RIVER RESEARCH AND APPLICATIONS

*River. Res. Applic.* **23**: 631–649 (2007)

Published online 3 April 2007 in Wiley InterScience
(www.interscience.wiley.com) DOI: 10.1002/rra.1005

# FLUORESCENCE ANALYSIS OF DISSOLVED ORGANIC MATTER IN NATURAL, WASTE AND POLLUTED WATERS—A REVIEW

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## ABSTRACT

Dissolved organic matter (DOM) in aquatic systems originates from a range of sources. Some is allochthonous, transported from the surrounding landscape to the water body, and is derived from and influenced by the geology, land use and hydrology of its origin. Some is created *in situ* through microbial activity, which may provide an independent source of organic matter, or a recycling mechanism for that which has been transported into the water body. The relative contribution of each source depends upon the location and environmental conditions within and without the water body. Human activity is also a source of DOM, much of which is believed to be labile, which can enter the aquatic system through direct point discharges, diffuse leaching and aerial dispersal. Fluorescence spectroscopy can provide an excellent tool to source DOM fractions, and to monitor and understand DOM transformations in aquatic systems, as much DOM has an intrinsic fluorescence. In particular, recent advances in optical technology, enabling rapid investigation of shorter wavelengths, have enabled more detailed characterization of organic material and its reactions in water. In this article, we review the use of fluorescence spectroscopic techniques to measure the intrinsic fluorescence of organic matter and the application of fluorescent DOM analysis in marine waters, freshwaters and wastewaters. Copyright © 2007 John Wiley & Sons, Ltd.

KEY WORDS: fluorescence spectroscopy; dissolved organic matter (DOM); review

*Received 22 March 2006; Revised 24 October 2006; Accepted 15 November 2006*

## INTRODUCTION

In the last 50 years, fluorescence has been used extensively in the water sciences. It has been applied to the investigation of the composition, concentration, distribution and dynamics of organic matter from various sources in a range of aquatic environments. In natural waters organic matter exists in dissolved, colloidal and particulate states with dissolved organic matter (DOM) being the most studied fraction, although some emphasis has been placed upon the colloidal fraction and its importance in water chemistry (Mopper *et al*., 1996; Mounier *et al*., 1999; Patel-Sorrentino *et al*., 2002). DOM may originate from a range of sources. Some is transported to the hydrological system and is derived from and influenced by the geology, land-use and hydrology of its origin. Some is created *in situ* through microbial activity which may be an independent source of organic matter or a recycling mechanism for that which is transported to the system. The contribution of each depends upon the location and environmental conditions within and without the water body. Of course, human activity is also a vast source of DOM much of which is believed to be labile, which enters water through direct discharge, indirect leaching into groundwater and aerial dispersal. DOM source and character have been extensively investigated (Coble, 1996; Mounier *et al*., 1999; Hautala *et al*., 2000; Parlanti *et al*., 2000; Clark *et al*., 2002; Katsuyama and Nobuhito, 2002; Her *et al*., 2003; Yamashita and Tanoue, 2003; Cammack *et al*., 2004; Jaffe *et al*., 2004; Elliott *et al*., 2006) as have its interactions (de Souza Sierra *et al*., 1997) and degradation (Amon and Benner, 1996; Skoog *et al*., 1996; Moran *et al*., 2000; Del Vecchio and Blough, 2002; Bertilsson *et al*., 2004) which occur on both spatial (Galapate *et al*., 1998; Del Castillo *et al*., 1999; Clark *et al*., 2002; Maurice *et al*., 2002; Baker *et al*., 2003; Baker and Spencer, 2004; Del Vecchio and Blough, 2004a) and temporal (Baker, 2002a,b, 2003) scales.

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All of this suggests that the DOM is ubiquitous, reactive and of variable structure and composition and further extends the understanding of the carbon cycle and the contribution of aquatic organic matter to the carbon budget (Battin, 1998; Benner and Ziegler, 1999). DOM was previously thought of as simply a relatively inert product of biological activity, however, the increasing use of fluorescence spectroscopy and advances in optical technology (enabling rapid investigation of shorter wavelengths) have enabled more detailed characterization of organic material and its reactions in water (Baker, 2002b; Clark *et al*., 2002; Cammack *et al*., 2004). The presence of DOM in water impacts on the biological and physico-chemical behaviour of the water body by affecting metal speciation and altering pH (Thacker *et al*., 2005). CDOM (coloured or chromophoric DOM) is thought to absorb radiant light from the water column, decreasing that available for photosynthesis (Ferrari *et al*., 1996). The presence of rapidly degradable, labile organic matter is of interest with a view to the analysis of river water quality (Baker and Inverarity, 2004). High levels of reactive organic matter cause oxygen depletion in the water body, through oxidation of the organic material, with associated impact upon aquatic life.

Despite the vast body of existing work relating to aquatic DOM there is still much to be learned. Work is ongoing to further examine the complex interactions of DOM; to determine the influence of DOM source and character on lability; to investigate the character and influence of the aquatic microbial community, its substrates, products and relationship with DOM; to identify what interactions occur within the varying DOM constituents in time and space and the relative importance of these parameters; and to further understand the processes of DOM cycling within the hydrological system.

In this article, we review the use of fluorescence spectroscopic techniques to measure the intrinsic fluorescence of organic matter and the application of fluorescent DOM analysis in marine waters, freshwaters and wastewaters.

## DISSOLVED ORGANIC MATTER FLUORESCENCE

Organic matter fluorescence occurs when a loosely held electron in an atom or a molecule is excited to a higher energy level by the absorption of energy, for example, a photon, and fluorescence occurs when energy is lost as light as the electron returns to its original energy level (ground state). Figure 1 shows a representation of the energy transfer involved in the process of fluorescence. Some energy is 'lost' from the excited electron by collision, non-radiative decay and other processes, prior to emission, so the energy of the emitted photon is lower than the excitation energy (the Stokes' Shift). The wavelength at which absorption (excitation) and emission occur is specific to the molecule (Lakowicz, 1999). Aromatic organic compounds provide particularly good subjects for study by fluorescence due to the energy sharing, unpaired electron structure of the carbon ring. In the study of fluorescent organic matter, those compounds that absorb light are called chromophores and those that absorb and re-emit light energy are called fluorophores (Mopper *et al*., 1996).

Fluorescence studies in the aquatic environment centre on the complex mixture of ubiquitous, poorly defined heterogeneous compounds (Westerhoff *et al*., 2001; Her *et al*., 2003) which comprise the fluorescent fractions of DOM. The most commonly studied fluorescent organic components of natural waters include humic substances, derived from the break-down of plant material by biological and chemical processes in the terrestrial and aquatic environments (Elkins and Nelson, 2001; Stedmon *et al*., 2003; Patel-Sorrentino *et al*., 2004), and amino acids in proteins and peptides. Humic substances can be sub-divided into three categories, chemically defined by solubility at different pH.

*Humic acids* are insoluble in aqueous solution at pH lower than 2, but soluble at higher pH. *Fulvic acids* are soluble in water under all pH conditions. *Humins* are insoluble in water under any pH conditions (Aiken *et al*., 1985).

Three fluorescent amino acids (tryptophan, tyrosine and phenylalanine) are indicative of proteins and peptides. The fluorescence of these specific amino acids is due to the presence of an indole group (a fused ring heterocycle containing both a benzene ring and a heterocyclic aromatic ring in which a nitrogen atom occurs as part of a ring) or some other aromatic ring structure in which electrons are 'shared' rather than occurring as opposite spin pairs and are therefore loosely held and available for promotion to the higher energy level. Debate continues about the origin of protein-like fluorescence, whether it is entirely from free amino acids in the DOM pool (Yamashita and Tanoue, 2003), or partially from aromatic amino acids bound in proteins or organism cell walls (Determann *et al*., 1998).



DOI: 10.1002/rra

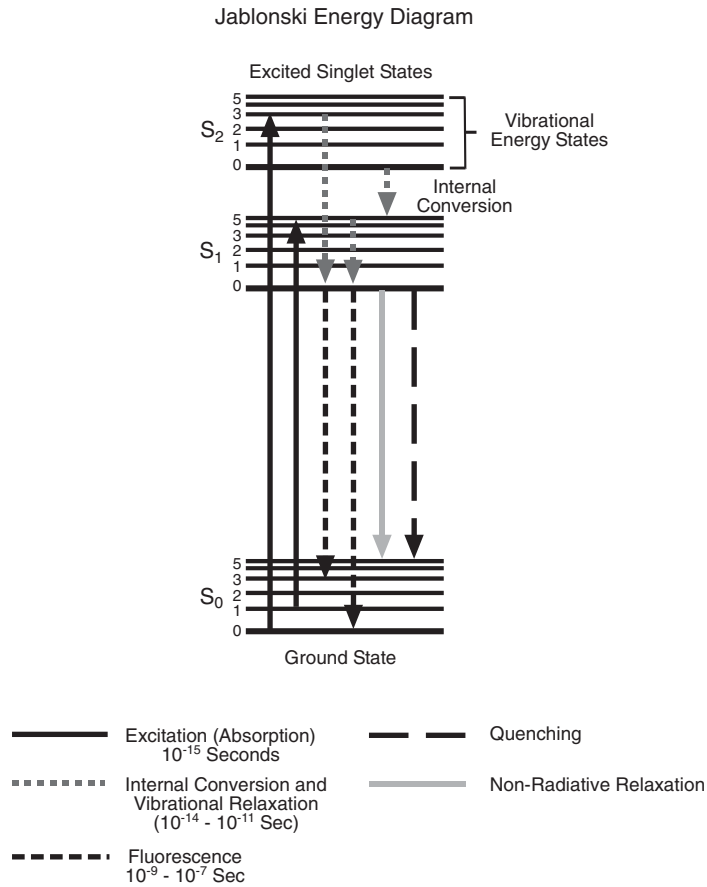


Figure 1. Jablonski diagram

There is clear evidence for a bacterial origin. Shelly *et al*. (1980a,b) and Dalterio *et al*. (1986) proposed identification of bacteria by their fluorescence characteristics. Determann *et al*. (1998) postulated that it may be possible to identify algal and bacterial tryptophan by emission wavelength, compared with that of tryptophan standard. Cammack *et al*. (2004) and Elliott *et al*. (2006) found that tryptophan-like fluorescence relates to the activity of a viable bacterial community being both a biological product of that community and a bioavailable substrate. Figure 2 demonstrates the structures of tryptophan, tyrosine and phenylalanine standards and theoretical humic and fulvic acids.

Table I lists the various naming formats of common aquatic fluorophores found in literature. This review will use the Coble (1996) names, as illustrated in Figure 3.

Due to the difficulties associated with definitively identifying individual fluorescent compounds in waters these groups of fluorophores are commonly named humic-like, fulvic-like and protein-like (specifically tryptophan- or tyrosine-like), so called because their fluorescence occurs in the same area of optical space as standards of these materials. However, fluorescence in the natural environment may be different to that observed in the laboratory under controlled conditions, using model compounds. Environmental protein fluorescence resembles that of amino acids, as observed in laboratory analysis of amino acid standards (Mayer *et al*., 1999), but with a blue shift to shorter emission wavelengths due to differences in the behaviour of amino acids in the different microenvironments present within proteins (Determann *et al*., 1998). International Humic Substances Society (IHSS) samples have been used as standards for the humic and fulvic fractions (Senesi *et al*., 1989) and while they provide valuable data, IHSS samples have been isolated and concentrated. These processes may change the structure of the organic material



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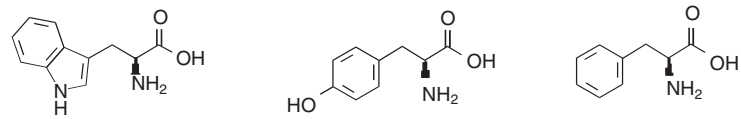
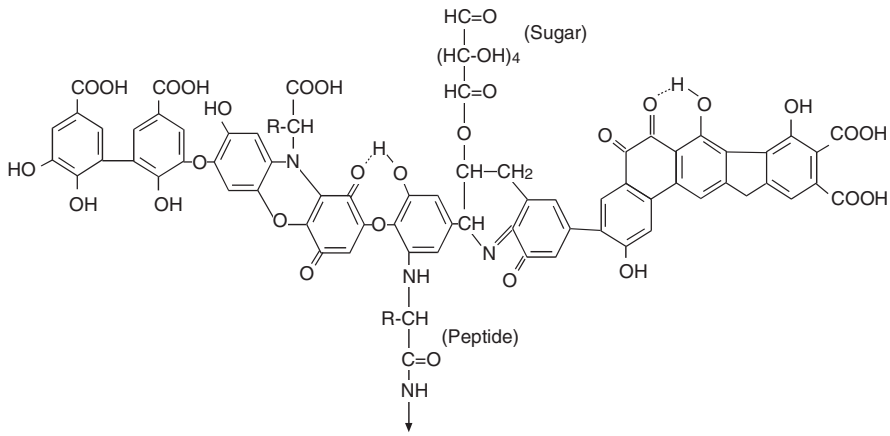
**Structure of tryptophan, tyrosine, phenylalanine**




**trp** w Tryptophan **tyr** y Tyrosin **phe** f Phenylalanin

**Theoretical humic acid**

*Stevenson, (1982) cited in Aitken et al., (1985)*




**Theoretical fulvic acid**

*Buffle, (1977) cited in Aitken et al., (1985)*

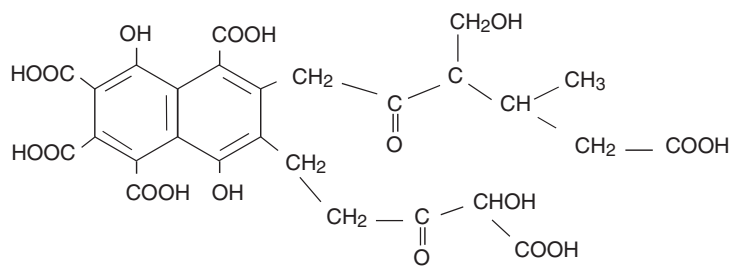



Figure 2. Actual and hypothetical structures of some common aquatic fluorophores

present through preferential removal of groups with certain ionic character, or destruction of functional groups with implications on the behaviour and character of the standards compared to unaltered organic matter in natural water samples. The fluorescence of humic and fulvic acids from a range of sources are examined in detail by Senesi *et al*. (1991) who determined that the molecular components of humic and fulvic acids differ with source and impart a specific spectral signature.

Another method of identifying fluorophores is by their chemical character after fractionation (Wu *et al*., 2003; Hautala *et al*., 2000; Marhaba and Lippincott, 2000; Marhaba *et al*., 2000) - hydrophobic or hydrophilic and acid, base or neutral. This naming system is less common than the Coble (1996) (A,C,B,T) or standard nomenclature (e.g. humic-like). It is, however, used in studies in which separation by retention on ion exchange columns are undertaken. Any information provided by fluorescence spectroscopy about molecular size, aromacity or aliphatic



DOI: 10.1002/rra

Table I. Naming formats of common aquatic fluorophores

| Fluorophore Name: (Coble, 1996) | Fluorophore Name: (Parlanti *et al.*, 2000) | Fluorophore Name: (Marhaba *et al.*, 2000; Marhaba and Lippincott, 2000) | Fluorophore type | Ex/Em wavelength (nm) at max fluorescence intensity | Author |
|---|---|---|---|---|---|
| A | $\alpha'$ | Hydrophobic acid fraction (HPOA) | Humic-like | 237–260/400–500 | |
| C | $\alpha$ | | Humic-like | 300–370/400–500 | |
| M | $\beta$ | | Marine Humic-like | 312/380–420 | |
| B | $\gamma$ | Hydrophobic neutral fraction (HPON) | Protein-like (Tyrosine-like) | 225–237/309–321 and 275/310 | |
| T | $\delta$ | Hydrophobic base fraction (HPOB) | Protein-like (Tryptophan-like) | 225–237/340–381 and 275/340 | |
| | | Hydrophilic acid fraction (HPIA) | | | |
| | | Hydrophilic neutral fraction (HPIN) | | | |
| Chlorophyll a | | | | 431/670 | (Moberg *et al.*, 2001) |
| Chlorophyll b | | | | 435/659 | (Moberg *et al.*, 2001) |
| Naphthalene | | | XOM—landfill leachate | 220–230/340–370 | (Baker and Curry, 2004) |
| | | | Fluorescent whitening agent | 260/430 260/540 400/460 | (Westerhoff *et al.*, 2001) |





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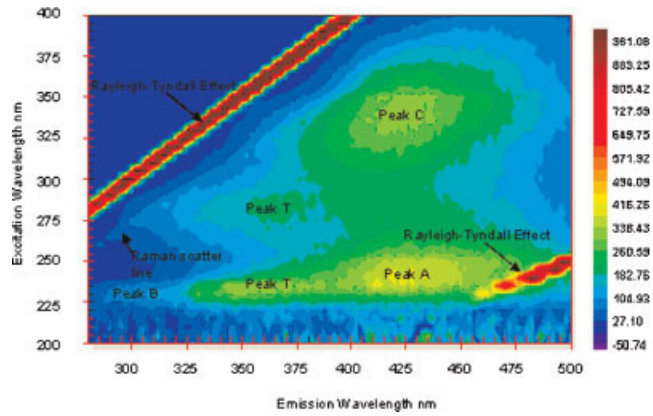


Figure 3. EEM showing common EEM features and the position of peaks A, C, B and T as named by Coble, 1996. This figure is available in colour online at www.interscience.wiley.com/journal/rra

properties allows additional chemical composition classification of the DOM, and better understanding of its bioavailability and reactions in natural waters.

## FLUORESCENCE SPECTROSCOPIC TECHNIQUES

Improvements in technology, particularly light-source wavelength range and stability, scanning speed and data processing capability, have enabled fluorescence spectroscopy to become a more flexible, rapid and portable diagnostic tool. It is possible, using simple equipment, to target a single excitation and emission wavelength pair, diagnostic of a specific molecule (Skoog *et al*., 1996; Petrenko *et al*., 1997; Determann *et al*., 1998; Chen, 1999; Thoss *et al*., 2000; Clark *et al*., 2002; Nagao *et al*., 2003; Del Vecchio and Blough, 2004a) which is useful in determining the presence or absence and character of a target compound. However, the technique is relatively slow and inflexible, particularly if a range of excitation and emission wavelength scans are required for the study of more than one fluorophore.

Other available techniques include fluorescence emission spectrometry, in which emission is scanned over a range of wavelengths for a fixed excitation wavelength (Ferrari *et al*., 1996; Hautala *et al*., 2000). This increases the range of fluorophores that might be found, but output is restricted to a linear scan, in which the choice of excitation wavelength determines the molecules that may be identified. Synchronous fluorescence scanning (SFS) has similar drawbacks. Despite scanning both excitation and emission wavelengths it is still a linear technique. Emission wavelength is measured at an offset from the excitation wavelength, commonly by 12–60 nm (Senesi *et al*., 1989, 1991; Yang and Zhang, 1995; Pullin and Cabaniss, 1997; Galapate *et al*., 1998; Lombardi and Jardim, 1999; Kalbitz *et al*., 2000; Marhaba and Lippincott, 2000; Marhaba *et al*., 2000; Piana and Zahir, 2000; Kalbitz and Geyer, 2001; Westerhoff *et al*., 2001; Reynolds, 2003; Bengraine and Marhaba, 2004; Jaffe *et al*., 2004).

Today, excitation emission matrix fluorescence spectroscopy (EEMS) is the state-of-the-art technique used. It was not until the mid-1990s (Coble, 1996) that EEMS became common in aquatic studies. The principal of EEMS is that excitation, emission and fluorescence intensity can be scanned over a range of wavelengths synchronously and plotted on a single chart, developing a 'map' of optical space, an excitation emission matrix (EEM). Such 'maps' can be seen in Figure 4, which includes a typical EEM of International Humic Substance Standards (IHSS) of a Suwannee River humic and fulvic acid and a tryptophan and tyrosine standard, at appropriate concentrations for obtaining a clear EEM. Figure 5 includes a typical EEM of marine, freshwater, untreated and treated sewage at appropriate dilutions.

EEMS is rapid (~1 min per sample (Baker, 2001). The production of a 3-D plot of fluorescence excitation wavelength, emission wavelength and intensity allows the visualization of a range of fluorophores in a given sample, in their relative positions in optical space. Relative concentrations can be determined based upon



DOI: 10.1002/rra

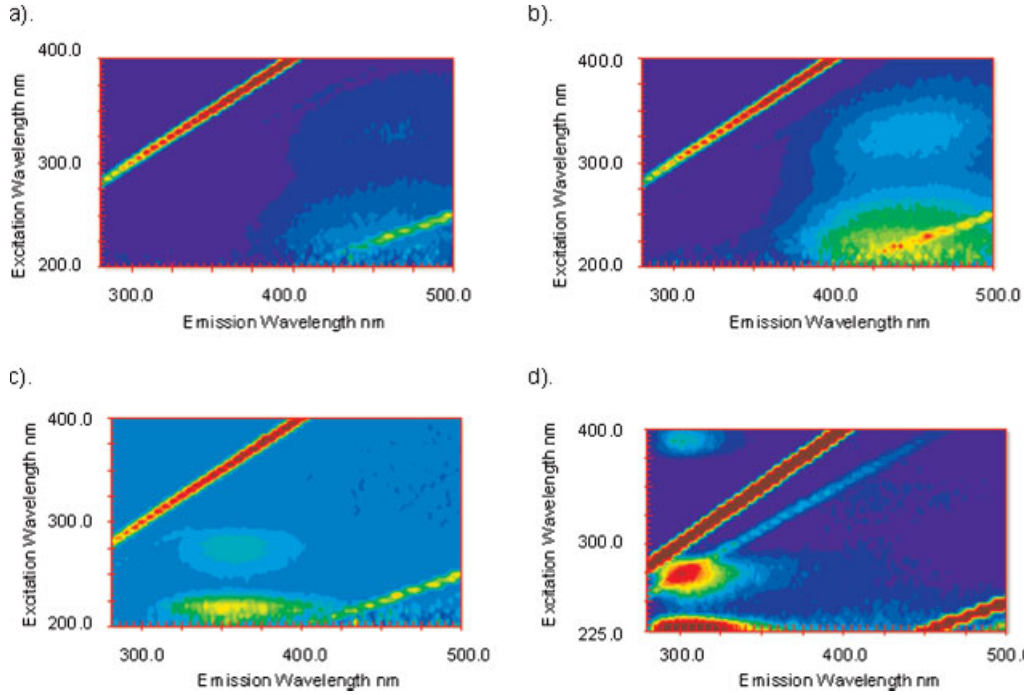


Figure 4. EEMs of (a) humic acid 12.5 ppm, (b) fulvic acid 12.5 ppm (c) tryptophan 0.1 ppm and (d) tyrosine standard 0.2 ppm (measured at enhanced sensitivity). Fluorescence intensity scale as Figure 3. This figure is available in colour online at www.interscience.wiley.com/journal/rra

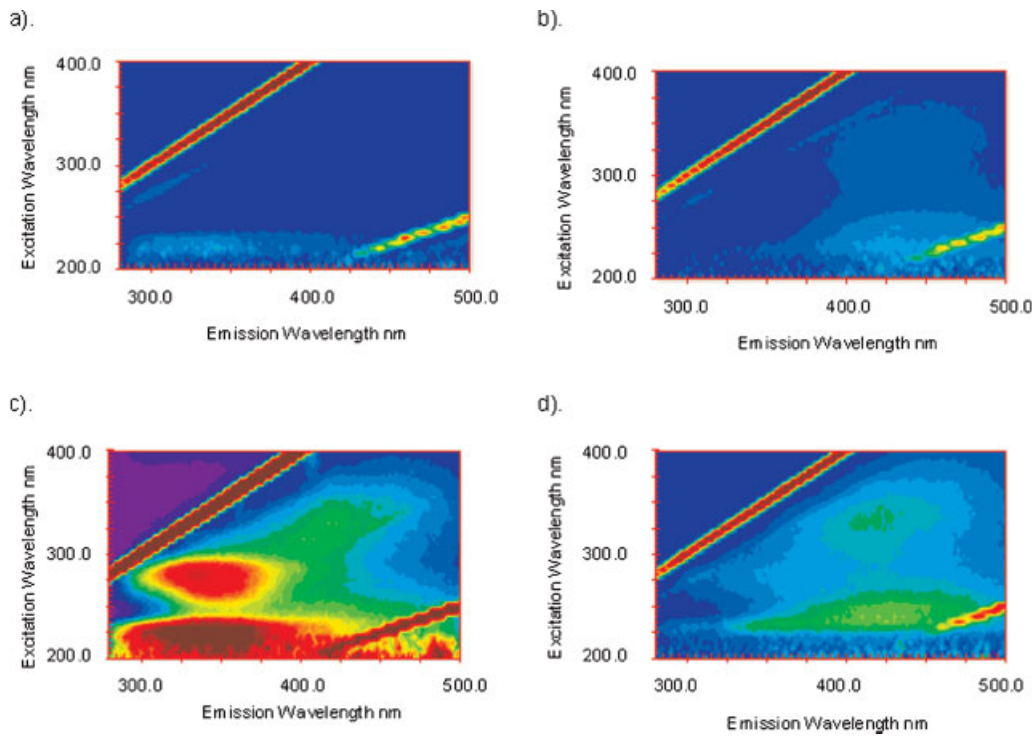


Figure 5. Typical EEMs of common natural waters. Fluorescence intensity scale as Figure 3. This figure is available in colour online at www.interscience.wiley.com/journal/rra



DOI: 10.1002/rra

calibration of fluorescence intensity against TOC (Baker, 2002c) or standards with detection limits at ppb or ppm levels depending on the fluorophore. The technique is non-destructive and requires little or no sample preparation. An additional feature of the EEMS approach is the vast array of data available for interpretation within an EEM (Lombardi and Jardim, 1999). Attempts have been made to extend the understanding of fluorophore character and concentration by utilizing this large amount of data, for example by the analysis of the shape under the peak, and the use of statistical techniques such as 'Analysis of Variance' (ANOVA) (Bertilsson *et al*., 2004; Jaffe *et al*., 2004; Smith *et al*., 2004), 'Parallel Factor Analysis' (PARAFAC) (Moberg *et al*., 2001; Brunsdon and Baker, 2002; Stedmon *et al*., 2003; Olivieri *et al*., 2004) and 'Partial Least Squares regression' (PLS) (Ferrer *et al*., 1998; Vasel and Praet, 2002; Bengraine and Marhaba, 2004) to analyse both individual and groups of EEM.

Fluorescence analyses of DOM have the potential to be constrained by a lack of understanding of the effects of inner filtering, the absorption and re-emission of emitted energy at a longer wavelength by surrounding molecules. This is particularly an issue in concentrated solutions. Inner filtering has been identified as a shift to a longer emission wavelength (red shift) in known fluorophores in model and natural solutions due to the concentration of fluorophores in the solution (Yang and Zhang, 1995; Mobed *et al*., 1996) and a shift to a shorter emission wavelength (blue shift) was observed with decreasing solution concentration (Hautala *et al*., 2000). There is a disagreement in the literature as to the concentration of DOM at which inner-filter effects interfere. Various suggestions have been made about the optimum concentration for DOM analysis in water to minimize the inner filtering effect, from 1 mg $L^{-1}$ (Hautala *et al*., 2000; Westerhoff *et al*., 2001) to 15 mg $L^{-1}$ (Yang and Zhang, 1995) and 100 mg $L^{-1}$ (Senesi *et al*., 1991). Vodacek and Philpot (1987) suggested that concentration effects should be negligible in natural waters as DOC concentration rarely exceeds 20 mg $L^{-1}$. However, high concentration solutions, such as untreated sewage, require dilution prior to fluorescence analysis (Mobed *et al*., 1996; Kalbitz and Geyer, 2001; Baker, 2002a; Baker *et al*., 2004). This reduces inner filtering by reducing the concentration of fluorophores in the sample while retaining the relative proportions in solution. The potential for absorption of emitted light by surrounding fluorophores is reduced with the reduction in fluorophores present, so emission wavelength becomes a direct value with no associated energy loss to surrounding molecules. Figure 6 illustrates the change in character of EEMs with dilution. Figure 7 shows that there is a wide variation in sample fluorescence per carbon, due to variations in DOM character, explaining the lack of a unique total organic carbon (TOC) concentration at which inner filtering is found to be negated (in freshwater and effluent samples from southwest England).

A further interference, scatter by particulates and larger colloids, is also reduced by dilution again by reduction in concentration. However scatter is only effectively reduced by filtering, which also ensures that only DOM contributes to the fluorescence signature.

Finally, linear features evident on fluorescence EEM are the Raman line and the Rayleigh–Tyndall features as illustrated in Figure 3. The Raman line is a faint linear trace at excitation wavelengths between 260 and 350 nm and emission wavelengths 280–400 nm which is an optical manifestation of the scattering properties of water due to the vibration of molecular O–H covalent bonds with the application of light energy. The position of the Raman line is dependent upon the wavelength of the incident radiation. Studies commonly correct for this effect by gross spectral subtraction. The shorter excitation wavelength forms of tyrosine-like fluorescence (Peak B, Coble, 1996) can be obscured by the position of the Raman line. Normalization of the fluorescence intensity data with the Raman data provides a useful internal standard. The Rayleigh–Tyndall effect is a visible feature which is source excitation energy reflected off the cuvette walls and which is visible in Figure 3 at emission wavelength = emission wavelength and at emission wavelength = 2 × excitation wavelength.

## ENVIRONMENTAL EFFECTS ON DOM FLUORESCENCE

The wavelengths at which molecules fluoresce and the intensity of the fluorescence does not just depend on the properties of the DOM but can be affected by a number of factors such as changes in pH (Patel-Sorrentino *et al*., 2002), quenching by chelation with metal ions (Reynolds and Ahmad, 1995) and changes in temperature (Baker, 2005).



DOI: 10.1002/rra

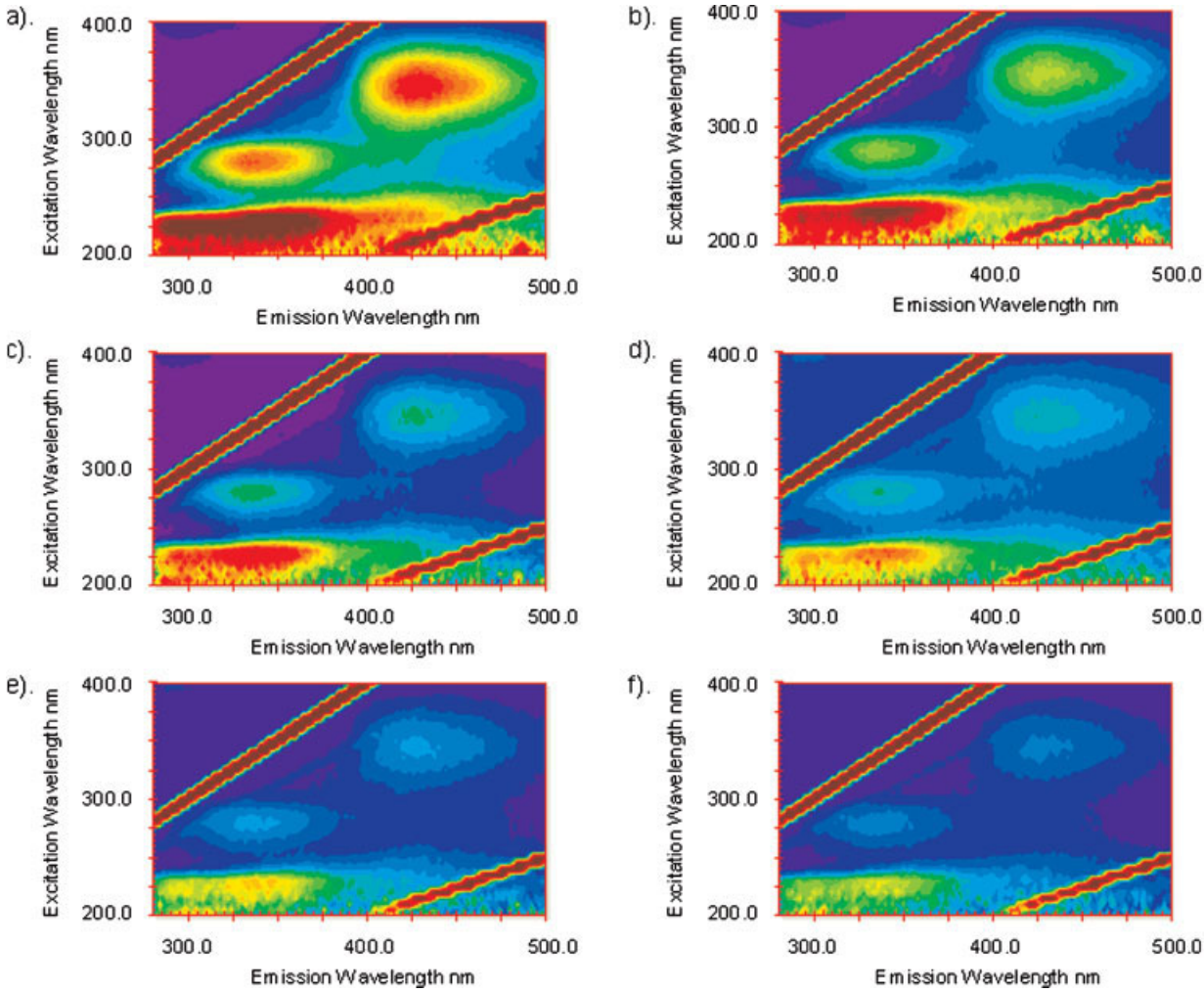


Figure 6. Sewage effluent dilution series by EEM. Fluorescence intensity scale as Figure 3. This figure is available in colour online at www.interscience.wiley.com/journal/rra

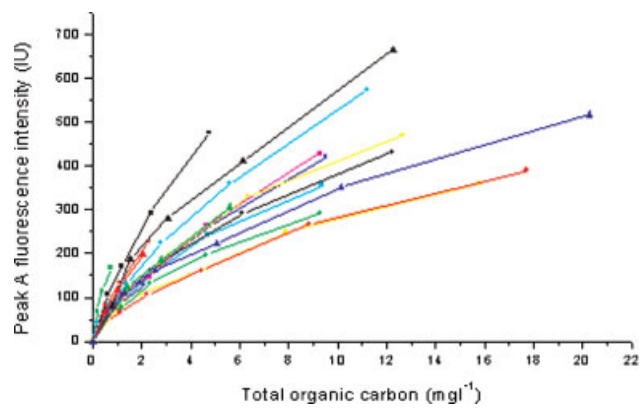


Figure 7. Peak A intensity change with dilution. Relationship with total organic carbon (TOC) at a range of dilutions showing a wide variation in sample fluorescence per carbon due to variations in DOM character with no unique TOC concentration at which inner filtering is found to be negated (in freshwater and effluent samples from southwest England). This figure is available in colour online at www.interscience.wiley.com/journal/rra



DOI: 10.1002/rra

*pH*

Changes in observed fluorescence intensity are a result of conformational changes in the molecules (Myneni *et al*., 1999; Westerhoff *et al*., 2001) exposing or hiding fluorescent parts of the molecule. Low pH causes the molecule to coil, while raising pH extends the molecule. The degree of pH effect depends upon the fluorophore. Fluorescence spectroscopy has been applied to solutions at a range of pH (2–12) although pH is sometimes altered to near neutral (pH 6–7) prior to fluorescence analysis (Yang and Zhang, 1995; Her *et al*., 2003). Vodacek and Philpot (1987) identified a trend of increasing fluorescence intensity as pH increased from pH 4–5.5 in humic substances, above which a continued, but less dramatic, increase was found. Westerhoff *et al*. (2001) identified a decrease in fluorescence intensity of 30–40% as pH decreased from pH 7–3 in fulvic acid standards and treated municipal wastewater and suggested an optimum fluorescence analysis pH of 3 for water samples. Reynolds (2003) determined that in tryptophan standards of pH<4.5 fluorescence decreased by up to 15%, pH 5–8 there was little impact, and at >pH 8 fluorescence was enhanced by up to 30%. It was also found that peak B (tyrosine-like) was more sensitive to pH changes than the other peaks. Patel-Sorrentino *et al*. (2002) investigated the impact of changing pH on A and C peaks in surface waters and found that fluorescence intensity generally increases with an increase from pH 2 to about pH 12 at which point a minor decrease is observed. It was also found that changes in fluorescence intensity as a result of pH change are reversible (Vodacek and Philpot, 1987; Patel-Sorrentino *et al*., 2002). In aquatic systems pH is normally between pH 5–9. Over this range fluorescence intensity of all fluorophores has been found to increase by about 10% although the scale of the increase is fluorophore specific. This is unlikely to have an impact on fluorescence analysis of most natural waters and is less than the variability caused by changes in DOM source or character.

*Metal ions*

In natural waters DOM fluorescence, particularly of humic substances (peaks A and C), are affected by the presence of metals through the formation of organo-metal complexes. Fluorescence may be quenched (Esteves da Silva *et al*., 1998) or enhanced in certain spectral regions (Blaser *et al*., 1999; Sharpless and McGown, 1999). Most studies analyse the effect of metals upon humic and fulvic acid fluorescence in controlled laboratory conditions at low concentrations of both, avoiding the creation of insoluble complexes which affect the optical character of the solution (Esteves da Silva *et al*., 1998; Elkins and Nelson, 2001).

In humic substance standards, fluorescence of fulvic acids is quenched in the presence of excess copper, iron and aluminium by varying degrees at concentration as low as 0.1 mg $L^{-1}$ (Vodacek and Philpot, 1987; Esteves da Silva *et al*., 1998). Aluminium and copper caused fluorescence to be quenched by up to 40% in concentrations up to 2 mg $L^{-1}$ (Reynolds and Ahmad, 1995). Iron quenches more efficiently than chromate, lead, copper and nickel at pH 4 and 8 for a range of humic substances (Piana and Zahir, 2000). Terrestrial humic acids were found to show a general decrease in fluorescence intensity, which could be a result of self-quenching in larger aggregates (Sharpless and McGown, 1999) while aquatic humic acids demonstrate enhancement in some regions of the spectra (Sharpless and McGown, 1999; Elkins and Nelson, 2001) with a related red-shift in excitation wavelength and blue-shift in emission wavelength. The wavelength changes are related to changes in the structure of the humic substances particularly on addition of aluminium (Elkins and Nelson, 2001). Despite the range of laboratory studies undertaken into the quenching effects of metals there is little evidence that extensive quenching by metals is common in most aquatic systems. The reason for this is, at present, unclear but suggests that in the natural environment other factors are more important controls on DOM fluorescence.

*Photodegradation*

The study of photodegradation, photobleaching or photo-oxidation of DOM is usually related to the creation of bioavailable substrate from DOM in aquatic environments, characterization of the optical and physical properties of humic substances and seasonal and temporal changes in these characteristics. The effects of photochemical degradation on DOM have been studied using fluorescence techniques in a range of aquatic environments. Table II lists the aquatic environments studied and relevant authors. The effects of photo-oxidation of organic matter are



DOI: 10.1002/rra

Table II. List of aquatic environments in which photodegradation tests have been undertaken using fluorescence techniques and the authors of those studies

| Environment | Author |
|---|---|
| Wetlands | Waiser and Robarts (2004) |
| Rivers | Gao and Zepp (1998); White *et al*. (2003); Patel-Sorrentino *et al*. (2004) |
| Marine | Ferrari *et al*. (1996); Skoog *et al*. (1996); Kieber *et al*. (1997); Miller *et al*. (2002); Bertilsson *et al*. (2004); Boehme *et al*. (2004); Del Vecchio and Blough (2004a); Zanardi-Lamardo *et al*. (2004) |
| Estuaries | Skoog *et al*. (1996); Moran *et al*. (2000) |
| Humic standards | Fukushima *et al*., 2001; Del Vecchio and Blough (2002); Del Vecchio and Blough (2004b); Uyguner and Bekbolet (2005) |

studied through analysis of changes in intrinsic fluorescence intensity (Skoog *et al*., 1996; Moran *et al*., 2000; Del Vecchio and Blough, 2002; Bertilsson *et al*., 2004) or peak position (Moran *et al*., 2000; Boehme *et al*., 2004).

Irradiation methodology is split between the use of high output artificial light filtered to provide the required wavelengths (Gao and Zepp, 1998; Moran *et al*., 2000; Del Vecchio and Blough, 2002; Miller *et al*., 2002; Uyguner and Bekbolet, 2005), natural sunlight (Amon and Benner, 1996; Skoog *et al*., 1996; Bertilsson *et al*., 2004) and *in situ* analysis (Skoog *et al*., 1996).

DOM may be degraded by direct or indirect means (direct alteration of the DOM structure or indirect chemical changes through reactions with free radicals created by the application of light) (Amon and Benner, 1996). The process is enhanced by the presence of iron and oxygen through photo-fenton reactions (light induced reactions catalysed by the presence of iron and oxygen) (Fukushima *et al*., 2001; White *et al*., 2003) while the presence of copper may inhibit the formation of some photolytic products.

There is general consensus between existing studies regarding the effect of photochemical reactions upon natural waters which are reviewed in greater detail by Benner and Ziegler (1999) with no comment on the use of fluorescence in analysis. In general a decrease in UV-absorbance (Ferrari *et al*., 1996), fluorescence intensity (Moran *et al*., 2000; White *et al*., 2003; Waiser and Robarts, 2004; Uyguner and Bekbolet, 2005), aromatic character (Benner and Ziegler, 1999), molecular size (Benner and Ziegler, 1999; Fukushima *et al*., 2001; Uyguner and Bekbolet, 2005) and DOC is seen (Moran *et al*., 2000; Clark *et al*., 2002). Photochemical products include dissolved inorganic carbon (DIC) (Benner and Ziegler, 1999), carbohydrates (Uyguner and Bekbolet, 2005) and volatile organic carbon compounds (Clark *et al*., 2002). Results are inconsistent regarding increasing bioavailability of photochemical products with Bertilsson *et al*. (2004) demonstrating an apparent balance in increasing DOM bioavailability and bacterial DOM production, with no net gain or loss of DOM.

The extent of photodegradation of DOM is related to the structure (Benner and Ziegler, 1999) and source (Skoog *et al*., 1996) of the DOM and the incidence of irradiation. Water from depth and waters with greater terrestrial input (Skoog *et al*., 1996) have a greater potential for photodegradation than surface waters. This may be due to the prior degradation of surface water DOM by UV exposure which is difficult to quantify, and the composition of the terrestrial organic matter. Other work concludes that shallow waters, for example, wetlands, coastal shelf regions (Del Vecchio and Blough, 2004a; Waiser and Robarts, 2004) show high DOM photochemical alteration as a result of greater UV-B penetration, mixing within the water body and input of terrestrial material. Humic-like compounds (particularly peak C) are found to be more likely to degrade than peaks A or T (Moran *et al*., 2000) and no work, other than Moran *et al*. (2000), has been found relating to the photodegradation of the B or T peaks. In natural waters photodegradation is found to have an impact on DOM structure and character with a change to smaller molecules, an associated effect on bioavailability and so is likely to change the fluorescence character by the presence or absence of peaks or changes in their relative intensities.

### *Temperature*

Early work by Wehry (1973) cited in Vodacek and Philpot (1987) states that fluorescence is inversely related to temperature due to increased collisional quenching at higher temperatures. Fluorescence changes caused by



DOI: 10.1002/rra

temperature should be reversible as no change is made to the structure of the DOM. However, experiments showed that non-reversible changes did occur, possibly as a result of the application of a light-source, which may be due to photodegradation or thermal decomposition (Vodacek and Philpot, 1987). Conventionally, temperature has been held constant during fluorescence analysis to avoid any interference from thermal quenching, although more recently (Baker, 2005) investigated the relative thermal quenching properties of the fluorophores present in DOM as a technique to probe DOM structure.

## APPLICATIONS OF DOM FLUORESCENCE IN NATURAL WATERS

### *Marine and Estuarine DOM*

Since initial work by Kalle (1949) cited in Coble (1996) tracing riverine dissolved organic carbon (DOC) in the ocean using fluorescence, fluorescence spectroscopy has been used increasingly in the study of DOM in marine and estuarine waters. Common applications include study of the fluorescence properties of DOM and CDOM (Ferrari *et al*., 1996; Mopper *et al*., 1996; Del Castillo *et al*., 1999; Lombardi and Jardim, 1999) as a tool for determining biological activity and associated protein fluorescence (Determann *et al*., 1998; Mayer *et al*., 1999; Parlanti *et al*., 2000; Yamashita and Tanoue, 2003; Jaffe *et al*., 2004), characterization of DOM from different sources (Coble, 1996; Clark *et al*., 2002; Jaffe *et al*., 2004), fluorescence of organics held in, and released from, sediment (Komada *et al*., 2002) and mixing of water bodies (de Souza Sierra *et al*., 1997).

Marine waters are rich in the marine humic peak (M) with intense tryptophan-like fluorescence (T) related to biological activity particularly in areas of high primary productivity, that is, surface waters and areas of upwelling. The marine humic fluorophore (M) was initially thought to be either an independent marine entity or an alteration product of terrestrial humic-like material C (Coble, 1996) (Table I). Further work by Parlanti *et al*. (2000) considered the importance of fluorophore $\beta$, which corresponded with Coble's marine humic (M) and which was associated with biological activity and elevated protein concentrations in areas of high primary productivity (Table I). This suggested that it is not an alteration product of the terrestrial originated C humic-like material, but a marine derived humic-like compound constituting 'fresh' marine humic material, prevalent in surface waters with a direct correlation with biological activity and salinity (Coble, 1996; Parlanti *et al*., 2000).

A change in humic substance character is commonly observed with depth and distance from shore. C-type fluorescence intensity is seen to decrease with an increase in marine influence (Del Castillo *et al*., 1999; Clark *et al*., 2002). Terrestrial type humic material is present in rivers and deep marine waters suggesting that this may represent older, more degraded and humified material (Komada *et al*., 2002).

Peaks T and B (protein-like fluorescence) in marine and estuarine environments are a result of a mixture of autochthonous (created *in situ*) and allochthonous (created elsewhere and transported) sources. Tryptophan-like fluorescence (peak T) is common in waters subject to anthropogenic influence such as bays, estuaries, coastal areas, also areas of high primary productivity and pore waters (Coble, 1996) and so is thought to derive directly from bacterial activity (Yamashita and Tanoue, 2003; Cammack *et al*., 2004). Tyrosine-like fluorescence (peak B) is present in all marine waters at all depths (Mayer *et al*., 1999; Yamashita and Tanoue, 2003). Peaks T and B represent either intrinsically fluorescent molecules which constitute bioavailable organic fractions of DOM or fluorescent products of microbial activity, existing on the bioavailable, labile organic fractions, or perhaps a mixture of both. Figure 8 shows the change in fluorescence demonstrated by EEMs along a transect of the River Tyne, Northwest England from freshwater, to estuarine and marine conditions.

### *Freshwater DOM*

The main applications of fluorescence spectroscopy to freshwaters have been in the determination of the optical properties of DOM (Battin, 1998), the influence of pH on fluorescence of organic matter (Patel-Sorrentino *et al*., 2002), characterization of DOM composition and source (Mounier *et al*., 1999; Hautala *et al*., 2000; Katsuyama and Nobuhito, 2002; Her *et al*., 2003), comparison of organic matter fluorescence with IHSS model compounds (Senesi *et al*., 1989; Wu *et al*., 2003; Kalbitz and Geyer, 2001) and determination of water source by fluorescence



DOI: 10.1002/rra

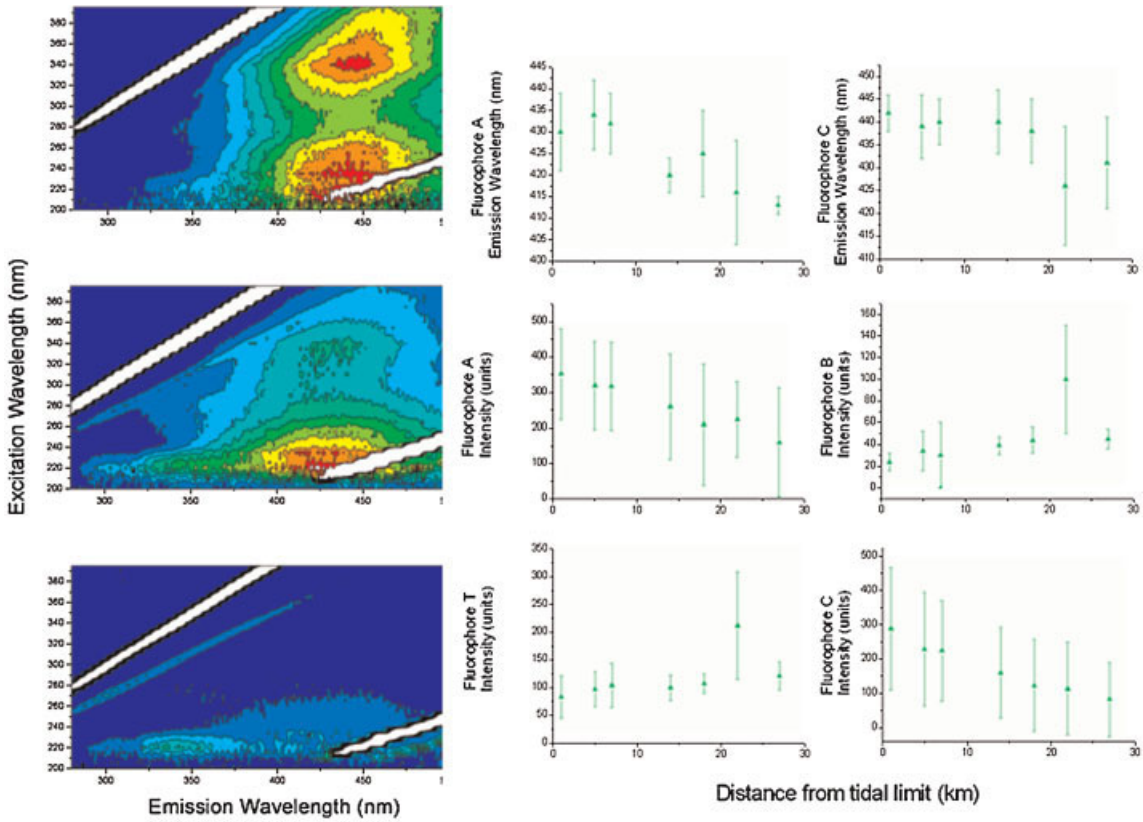


Figure 8. Change in DOM fluorescence through an estuary; River Tyne, NE England. Left: Fluorescence EEMs (all to the same scale) from tidal limit (top), mid estuary (middle) and North Sea (bottom). Right: changes in fluorescence properties along an estuarine transect. Note the impact of an STW discharge at 22 km from tidal limit. Fluorescence intensity scale as Figure 3. This figure is available in colour online at www.interscience.wiley.com/journal/rra

fingerprinting (Thoss *et al*., 2000; Yan *et al*., 2000; Newson *et al*., 2001; Baker, 2002c). However, the study of organic matter fluorescence in the freshwater environment is not yet as widespread as that in the marine sciences.

Naturally occurring, fluorescent, freshwater DOM is predominantly composed of humic (C and A) fractions from the breakdown of organic material in water, riparian zones (Katsuyama and Nobuhito, 2002) and other soils. Increasing urbanization is observed through the presence of peak T (tryptophan-like fluorescent material) with minor peak-B (tyrosine-like material) (see Fluorescent DOM in polluted waters section). Unlike marine studies, changes to longer or shorter emission wavelengths (red or blue shifts) have not been widely identified as a result of change in environmental conditions, but have been discussed with regard to the degradation or aggregation of material. A red shift in the peak position may relate to increasing hydrophobicity (humic-like character) and a blue shift to decreasing aromacity (Wu *et al*., 2003) or be an artefact of the concentration of DOM in solution.

Freshwater fluorescence has been investigated with an emphasis on spatial variation (Galapate *et al*., 1998; Katsuyama and Nobuhito, 2002, Baker *et al*., 2003; Baker and Spencer, 2004). Baker and Spencer (2004) identified a change from natural, humic-rich waters in upland regions (peaks A and C) to 'peak-T'-rich waters downstream with increasing anthropogenic input and urbanization. Similar patterns were observed on a catchment scale, relating changes in fluorescence spectrum to upstream and downstream character and known anthropogenic inputs, for example, sewage treatment effluents, combined sewer overflows (CSOs), airport wash off (Baker *et al*., 2003).

Organic matter in rivers also shows seasonally differential fluorescence intensity. Peak T, associated with CSO discharges, is observed to be more intense in summer, probably due to reduced base flows and lower dilution (Baker, 2002b; Baker *et al*., 2003). Organic matter character in rivers shows clear seasonal trends due to flow rate and the impact of wetting and drying on surrounding land (Katsuyama and Nobuhito, 2002). Figure 9 illustrates the temporal and spatial variation in DOM character across a range of rivers from upland catchments.



DOI: 10.1002/rra

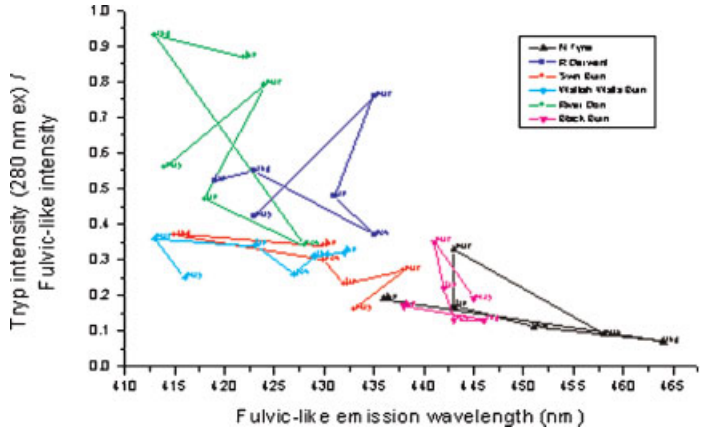


Figure 9. Spatial and temporal variation in fluorescence properties of DOM in the Tyne catchment, NE England over an annual sampling campaign 2002–2003. Upland, peat rich catchments are the North Tyne and Black Burn, a lowland catchment with intensive agricultural land cover is the Wallish Walls Burn, and lowland urbanized catchments are the Rivers Don and Derwent. This figure is available in colour online at www.interscience.wiley.com/journal/rra

*Wastewater fluorescence*

Crude sewage is composed of heterogeneous mixture of compounds including fulvic acids, proteins, carbohydrates and lipids with varying contributions from organic surfactants, nucleic acids and volatile fatty acids (Ahmad and Reynolds, 1995). It is a mixture of domestic waste, industrial (consented and unconsented) discharges and a domestic element from industrial premises (kitchens and toilets), in addition to surface runoff and storm flow. Composition varies depending upon the age and type of sewerage system in the catchment (separate or combined), time of day (Reynolds and Ahmad, 1997), prevailing and prior weather conditions and type of incoming sewer (gravity or pumped).

In line with the work carried out over the last 20 years in the field of DOM analysis in natural waters, research has been undertaken into the use of fluorescence as a tool for water treatment process optimization, water quality assessment and pollution monitoring. These applications have important ramifications for the water industry and environmental regulators. By monitoring DOM levels through a treatment works the operation of the works may be optimized, and up to 40% of energy costs could be saved by optimizing process efficiency (particularly aeration) (Ahmad and Reynolds, 1999). Field-based fluorescence determination of water quality would allow rapid assessment of the organic matter content and potential polluting load of a discharge or water-body, the identification and tracing of pollution incidents and therefore more rapid remedial action could be taken, avoiding statutory fines. Figure 10 illustrates the difference in fluorescence character between river waters and sewage effluents from southwest England related to TOC concentration.

Studies of EEMs of untreated wastewaters show that they commonly comprise a broad humic-type peak C with intense T peaks and B peaks which occur at the same position in optical space as standard solutions of tryptophan and tyrosine, respectively (Baker *et al*., 2004). Also present are peak A and, occasionally, other peaks which are likely to be related to the presence of fluorescent whitening agents (FWAs) from detergents (Westerhoff *et al*., 2001).

Peak T generally contributes the highest intensity peaks in wastewaters (Reynolds and Ahmad, 1997). Galapate *et al*. (1998), Baker *et al*. (2003, 2004) and Reynolds (2003) showed that peak T can be considered as a tracer and relic of anthropogenic material in natural waters due to its peak intensity, even in treated effluents, compared with the background levels of the natural water (see Fluorescent DOM in polluted waters section). A number of articles by Ahmad and Reynolds (Ahmad and Reynolds, 1995, 1999; Reynolds and Ahmad, 1997; Reynolds, 2002) have determined that a clear decline in fluorescence intensity of peak T is observed from influent to effluent across a treatment process. Peak T at Ex/Em 280/340 nm was identified as being most likely to relate to the biodegradable



DOI: 10.1002/rra

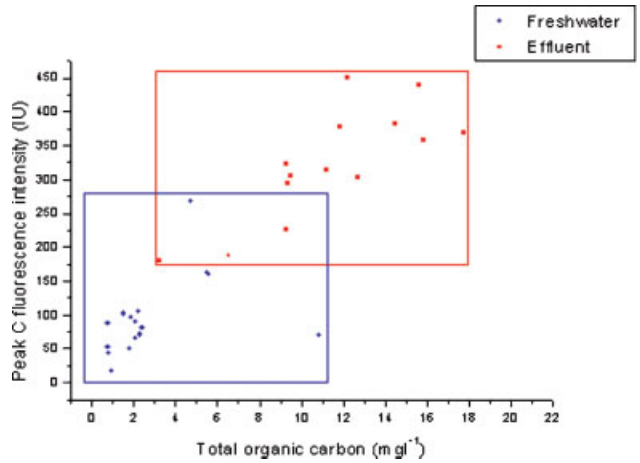


Figure 10. Illustration of fundamental differences between freshwaters and effluents from southwest England. Relationship between Peak C fluorescence intensity and total organic carbon (TOC). This figure is available in colour online at www.interscience.wiley.com/journal/rra

fraction of wastewater with up to 90% reduction observed across a treatment works. The work of Cammack *et al*. (2004) and Elliott *et al*. (2006) showed peak T fluorescence to be directly associated with the growth stage of bacterial communities. For this reason, it is believed that peak T is a more labile fraction of DOM which is preferentially degraded through the treatment process.

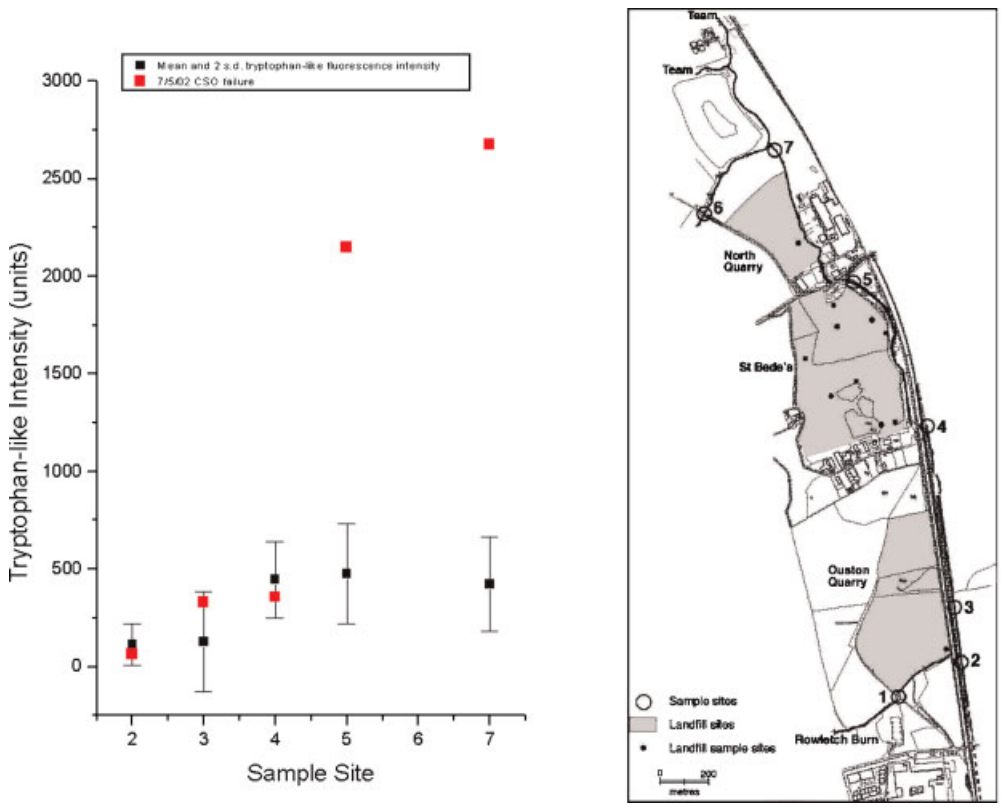


Figure 11. Peak T fluorescence intensity variations downstream in the Rowletch Burn, NE England. Note an increase in mean peak T intensity downstream of site 34, indicative of landfill leachate pollution (open circles), and the impact of a CSO failure between sites 4 and 5 (red squares) during one sampling campaign. This figure is available in colour online at www.interscience.wiley.com/journal/rra



DOI: 10.1002/rra

*Fluorescent DOM in polluted waters*

A number of studies have applied fluorescence spectroscopy to the tracking and characterization of wastewater in rivers using the fluorescence signature, particularly of peak T which is associated with readily biodegradable material (see Wastewater fluorescence section), for example, in Japanese rivers (Galapate *et al*., 1998) and Japanese lakes (Reynolds, 2003), and British rivers (Baker *et al*., 2003, 2004) in which EEMs and field-based fluorescence were used, to characterize the waters of the Ouseburn, an urban river in North East England. Seasonal variations in inputs from storm overflows were identified, as were failed CSOs and cross-connected sewers. Field-based fluorescence has also been used to investigate the effect of marine sewage plumes on the biology, optical character and particle-size distribution of coastal waters around an outfall diffuser using peak T fluorescence to track the extent of the plume (Petrenko *et al*., 1997).

Fluorescent compounds of anthropogenic origin with the potential to enter the freshwater system, which may be identified and traced by their fluorescence signature, include FWAs from tissue mills and laundry products (Baker, 2002c), landfill leachate components such as naphthalene (Baker and Curry, 2004), material from agricultural effluents (Baker, 2002a) and treated sewage effluents and sewer discharges (Galapate *et al*., 1998; Baker *et al*., 2003, 2004; Her *et al*., 2003; Reynolds, 2003) represented by fluorescence peaks B and T. Baker *et al*. (2004) used fluorescence spectrometry to identify pollution events from CSOs and sewer discharges using a portable, field spectrofluorimeter. Figure 11 demonstrates the variation in peak T (tryptophan-like) intensity with increasing pollution from diffuse source landfill leachates and a point source CSO failure.

## CONCLUSIONS

Fluorescence spectroscopy, particularly excitation emission matrices (EEMs), is a flexible tool which has potential for wide application in studies of aquatic organic matter. Investigation of the source, character and reactions of marine organic matter is common, but investigation into the behaviour of organic matter in freshwater is still lags marine sources. Protein-like peaks T and B are less well understood than humic-like peaks A and C in both source and fluorescence but have been found to be linked to bacterial activity, sewage treatment process efficiency and therefore organic matter bioavailability. Clarification is still required into the source of the protein-like material, to determine whether it is a naturally occurring bioavailable substrate, a product of microbial activity or a mixture of both. In addition, the position of tryptophan within proteins requires investigation as this may have a great impact on the fluorescence of the molecule (Engelborghs, 2003). However, the potential for characterization and quantification of organic matter in natural waters, of allochthonous and autochthonous origin, is becoming more common in freshwater analysis. Of particular interest are the links between fluorescence analysis and current chemical and biological water quality monitoring techniques. If valid correlations can be found, fluorescence could be used as a rapid, on-site tool for water quality testing and pollution monitoring.

## ACKNOWLEDGEMENTS

Thanks to Ian Boomer, David Ward and Cynthia Carliell-Marquet for reading the article draft and suggesting improvements. Catherine Muller of Birmingham, University for Tyrosine standard EEM and Anne Ankorn of Birmingham University for drawing figures. The Environment Agency at Starcross, Exeter, UK for provision of samples used in Figures 3, 5, 6, 7, 10. The work is funded by NERC CASE studentship, ref: NER/S/C/2004/12659. Andy Baker acknowledges the support of the Philip Leverhulme Prize.

## REFERENCES

Ahmad SR, Reynolds DM. 1995. Synchronous fluorescence spectroscopy of wastewater and some potential constituents. *Water Research* **29**(6): 1599–1602.

Ahmad SR, Reynolds DM. 1999. Monitoring of water quality using fluorescence technique: prospect of on-line process control. *Water Research* **33**(9): 2069–2074.



DOI: 10.1002/rra

Aiken GR, McKnight D, Weshaw RL, MacCarthy P. 1985. An introduction to humic substances in soil, sediment and water. *Humic Substances in Soil, Sediment and Water*, Aiken GR, McKnight D, Wershaw RL, MacCarthy P (eds). John Wiley & Sons: New York; 203.

Amon RMW, Benner R. 1996. Photochemical and microbial consumption of dissolved organic carbon and dissolved oxygen in the Amazon River system. *Geochimica et Cosmochimica Acta* **60**(10): 1783–1792.

Baker A. 2001. Fluorescence excitation-emission matrix characterization of some sewage-impacted rivers. *Environmental Science & Technology* **35**(5): 948–953.

Baker A. 2002a. Fluorescence properties of some farm wastes: implications for water quality monitoring. *Water Research* **36**(1): 189–195.

Baker A. 2002b. Spectrophotometric discrimination of river dissolved organic matter. *Hydrological Processes* **16**(16): 3203–3213.

Baker A. 2002c. Fluorescence excitation—emission matrix characterization of river waters impacted by a tissue mill effluent. *Environmental Science & Technology* **36**(7): 1377–1382.

Baker A. 2005. Thermal fluorescence quenching properties of dissolved organic matter. *Water Research* **39**(18): 4405–4412.

Baker A, Curry M. 2004. Fluorescence of leachates from three contrasting landfills. *Water Research* **38**(10): 2605–2613.

Baker A, Inverarity R. 2004. Protein-like fluorescence intensity as a possible tool for determining river water quality. *Hydrological Processes* **18**(15): 2927–2945.

Baker A, Spencer RGM. 2004. Characterization of dissolved organic matter from source to sea using fluorescence and absorbance spectroscopy. *Science of the Total Environment* **333**(1–3): 217–232.

Baker A, Inverarity R, Charlton M, Richmond S. 2003. Detecting river pollution using fluorescence spectrophotometry: Case studies from the Ouseburn, NE England. *Environmental Pollution* **124**(1): 57–70.

Baker A, Ward D, Lieten SH, Periera R, Simpson EC, Slater M. 2004. Measurement of protein-like fluorescence in river and waste water using a handheld spectrophotometer. *Water Research* **38**(12): 2934–2938.

Battin TJ. 1998. Dissolved organic matter and its optical properties in a blackwater tributary of the upper Orinoco river, Venezuela. *Organic Geochemistry* **28**(9–10): 561–569.

Bengraine K, Marhaba TF. 2004. Predicting organic loading in natural water using spectral fluorescent signatures. *Journal of Hazardous Materials* **B108**(3): 207–211.

Benner R, Ziegler S. 1999. Do photochemical transformations of dissolved organic matter produce biorefractory as well as bioreactive substrates? 8th International Symposium on Marine Ecology.

Bertilsson S, Carlsson P, Granéli W. 2004. Influence of solar radiation on the availability of dissolved organic matter to bacteria in the Southern Ocean. *Deep Sea Research II* **51**(22–24): 2557–2568.

Blaser P, Heim A, Luster J. 1999. Total luminescence spectroscopy of NOM-typing samples and their aluminium complexes. *Environment International* **25**(2–3): 285–293.

Boehme J, Coble PG, Conmy RN, Stovall-Leonard A. 2004. Examining CDOM fluorescence variability using principal component analysis: seasonal and regional modelling of three-dimensional fluorescence in the Gulf of Mexico. *Marine Chemistry* **89**(1–4): 3–14.

Brunsdon C, Baker A. 2002. Principal filter analysis for luminescence excitation emission data. *Geophysical Research Letters* **29**(24): Art. No. 2156.

Cammack WKL, Kalf J, Prairie YT, Smith EM. 2004. Fluorescent dissolved organic matter in lakes: relationship with heterotrophic metabolism. *Limnology and Oceanography* **49**(6): 2034–2045.

Chen RF. 1999. *In situ* fluorescence measurements in coastal waters. *Organic Geochemistry* **30**(6): 397–409.

Clark CD, Jiminez-Morais J, Jones G II, Zanardi-Lamardo E, Moore CA, Zika RG. 2002. A time-resolved fluorescence study of dissolved organic matter in a riverine to marine transition zone. *Marine Chemistry* **78**(2–3): 121–135.

Coble PG. 1996. Characterization of marine and terrestrial DOM in seawater using excitation-emission matrix spectroscopy. *Marine Chemistry* **51**(4): 325–346.

Dalterio RA, Nelson WH, Britt D, Sperry J, Psaras D, Tanguay JF, Suib SL. 1986. Steady-state and decay characteristics of protein tryptophan fluorescence from bacteria. *Applied Spectroscopy* **40**(1): 86–90.

de Souza Sierra MM, Donard OFX, Lamotte M. 1997. Spectral identification and behaviour of dissolved organic fluorescent materials during estuarine mixing processes. *Marine Chemistry* **58**(1–2): 51–58.

Del Castillo CE, Coble PG, Morell JM, Lopez JM, Corredor JE. 1999. Analysis of the optical properties of the Orinoco River plume by absorption and fluorescence spectroscopy. *Marine Chemistry* **66**(1–2): 35–51.

Del Vecchio R, Blough NV. 2002. Photobleaching of chromophoric dissolved organic matter in natural waters: kinetics and modelling. *Marine Chemistry* **78**(4): 231–253.

Del Vecchio R, Blough NV. 2004a. Spatial and seasonal distribution of chromophoric dissolved organic matter and dissolved organic carbon in the Middle Atlantic Bight. *Marine Chemistry* **89**(1–4): 169–187.

Del Vecchio R, Blough NV. 2004b. On the origin of the optical properties of humic substances. *Environmental Science & Technology* **38**(14): 3885–3891.

Determann S, Lobbes JM, Reuter R, Rullkötter J. 1998. Ultraviolet fluorescence excitation and emission spectroscopy of marine algae and bacteria. *Marine Chemistry* **62**(1–2): 137–156.

Elkins KM, Nelson DJ. 2001. Fluorescence and FT-IR spectroscopic studies of Suwannee river fulvic acid complexation with aluminium, terbium and calcium. *Journal of Inorganic Biochemistry* **87**(1–2): 81–96.

Elliott S, Lead JR, Baker A. 2006. Thermal quenching of fluorescence of freshwater, planktonic bacteria. *Analytica Chimica Acta* **564**: 219–225.

Engelborghs Y. 2003. Correlating protein structure and protein fluorescence. *Journal of Fluorescence* **13**(1): 9–16.

Esteves da Silva JCG, Machado ASC, Oliveira CJS, Pinto MSSDS. 1998. Fluorescence quenching of anthropogenic fulvic acids by Cu(II), Fe(III) and $UO_2^{2+}$. *Talanta* **45**(6): 1155–1165.



DOI: 10.1002/rra

Ferrari GM, Dowell MD, Grossi S, Targa C. 1996. Relationship between the optical properties of chromophoric dissolved organic matter and total concentration of dissolved organic carbon in the southern Baltic Sea region. *Marine Chemistry* **55**(3–4): 299–316.
Ferrer R, Beltran JL, Guiteras J. 1998. Multivariate calibration applied to synchronous fluorescence spectrometry. Simultaneous determination of polycyclic aromatic hydrocarbons in water samples. *Talanta* **45**(6): 1073–1080.
Fukushima M, Tatsumi K, Nagao S. 2001. Degradation characteristics of humic acid during photo-fenton processes. *Environmental Science & Technology* **35**(18): 3683–3690.
Galapate RP, Baes AU, Ito K, Mukai T, Shoto E, Okada M. 1998. Detection of domestic wastes in Kurose River using synchronous fluorescence spectroscopy. *Water Research* **32**(7): 2232–2239.
Gao H, Zepp RG. 1998. Factors influencing photoreactions of dissolved organic matter in a coastal river of the Southeastern United States. *Environmental Science & Technology* **32**(19): 2940–2946.
Hautala K, Peuravuori J, Pihlaja K. 2000. Measurement of aquatic humus content by spectroscopic analysis. *Water Research* **34**(1): 246–258.
Her N, Amy G, McKnight D, Sohn J, Yoon Y. 2003. Characterization of DOM as a function of MW by fluorescence EEM and HPLC-SEC using UVA, DOC, and fluorescence detection. *Water Research* **37**(17): 4295–4303.
Jaffe R, Boyer JN, Lu X, Maie N, Yang C, Scully NM, Mock S. 2004. Source characterization of dissolved organic matter in a subtropical mangrove-dominated estuary by fluorescence analysis. *Marine Chemistry* **84**(3–4): 195–210.
Kalbitz K, Geyer W. 2001. Humification indices of water-soluble fulvic acids derived from synchronous fluorescence spectra—effects of spectrometer type and concentration. *Journal of Plant Nutrition and Soil Science-Zeitschrift Fur Pflanzenernahrung Und Bodenkunde* **164**(3): 259–265.
Kalbitz K, Geyer S, Geyer W. 2000. A comparative characterization of dissolved organic matter by means of original aqueous samples and isolated humic substances. *Chemosphere* **40**(12): 1305–1312.
Kalle K. 1949. Fluoreszenz und Gelbstoff im Bottnischen und Finnischen Meerbusen. *Deutsche Hydrographische Zeitschrift* **2**: 117–124.
Katsuyama M, Nobuhito O. 2002. Determining the sources of stormflow from the fluorescence properties of dissolved organic carbon in a forested headwater catchment. *Journal of Hydrology* **268**(1–4): 192–202.
Kieber RJ, Hydro LH, Seaton PJ. 1997. Photooxidation of triglycerides and fatty acids in seawater: implication toward the formation of marine humic substances. *Limnology and Oceanography* **42**(6): 1454–1462.
Komada T, Schofield OME, Reimers CE. 2002. Fluorescence characteristics of organic matter released from coastal sediments during resuspension. *Marine Chemistry* **79**(2): 81–97.
Lakowicz. 1999. *Principle of Fluorescence Spectroscopy* 2nd Edition. Kluwer Academic/Plenum Publishers: New York.
Lombardi AT, Jardim WF. 1999. Fluorescence spectroscopy of high performance liquid chromatography fractionated marine and terrestrial organic materials. *Water Research* **33**(2): 512–520.
Marhaba TF, Lippincott RL. 2000. Application of fluorescence technique for rapid identification of DOM fractions in source waters. *Journal of Environmental Engineering* **126**(11): 1039–1044.
Marhaba TF, Van D, Lippincott RL. 2000. Rapid identification of dissolved organic matter fractions in water by spectral fluorescent signatures. *Water Research* **34**(14): 3543–3550.
Maurice PA, Cabaniss SE, Drummond J, Ito E. 2002. Hydrogeochemical controls on the variations in chemical characteristics of natural organic matter at a small freshwater wetland. *Chemical Geology* **187**(1–2): 59–77.
Mayer LM, Schick LL, Loder TC. 1999. Dissolved protein fluorescence in two Maine estuaries. *Marine Chemistry* **64**(3): 171–179.
Miller WL, Moran MA, Sheldon WM, Zepp RG, Opsahl S. 2002. Determination of apparent quantum yield spectra for the formation of biologically labile photoproducts. *Limnology and Oceanography* **47**(2): 343–352.
Mobed JJ, Hemmingsen SL, Autry JL, McGown LB. 1996. Fluorescence characterization of IHSS humic substances: total luminescence spectra with absorbance correction. *Environmental Science & Technology* **30**(10): 3061–3065.
Moberg L, Robertsson G, Karlberg B. 2001. Spectrofluorimetric determination of chlorophylls and pheopigments using parallel factor analysis. *Talanta* **54**(1): 161–170.
Mopper K, Feng Z, Bentjen SB, Chen RF. 1996. Effects of cross-flow filtration on the absorption and fluorescence properties of seawater. *Marine Chemistry* **55**(1–2): 53–74.
Moran MA, Sheldon WM, Zepp RG. 2000. Carbon loss and optical property change during long-term photochemical and biological degradation of estuarine organic matter. *Limnology and Oceanography* **45**(6): 1254–1264.
Mounier S, Patel N, Quilici L, Benaim JY, Benamou C. 1999. Three-dimensional fluorescence of the dissolved organ carbon in the Amazon River. *Water Research* **33**(6): 1523–1533.
Myneni SCB, Brown JT, Martinez GA, Meyer-Ilse W. 1999. Imaging of humic substance macromolecular structures in water and soils. *Science* **286**(5443): 1335–1337.
Nagao S, Matsunaga T, Suzuki Y, Ueno T, Amano H. 2003. Characteristics of humic substances in the Kuji River waters as determined by high-performance size exclusion chromatography with fluorescence detection. *Water Research* **37**(17): 4159–4170.
Newson M, Baker A, Mounsey S. 2001. The potential role of freshwater luminescence measurements in exploring runoff pathways in upland catchments. *Hydrological Processes* **15**(6): 989–1002.
Olivieri AC, Arancibia JA, Munoz de la Peña A, Durán-Merás I, Mansilla AE. 2004. Second-order advantage achieved with four-way fluorescence excitation-emission-kinetic data processed by parallel factor analysis and trilinear least-squares. Determination of methotexate and leucovorin in human urine. *Analytical Chemistry* **76**(19): 5657–5666.
Parlanti E, Wörz K, Geoffroy L, Lamotte M. 2000. Dissolved organic matter fluorescence spectroscopy as a tool to estimate biological activity in a coastal zone submitted to anthropogenic inputs. *Organic Geochemistry* **31**(12): 1765–1781.



DOI: 10.1002/rra

Patel-Sorrentino N, Mounier S, Benaim JY. 2002. Excitation-emission fluorescence matrix to study pH influence on organic matter fluorescence in the Amazon basin rivers. *Water Research* **36**(10): 2571–2581.

Patel-Sorrentino N, Mounier S, Lucas Y, Benaim JY. 2004. Effects of UV-visible irradiation on natural organic matter from the Amazon basin. *Science of the Total Environment* **321**(1–3): 231–239.

Petrenko AA, Jones BH, Dickey TD, LeHaitre M, Moore C. 1997. Effects of a sewage plume on the biology, optical characteristics, and particle size distributions of coastal waters. *Journal of Geophysical Research* **102**(C11): 25,061–25,071.

Piana MJ, Zahir KO. 2000. Investigation of metal ions binding of humic substances using fluorescence emission and synchronous-scan spectroscopy. *Journal of Environmental Science and Health: Part B* **35**(1): 87–102.

Pullin MJ, Cabaniss SE. 1997. Physicochemical variations in DOM-synchronous fluorescence: implications for mixing studies. *Limnology and Oceanography* **42**(8): 1766–1773.

Reynolds DM. 2002. The differentiation of biodegradable and non-biodegradable dissolved organic matter in wastewaters using fluorescence spectroscopy. *Journal of Chemical Technology and Biotechnology* **77**(8): 965–972.

Reynolds DM. 2003. Rapid and direct determination of tryptophan in water using synchronous fluorescence spectroscopy. *Water Research* **37**(13): 3055–3060.

Reynolds D, Ahmad SR. 1995. Effect of metal ions on the fluorescence of sewage wastewater. *Water Research* **29**(9): 2214–2216.

Reynolds DM, Ahmad SR. 1997. Rapid and direct determination of wastewater BOD values using a fluorescence technique. *Water Research* **31**(8): 2012–2018.

Senesi N, Miano TM, Provenzano MR, Brunetti G. 1989. Spectroscopic and compositional comparative characterization of I.H.S.S. reference and standard fulvic and humic acids of various origin. *The Science of the Total Environment* **81–82**: 143–156.

Senesi N, Miano TM, Provenzano MR, Brunetti G. 1991. Characterization, differentiation and classification of humic substances by fluorescence spectroscopy. *Soil Science* **152**(4): 259–271.

Sharpless CM, McGown LB. 1999. Effects of aluminium-induced aggregation on the fluorescence of humic substances. *Environmental Science & Technology* **33**(18): 3264–3270.

Shelly DC, Quarles JM, Warner IM. 1980. Identification of fluorescence pseudomonas species. *Clinical Chemistry* **26**(8): 1127–1132.

Shelly DC, Warner IM, Quarles JM. 1980. Multiparameter approach to the ''fingerprinting'' of fluorescent pseudomonads. *Clinical Chemistry* **26**(10): 1419–1424.

Skoog A, Wedborg M, Fogelqvist E. 1996. Photobleaching of fluorescence and the organic carbon concentration in a coastal environment. *Marine Chemistry* **55**(3–4): 333–345.

Smith CB, Anderson JE, Webb SR. 2004. Detection of Bacillus endospores using total luminescence spectroscopy. *Spectrochimica Acta Part A* **60**(11): 2517–2521.

Stedmon CA, Markager S, Bro R. 2003. Tracing dissolved organic matter in aquatic environments using a new approach to fluorescence spectroscopy. *Marine Chemistry* **82**(3–4): 239–254.

Thacker SA, Tipping E, Baker A, Gondar D. 2005. Development and application of functional assays for freshwater dissolved organic matter. *Water Research* **39**(18): 4559–4573.

Thoss V, Baird MS, Lock MA. 2000. The development of a chemical 'fingerprint' to characterise dissolved organic matter in natural waters. *Journal of Environmental Monitoring* **2**(5): 398–403.

Uyguner CS, Bekbolet M. 2005. Evaluation of humic acid photocatalytic degradation by UV-vis and fluorescence spectroscopy. *Catalysis Today* **101**(3–4): 267–274.

Vasel JL, Praet E. 2002. On the use of fluorescence measurements to characterize wastewater. *Water Science and Technology* **45**(4–5): 109–116.

Vodacek A, Philpot WD. 1987. Environmental effects on laser-induced fluorescence spectra of natural waters. *Remote Sensing of Environment* **21**(1): 83–95.

Waiser MJ, Robarts RD. 2004. Photodegradation of DOC in a shallow prairie wetland: evidence from seasonal changes in DOC optical properties and chemical characteristics. *Biogeochemistry* **69**(2): 263–284.

Westerhoff P, Chen W, Esparza M. 2001. Fluorescence analysis of a standard fulvic acid and tertiary treated wastewater. *Journal of Environmental Quality* **30**(6): 2037–2046.

Wehry EL. 1973. Effects of molecular structure and molecular environment on fluorescence. In *Practical Fluorescence: Theory, Methods, and Techniques*, Guilbault GG (ed). Marcel Dekker: New York; 79–136.

White EM, Vaughan PP, Zepp RG. 2003. Role of the photo-Fenton reaction in the production of hydroxyl radicals and photobleaching of colored dissolved organic matter in a coastal river of the southeastern United States. *Aquatic Sciences* **65**(4): 402–414.

Wu FC, Evans RD, Dillon PJ. 2003. Separation and characterization of NOM by high-performance liquid chromatography and on-line three-dimensional excitation emission matrix fluorescence detection. *Environmental Science & Technology* **37**(16): 3687–3693.

Yamashita Y, Tanoue E. 2003. Chemical characterization of protein-like fluorophores in DOM in relation to aromatic amino acids. *Marine Chemistry* **82**(3–4): 255–271.

Yan Y, Li H, Myrick ML. 2000. Fluorescence fingerprint of waters: excitation-emission matrix spectroscopy as a tracking tool. *Applied Spectroscopy* **54**(10): 1539–1542.

Yang Y, Zhang D. 1995. Concentration effect on the fluorescence spectra of humic substances. *Communications in Soil Science and Plant Analysis* **26**(15–16): 2333–2349.

Zanardi-Lamardo E, Moore C, Zika RG. 2004. Seasonal variation in molecular mass and optical properties of chromophoric dissolved organic material in coastal waters of southwest Florida. *Marine Chemistry* **89**(1–4): 37–54.



DOI: 10.1002/rra