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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

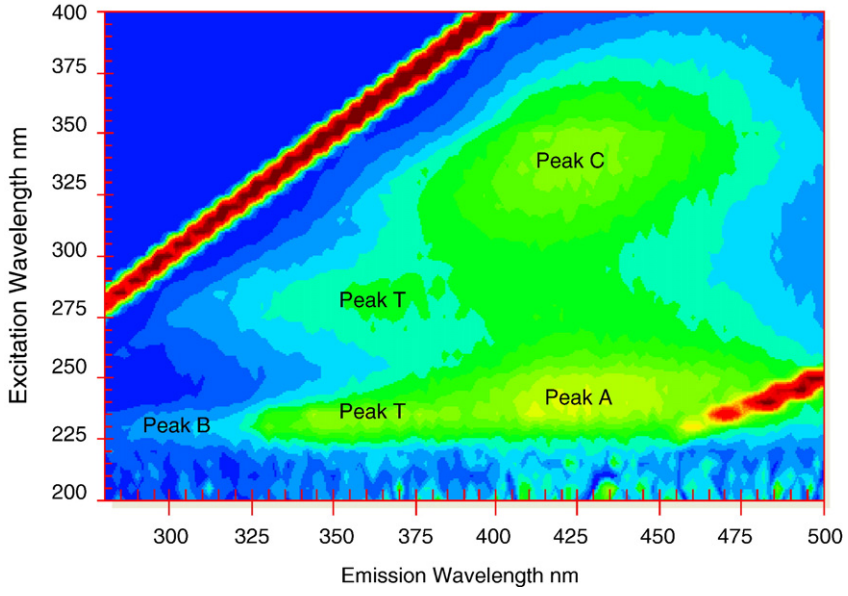

Fig. 1 – Example EEM illustrating positions of T_1, T_2, *C* and *A* peaks.