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Measurement of protein-like fluorescence in river and waste water using a handheld spectrophotometer

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Abstract

Protein-like fluorescence intensity in rivers increases with increasing anthropogenic DOM inputs from sewerage and farm wastes. Here, a portable luminescence spectrophotometer was used to investigate if this technology could be used to provide both field scientists with a rapid pollution monitoring tool and process control engineers with a portable waste water monitoring device, through the measurement of river and waste water tryptophan-like fluorescence from a range of rivers in NE England and from effluents from within two waste water treatment plants. The portable spectrophotometer determined that waste waters and sewerage effluents had the highest tryptophan-like fluorescence intensity, urban streams had an intermediate tryptophan-like fluorescence intensity, and the upstream river samples of good water quality the lowest tryptophan-like fluorescence intensity. Replicate samples demonstrated that fluorescence intensity is reproducible to $\pm 20\%$ for low fluorescence, 'clean' river water samples and $\pm 5\%$ for urban water and waste waters. Correlations between fluorescence measured by the portable spectrophotometer with a conventional bench machine were 0.91; (Spearman's rho, $n = 143$), demonstrating that the portable spectrophotometer does correlate with tryptophan-like fluorescence intensity measured using the bench spectrophotometer.

Keywords: Fluorescence; Waste water; River water quality; Sewage; Luminescence; Spectrophotometry

1. Introduction

River and waste waters have distinctive spectrophotometric properties in terms of both absorption of light and fluorescence. As well as strong absorption in the ultra-violet region, much dissolved organic matter (DOM) present in river and waste water fluoresces [19,2]. Recent advances in fluorescent spectrophotometry have permitted the rapid (~1 min) detection of DOM fluorescence at a wide range of both excitation and emission wavelengths to produce an excitation–emission matrix or EEM. An EEM will typically cover a range of excitation and emission wavelengths from ~200 nm (short wavelength UV) through to ~500 nm (visible blue–green light), and may contain fluorescence centres that are attributed to both natural DOM groups such as humic and fulvic-like material, as well as fluorescent proteins [2,3]. Studies of DOM EEM fluorescence properties have included waste water characterisation within the treatment process [1,4,5], DOM characterisation in marine and estuarine waters [3,6–9], and riverine DOM fingerprinting [2,3,10–16].

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Previous research [2,11] has demonstrated that protein-like fluorescence intensity is increased with increasing anthropogenic DOM inputs from sewerage and farm wastes. Protein-like fluorescence centres observed in EEMs occur at the same locations in optical space as tryptophan and tyrosine standard solutions, and are therefore classified as tryptophan and tyrosine-like. Tryptophan-like fluorescence centres occur at two wavelength pairs—220 nm excitation/350 nm emission and 280 nm excitation/350 nm emission, whereas tyrosine-like is predominantly observed at wavelengths of 220 nm excitation/305 nm emission (a second centre at 280 nm excitation is obscured by the Raman line of water). All farm wastes exhibited high intensities of tryptophan-like fluorescence. Silage liquor was characterised by a very high tryptophan-like fluorescence intensity and an initial tryptophan-like : fulvic-like fluorescence intensity ratio of greater than 20–1. Cattle and pig slurries exhibited a lower tryptophan-like: fulvic-like fluorescence intensity ratio ($\sim$2–5) and lower tryptophan-like fluorescence intensity. The ratios of tryptophan-like: fulvic-like fluorescence intensity observed from the farm wastes investigated are significantly higher than those observed in river waters of 'good' chemical water quality (as defined by the England and Wales Environment Agency), suggesting that farm waste pollution events could leave a signature in river waters due to their distinctively high tryptophan-like fluorescence intensity. Most recently, Baker et al. [17] demonstrate that fluorescence spectrophotometry can be used in environmental monitoring programs through the detection of sewerage pollution events in rivers.

Therefore fluorescence can detect river pollution derived from fluorescent organic matter, in particular tryptophan-like fluorescence found in farm and human wastes. However, this is somewhat limited by the necessity to return samples to the laboratory for analysis which is both time consuming and can lead to a degradation of water samples prior to analysis. A portable field-based spectrophotometer, coupled with the rapidity of the technique (less than 1 min to obtain a result) would provide field scientists with a rapid pollution monitoring tool, and process control engineers with a portable waste water-monitoring device. Recent technological advances now make this possible, and here we present the first results of river and waste water fluorescence from a range of rivers in NE England and from effluents from within two waste water treatment plants, using a portable spectrophotometer.

2. Methods

River water samples were collected from a range of rivers in NE England, reflecting the widest range of water quality on the rivers, as part of ongoing research programs. The rivers include the River Tyne, a large catchment of 2935 km^2 from which waters were sampled in July 2003 from upstream sites with water quality that is classified as 'good' or 'very good' in the Environment Agency General Quality Assessment of 2002. Several small urban catchments (River Don, Seaton Burn and Ouseburn) were also sampled in the sample month. All are impacted by sewerage problems from failing combined sewerage overflows (CSOs) and cross connected sewers (CCSs), [17] and have water quality that is classified as 'average' to 'poor' quality. On the Seaton Burn, water samples were also taken of sewerage effluents. One CCS and one failing CSO was sampled repeatedly over the course of two weeks in July 2003. Finally, waste water samples were taken from within two waste water treatment plants in NE England. The sites had primary and secondary treatment. The secondary treatment was aerated sludge reactor in one case and trickling filter in the other. Samples were taken throughout the treatment process from influent through to effluent.

Samples were collected in the field using cleaned (in 10% HCl and distilled deionised water) glass or polypropylene sample bottles, and a subsample taken for analysis using the portable SMF2 spectrophotometer (SafeTrainingSystems Ltd, Wokingham, UK). The SMF2 has a xenon flash lamp as excitation source, with the flash focused through a bandpass filter to select the required excitation wavelength. For tryptophan, a combination of three interference filters was used with a peak excitation wavelength of 280 nm and a full-width half-maximum of 60 nm. No cut off filter was used as the 280 excitation peak was well separated from the 350–360 nm measuring area for the fluorescence signal. The SMF2 has a spectral display on the instrument which allows the operator to observe the peak shape of the tryptophan-like fluorescence centre and to observe any other unexpected fluorescence which could affect the quantification of the tryptophan-like fluorescence. The instrument also has incorporated a 9 V rechargeable battery which allows 4–8 h of operation. A standard 1 cm quartz cuvette was used for the water samples and sets of 3 repeat analyses made for each sample. Calibration samples were run before the start of any analysis of water samples by analyzing distilled water. For these samples the intensity measured was greater than zero as it includes a contribution from Raman emission within the range of the filter sets used. Analyses were adjusted to a constant intensity of 22 units, and this value subtracted from subsequent water analyses.

The remaining sample was returned to the laboratory and analysed within 24 h (and usually within 3 h) using a bench Varian Cary Eclipse luminescence spectrophotometer using published methods [2]. Fluorescence was excited from 200 to 370 nm and emission detected from

280 to 500 nm with slits set to 5 nm and a scan speed of 9600 nm/min. Calibration samples were regularly taken using distilled water and measuring the Raman intensity at 348 nm excitation wavelength: results were adjusted to a value to 20 intensity units. Using the EEMs generated, tryptophan-like fluorescence intensity was measured at both of its fluorescence centres (at 220 and 280 nm excitation wavelengths and 350 nm emission wavelength).

For waste water samples, the sample was diluted by $\times 5$ to $\times 100$ with distilled deionised water before analysis with either spectrophotometer to avoid the effects of inner-filtering (where with high concentrations of organic matter some fluorescence is reabsorbed within the cuvette; Ohno [18]). The amount of dilution required had been previously determined by serial dilutions performed on samples from each of the waste water treatment works sample sites, using the bench spectrophotometer to determine a dilution amount such that the fluorescence intensity–concentration relationship was linear and above detection limit.

3. Results

Typical fluorescence EEMs are shown in Fig. 1, and the results for both spectrophotometers are presented in Fig. 2. The tryptophan-like fluorescence measured in river and waste water by the portable SMF2 spectrophotometer ranges from 20 to 10,000 intensity units, with waste waters and sewerage effluents having the highest fluorescence intensity, urban streams have intermediate fluorescence intensity, and the upstream River Tyne samples the lowest fluorescence intensity. These results are as might be expected from previous studies which suggest that tryptophan-like fluorescence intensity increases with waste water concentration. Replicate samples demonstrated that the SMF2 fluorescence intensity is reproducible to ± 3 units: for low fluorescence intensity, good quality river waters this amounts to $\sim 20\%$ error, whereas for urban rivers and waste waters it is a $\sim 5\%$ error. Error bars on the waste water samples are derived from this error but are larger due to the multiplication of any error term due to the dilutions used.

Comparative results from the bench Cary Eclipse spectrophotometer demonstrates that samples exhibit a similar range of tryptophan-like fluorescence intensities from 10 to 100,000 intensity units, and that the river and waste water sites have a similar distribution of fluorescence intensities. Replicate samples demonstrated that fluorescence intensities duplicated within ± 10 intensity units, given an error of $\sim 30\%$ in the cleanest samples and $< 5\%$ for urban river and waste water samples.

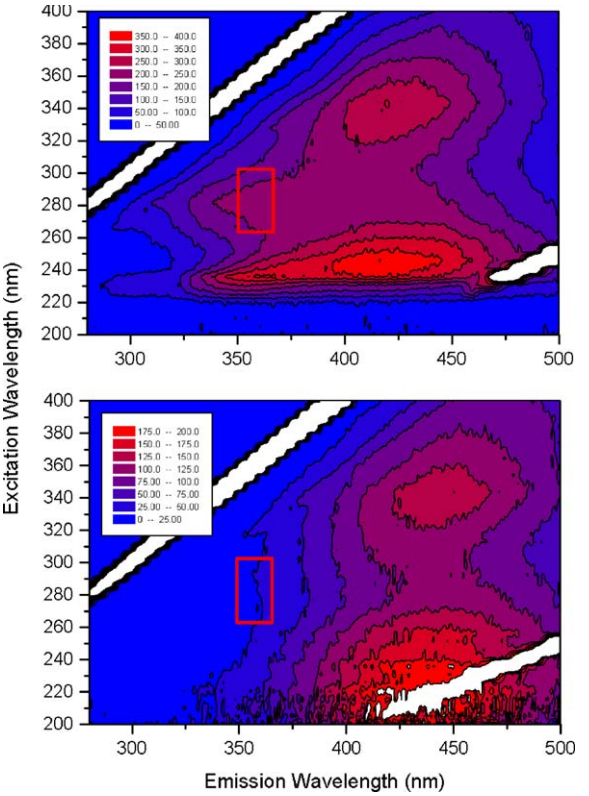

Fig. 1. Typical river water excitation emission matrices. Top: Sample downstream of a secondary treated waste water discharge. Bottom: Sample from a good water quality site on the river Tyne. The area of analysis of the portable SMF2 spectrophotometer is shown by the red box.

The fluorescence intensities measured on the portable SMF-2 with both the 220 nm and 280 nm tryptophan-like fluorescence centres determined on the bench Cary Eclipse spectrophotometer were correlated. Only the correlation with the 220 nm centre is shown in Fig. 2, although the correlation with the 280 nm centre is equally strong. The correlation between the two machines for the whole data set is 0.91; (Spearman's rho due to non-normal data distribution, $n = 143$). If just the river and sewerage (CSO, CCS) samples are considered (to remove any possible leverage effect of the high fluorescence intensity waste water samples on the correlation coefficient), $r = 0.87$ (Pearson's $r, n = 126$), and on the river water samples alone $r = 0.86$ (Person's $r, n = 91$). These results demonstrate that the portable spectrophotometer does correlate with tryptophan-like fluorescence intensity measured using the bench spectrophotometer, and suggests that it can be used to determine both river and waste water tryptophan-like fluorescence intensity.

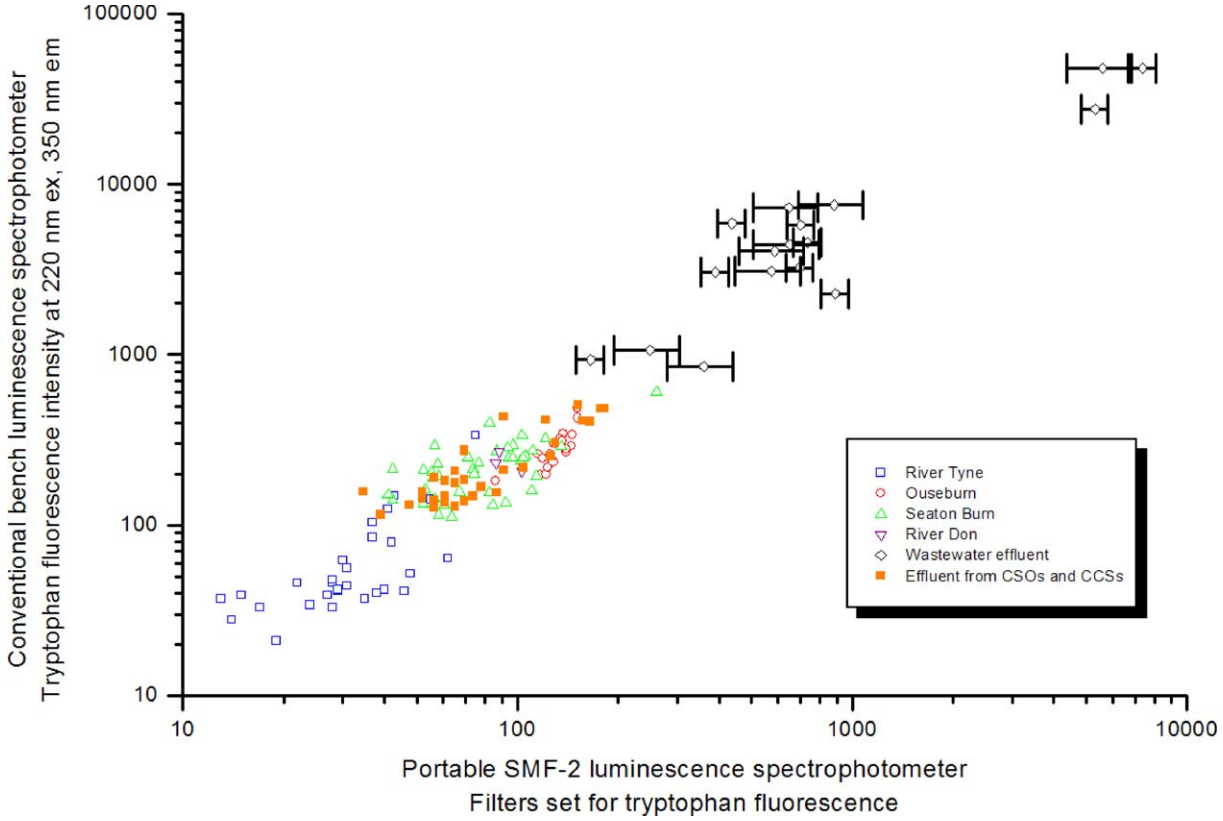

Fig. 2. Comparison of fluorescence intensity measured by the two spectrophotometers for river and waste water samples.

4. Conclusions

Portable spectrophotometric detection of tryptophan-like fluorescence in river and waste waters is now possible. Portability has widespread applications in the water industry. Tryptophan-like fluorescence is widely associated [2,11] with pollution from human and animal wastes: a field based spectrophotometer permits the in-situ sourcing of pollution and rapid remediation. In our study, samples with fluorescence intensity of >100 on the Cary Eclipse and >60 on the SMF-2 are all from rivers that have water quality issues (defined as failing their chemical water quality targets as defined by the Environment Agency of England and Wales) or from waste water samples. Within process control, portable spectrophotometry can be used to investigate variations in waste water quality at different stages of the treatment process. These samples were the more time consuming to analyse, as these required a dilution step. Further research is needed to investigate a wider range of applications using different filter sets to determine other fluorescent organic matter of interest to water science. Tryptophan-like fluorescence, as reported in this paper, was the first field application to be investigated for the portable spectrophotometer, but it is likely that many other applications may be developed, for instance, polycyclic aromatic hydrocarbons (PAHs) are routinely measured by laboratory-based instruments and long wavelength ultra violet excitation, but direct field measurement with short wavelength ultra violet excitation may have an advantage.

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