) is geographical location and error is a normally distributed error term.

Fig. 6 shows the regression coefficient (R^2) of the T_1/BOD_5 relationship. In this figure the shade of the sample marker indicates the strength of the relationship by site. This figure illustrates that, within a geographical region, sites demonstrate individual T_1/BOD_5 relationships and that, locally, the correlation between BOD and T_1 may be stronger than that observed in the whole dataset. GWR suggests that the development of site specific and geographical T_1/BOD_5 relationships would further clarify and support the application of fluorescence in surface water quality assessment.

4.3. *Low BOD_5 samples*

The relationship between BOD_5 and fluorescence for very low BOD_5 samples is difficult to determine as BOD_5 is commonly reported as "<1". In this instance the study of fluorescence EEMs is more informative of the nature of the organic material than BOD_5, giving an indication of the types of material present (humic-like or "fresh" microbial derived) and the relative proportions of each type. In addition, analysis of the T_1 peak fluorescence intensities which correlate well with the biodegradable fraction of organic matter present, may develop an understanding of the proportion of more bioavailable material present, to very low concentrations, and the potential oxygen depleting potential of this load in natural waters.

4.4. *Comparison with previous work*

This study incorporates a greater number of samples with paired BOD_5 and fluorescence data with greater geographical diversity than previous work on the subject. Table 1 lists the details of previous attempts to relate fluorescence to BOD_5, many of which use synthetic wastewaters, small sample sizes, and unpaired analysis, and the correlations from these studies. Despite the great variation inherent in this data set a good correlation between fluorescence and BOD_5 is demonstrated for the T_1 peak. The relationship found in this study is clearly of similar significance to previous studies, despite the large number of samples, their geographical distribution and the range of sample types being significantly greater than in previous studies.

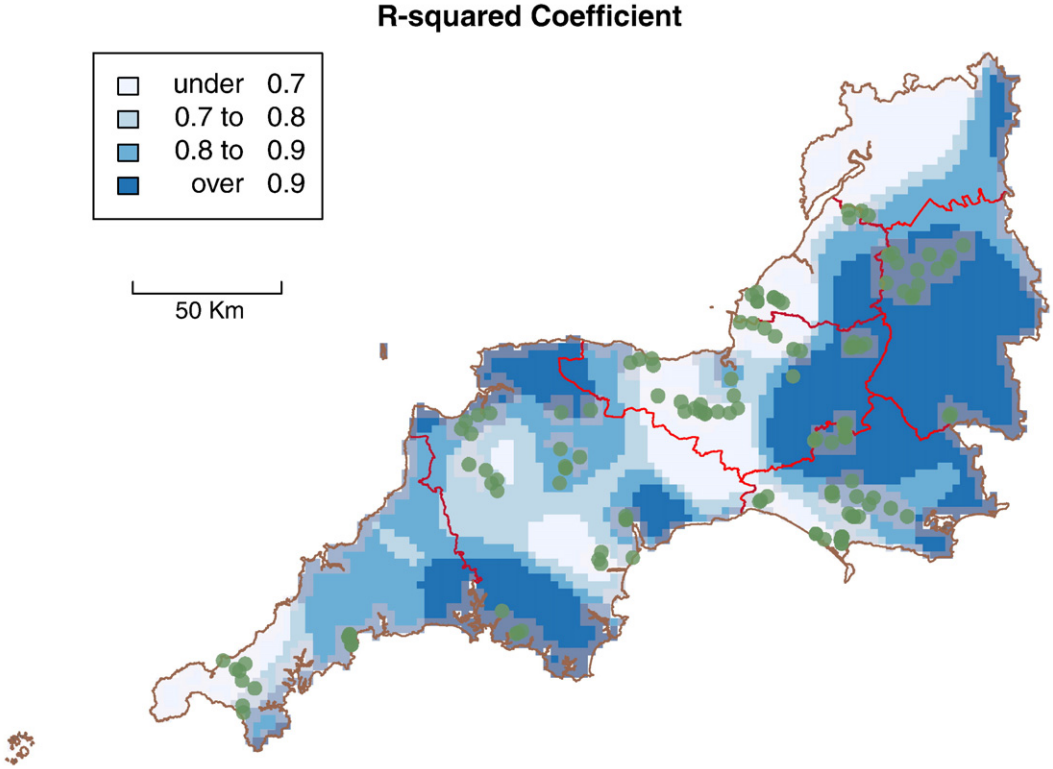

Fig. 6 – Map showing relationship (R^2) between T_1 and BOD_5 for surface water samples using geographically weighted regression (GWR).

5. Conclusions

Fluorescence spectroscopy has the potential to be a powerful laboratory or field tool in the measurement and characterisation of bioavailable organic matter in water, with particular application in effluent quality monitoring, pollution event investigation and regular on-site testing. The SMF-2 is capable of field measurement of BOD_5 equivalent.

The relationship between fluorescence and BOD_5 has been shown to be strong for a highly variable set of samples. The application of GWR to surface water analysis shows that strong local relationships can be found and that, with a catchment specific correlation, fluorescence can be used in water quality assessment. Further work is required to develop a greater understanding of local sample heterogeneity, the importance of geographical/spatial influences on sample character, and other factors which may influence the strength of the correlation.

For samples of low BOD_5, fluorescence analysis of sample fluorescence could more accurately indicate of the type of organic material present (natural or anthropogenic), the relative proportions of these different types, and could give an understanding of the proportion of more bioavailable or labile material and its' oxygen depleting potential in natural water.

To make the most of the technique it is necessary to consider fluorescence spectroscopy as a more accurate and flexible indicator of bioavailability than BOD_5. To maximise its potential as an analytical tool it should be "un-coupled" from BOD_5 and, instead, used as an independent indicator test for bioavailable organic matter presence, associated biological activity and oxidising potential with associated impacts on water quality.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.scitotenv.2007.10.054.

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