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Fluorescence Spectroscopy as a Potential Tool for *In-Situ* Monitoring of Dissolved Organic Matter in Surface Water Systems

Elfrida M. Carstea
National Institute of Research and Development for Optoelectronics INOE 2000, Magurele, Ilfov
Romania

1. Introduction

Water is a common substance, yet life cannot exist without it, being the major component of all living things. Considering the tremendous impact water has on life health, it is always an imperative task to study its quality. During the past decades, more advanced techniques were developed not only to generally characterise the water quality, but also to analyse DOM fractions.

Organic matter is present in every type of aquatic system and, due to the influence that it has on their ecological health, it can be used as a useful water quality indicator. The organic matter fraction from natural waters can be autochthonous, formed in situ through microbial activity, algal productivity, invertebrate grazing, etc., and allochthonous, formed externally and brought into the water system through soil leaching, geological activities or degradation of terrestrial vegetation (Winter et al., 2007). Human activities can influence both of these fractions: increased algal - derived organic matter due to eutrophication increased microbially - derived organic matter from human and animal wastes, and changes in allochthonous organic matter from changes in land use.

An emerging technique, fluorescence spectroscopy, which was successfully used in biology, medicine or chemistry, became a promising approach to the assessment of organic aquatic components and organic pollutants, due to its rapid analysis and high sensitivity. Fluorescence spectroscopy, in the form of three dimensional excitation-emission matrix (EEM), synchronous fluorescence spectrum (SFS) and laser induced fluorescence spectrum (LIFS) can be used to estimate water pollution and to probe the composition of DOM in watersheds. Although the fluorescence technique have been in the attention of those who are interested in real-time monitoring of water pollution, only few studies have been made in this field (Carstea et al., 2010; Downing et al., 2009; Spencer et al., 2007).

This paper proposes to review some of the methods potential to characterise different water systems that have dissimilar hydrological and geographical features and different sources of water pollution. Prior to this, theoretical aspects of fluorescence principles and dissolved organic matter properties will be shortly described.

2. Principles of fluorescence spectroscopy

Fluorescence is a special type of luminescence that describes the emission of light from molecules, named fluorophores, in electronically excited states. The fluorophores absorb energy in the form of light, at a specific wavelength, and release it in the form of emission of light, at a specific higher wavelength (i.e., with lower energy). The general principles of light absorption and emission can be illustrated by a Jablonski diagram, as seen in figure 1.

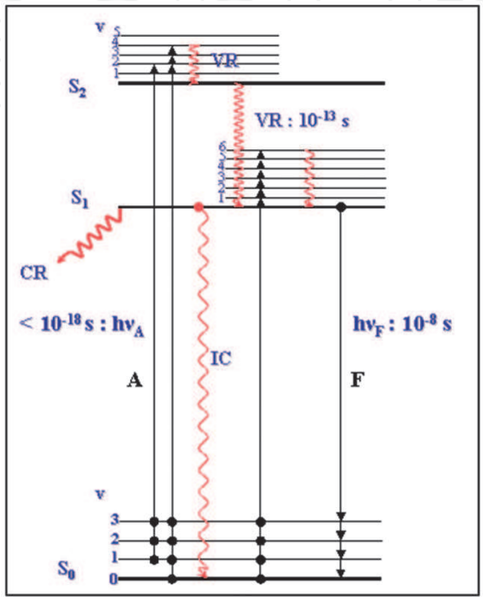


Fig. 1. Jablonski diagram presenting the processes of absorption (A) and fluorescence (F); VR – vibrational relaxation, IC – internal conversion.

When a molecule is found in the ground singlet state, S_0, and absorbs light, the light energy is transferred to the electronically excited states: singlet states, S_1 or S_2. Afterwards, the molecule is subjected to internal conversion or vibrational relaxation, which implies the transition from an upper electronically excited state to a lower one, lasting from 10^{-14} to 10^{-11} s. In the final stage, emission occurs when the molecule returns to the ground state, S_0, in 10^{-9} to 10^{-7} s, emitting light at a greater wavelength, according to the difference in energy between the two electronic states (Lakowitz, 2006; Valeur, 2001). This process is known as fluorescence. When excitation source is a laser, the fluorescence is called laser induced fluorescence.

The Jablonski diagram shows that the energy of the emission is generally less than that of absorption. Thus, fluorescence typically occurs at lower energies or longer wavelengths. This effect is called Stokes' shift, which is caused by several factors: the rapid decay to the lowest vibrational level of S_1, further decay of fluorophores to higher vibrational levels of S_0, solvent effects, excited-state reactions, complex formation, and/or energy transfer (Lakowicz, 2006).

Generally, the emission spectrum for a given fluorophore is a mirror image of the excitation spectrum. The symmetry is a result of the same transitions, which are involved in absorption and emission and the similarities of the vibrational levels of S_0 and S_1 (Christensen, 2005).

2.1 Factors affecting fluorescence intensity

The fluorescence excitation and emission spectra comply with the above-mentioned rule and properties, but several environmental factors can change the characteristics of the fluorescence signal. The fluorescence response is highly affected by solution temperature, composition, concentration, pH and salinity. These factors are presented in the following sections.

2.1.1 Fluorescence quenching

Fluorescence quenching is a term, which covers any process that leads to a decrease in fluorescence intensity of a sample. It is a deactivation of the excited molecule either by intra- or intermolecular interactions. Quenching can be divided into two main categories: static and dynamic quenching.

When the environmental influence (quencher) inhibits the excited state formation, the process is referred to as static quenching. Static quenching is caused by ground state complex formation, where the fluorophore forms non-fluorescent complexes with a quencher molecule. Dynamic quenching or collisional quenching refers to the process when a quencher (e. g. oxygen) interferes with the behaviour of the excited state after its formation. The excited molecule will be deactivated by contact with other molecules or by intermolecular interactions (collision). A wide variety of substances can act as quenchers of fluorescence for different fluorophores (Christensen, 2005; Lakowicz, 2006). In table 1, the quenchers of typical fluorphores are presented.

Typical fluorophore(s)	**Quencher(s)**
Tryptophan	Acrylamide, halogen anesthetics, hydrogen peroxide, imidazole, histidine, picolinium nicotinamide, succinimide, trifluoroacetamide
Anthracene	Amines, halogens, iodide, thiocyanate
Tyrosine	Disulfides
Polycyclic aromatic hydrocarbons	Nitromethane and nitro compounds
Aromatic hydrocarbons, chlorophyll	Quinones
Naphthalene	Nitroxides, nitric oxide, halogens
Most fluorophores	Oxygen

Table 1. Fluorescence quenchers of typical fluorophores (adapted from Lakowicz, 2006).

Generally, in water the most important fluorescence quencher with high impact on the fluorescence response is temperature. Quenching is enhanced with increasing temperature determining the electrons within a molecule to return to the ground state by a radiationless process. In a study on dissolved organic matter (DOM) thermal fluorescence quenching, Baker (2005) showed that by decreasing the temperature from 45^0 C to 10^0 C the DOM fluorescence intensity increased with ~ 48 %. According to Baker's study (2005) the most affected fluorophore is tryptophan in comparison with fulvic acid.

Fluorescence quenching of dissolved organic matter (DOM) can also be induced using certain metal ions, like Cu^{2+}, Fe^{2+}/Fe^{3+}, Al^{3+}, etc. by the process of complex formation. Metal quenching affects mostly the humic substances and less the amino acids. Most studies have been performed in laboratories, under controlled conditions and little is known about the effects on natural organic matter (Kelton et al., 2007; Reynolds & Ahmad,1995).

2.1.2 Concentration and inner filter effect

Within the context of fluorescence measurements, the inner filtering effect (IFE) represents an apparent decrease in emission quantum yield and/or a distortion of band shape as a result of the absorption and emitted radiation by the sample matrix (Henderson et al, 2009). The fluorescence intensity is attenuated by:

- **Primary inner-filter effect,** referring to the absorption of the excitation beam prior to reaching the interrogation zone;
- **Secondary inner filter effect**, which refers to the absorption of the emitted fluorescence photons (Ohno, 2002);
- **Inner filter effects due to the presence of other substances**. When the solution contains other chromophores that absorb in the same wavelength range as the fluorescent compound under study, the chromophores act as filters at the excitation wavelength and the fluorescence intensity must be multiplied by a correction factor (Valeur, 2001).

In order to be easily understood, the primary and secondary IFE are graphically presented in figure 2.

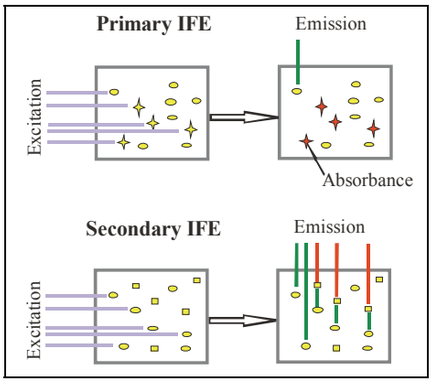


Fig. 2. Diagram of the mechanisms of inner filter effect: primary IFE and secondary IFE.

Various authors have suggested different approaches to correct for IFE, the two most common being an empirical correction based on the Raman scatter peak and a mathematical one based on absorbance profile of the same sample (Parker and Barnes, 1957; Lakowicz, 2006). An alternative approach is to leave the data uncorrected, and utilise the resulting wavelength-dependent non-linear relationship between fluorescence intensity and concentration (Henderson et al., 2009).

The IFE can also be avoided by using front-face illumination because it offers the advantage of being much less sensitive to the excitation inner filter effect. The illuminated surface is better oriented at 30^0 than at 45^0, because at 45^0 the unabsorbed incident light is partially reflected towards the detection system, which may increase the stray light interfering with the fluorescence signal (Valeur, 2001).

Another technique to minimise the IFE is to reduce the path length of the excitation light through the cuvette, but only the primary IFE is reduced. Simple sample dilution to a concentration at which IFE effects are negligible has also been suggested (Baker and Curry, 2004; Baker et al., 2004). There have also been recommendations towards the appropriate concentration quantified as absorbance values. Kubista et al. (1994) suggested that IFE does not occur if the samples show absorbance values lower than 0.05, while Pagano and Kenny (1999) indicate a threshold of 0.01.

2.1.3 Influence of pH on the fluorescence

The pH value of the sample affects the fluorescence of a fluorophore. The pH influence on fluorescence intensity of DOM components always presents the same trend: intensities increase with higher pH until 10, as observed by Reynolds and Ahmad (1995) at raw sewage samples. A small plateau is seen at pH from 5 to 7. Generally, it is not recommended to alter the pH of a sample, but special attention should be paid and the fluorescence spectra should be corrected (Baker, 2007). Similar results have been obtained by Patel-Sorentino et al. (2002) for the humic substances, only that after a fluorescence intensity increase until 10, a slight decrease of its intensity occurs at pH 12.

According to Patel-Sorentino et al. (2002), there are three possible hypotheses:

- **Alteration of the molecular orbital of the excitable electrons**, as a consequence of ionization of the fluorescent molecules after modifications of pH.
- **Macromolecular configuration of humic substances**: the more rigid structures are giving better fluorescent yields. Ghosh and Schnitzer (1980) observed that the structure of humic substances varied with pH changes. Their conclusion is that humic substances have linear structure at high pH and a coil one when pH decreases. Patel-Sorentino et al. (2002) also explained that a spherocolloied configuration could mask some fluorophores inside their structure. At higher pH, the configuration becomes linear and some fluorophores, which are not anymore masked, can fluoresce, increasing the fluorescence intensity.
- **Metal ions present in freshwaters**. This implies that there are some competition phenomena between H^+ ions and metal ions to complex DOM in freshwater, leading to complexation-decomplexation processes which directly affect fluorescence intensity. However, Patel-Sorentino et al. (2002), also note that the metal ions concentration in freshwater samples would be lower than the concentration of metals that quench the fluorescence of DOM. Therefore, the metal ions, which can increase the fluorescence, would have a too weak effect to produce a significant variation in DOM fluorescence intensity.

2.1.4 Salinity influence on the fluorescence

Salinity can affect DOM fluorescence by altering intramolecular reactions, such as conformational change and charge transfer. This results in an increased photoreactivity and fluorescence loss in certain fluorescence compounds (Osburn, 2001; Chen et al., 2002). The relationship between salinity and fluorescence intensity could help detect the source of natural organic matter in marine waters (Elliot, 2006; PhD Thesis).

Del Castillo et al. (1999) investigated changes in chromophoric DOM composition by studying the shifts in the fluorescence maxima. Where the salinity at a site was high, the position of the emission maximum at 350 nm excitation wavelength was shifted to shorter wavelengths, suggesting that high salinity leads to changes in chromophoric DOM.

As shown in previous sections, fluorescence technique is a powerful tool in analysing different samples, but several environmental factors must be taken into account. Most problems arise at highly polluted samples, which imply high concentration of the contaminant and in this case filtration, dilution and absorption to check for IFE are recommended. The pH, salinity and temperature of the sample should be measured, if possible, and the excitation and emission spectra should be corrected, if parameters values are above or under the normal domain. No correction is needed if the sample temperature, at the time of measurement, is between 20^0 C and 25^0 C, or if the pH is between 6 and 8. In

conclusion, the general recommendation is that all these parameters need to be measured before the fluorescence analysis are performed and reported in the scientific literature.

2.2 Techniques for fluorescence spectra recording

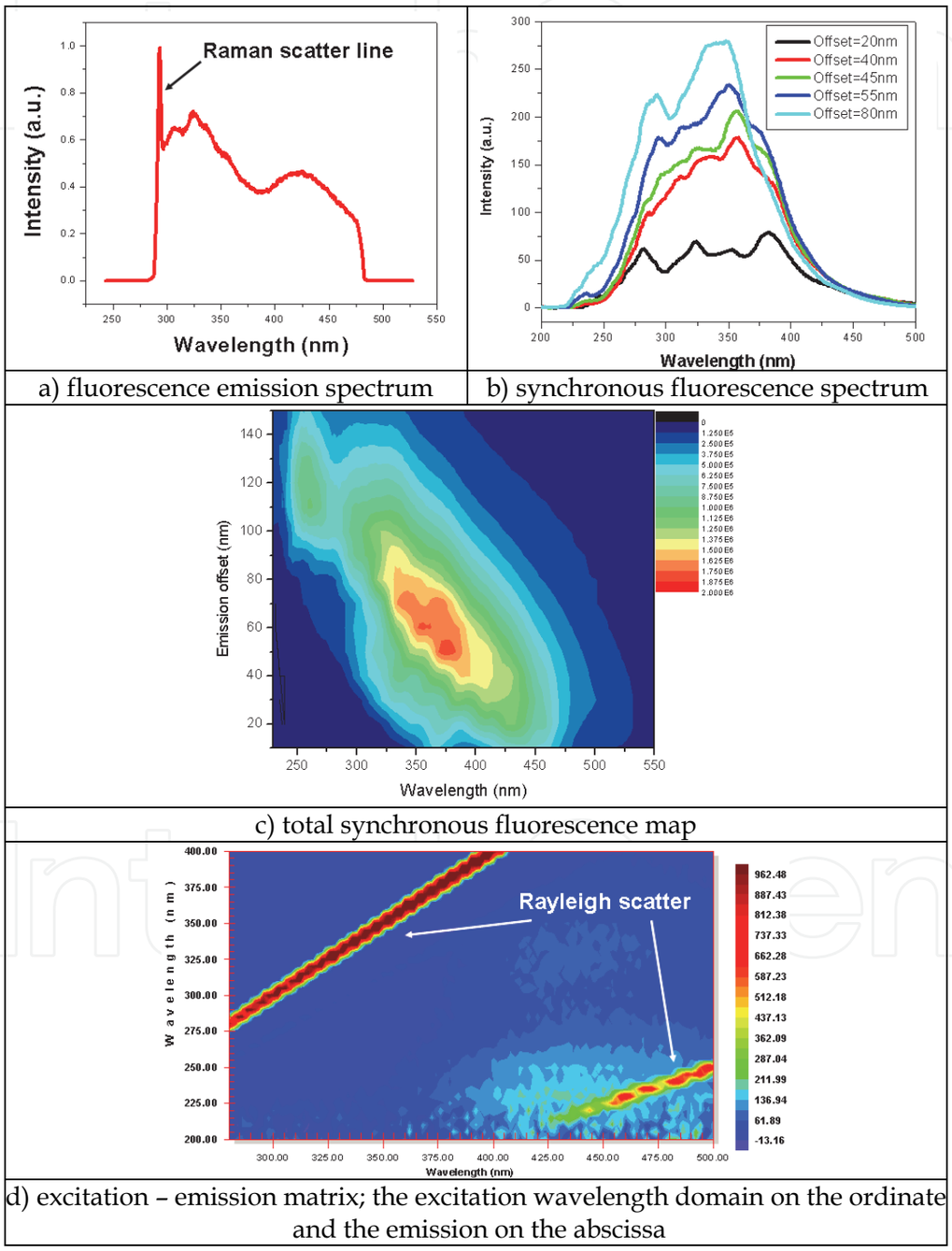
The fluorescence signal is typically recorded as a: fluorescence emission spectrum, fluorescence excitation spectrum, synchronous fluorescence spectrum, total synchronous fluorescence spectrum or excitation – emission spectrum (Figure 3). An emission spectrum consists in the wavelength distribution of the light emission, measured at a single constant excitation wavelength (Figure 3a). Conversely, an excitation spectrum represents the dependence of emission intensity, measured at a single emission wavelength, upon the excitation wavelengths.

In most cases, the analysed samples contain complex multi-component mixtures which cannot be resolved satisfactorily by conventional fluorescence methods. Due to these gaps, for rapid, sensitive, and selective fluorescence analysis, three state-of–the-art methods have been introduced: synchronous fluorescence spectroscopy (SFS), total synchronous fluorescence spectroscopy (TSFS) and excitation-emission matrix (EEM) (Coble, 1996, Deepa and Mishra, 2006; Hudson et al., 2007). As mentioned earlier, an emission or excitation spectrum is recorded by separately scanning the excitation, respectively emission monochromator at various wavelengths. SFS spectra are recorded by scanning both monochromators simultaneously (Deepa and Mishra, 2006) (Figure 3b). Using SFS, the spectral band is narrowed and sharper peaks can be obtained by applying the optimum wavelength offset ($\Delta\lambda$) between excitation and emission. A SFS spectrum is illustrated as fluorescence intensity function of excitation wavelength, for a certain $\Delta\lambda$ (Figure 3c). Total synchronous fluorescence spectrum offers more selectivity and sensitivity to multi-fluorophores mixture analysis. It is presented as a contour map, containing numerous synchronous spectra at different offsets gathered into one bi-dimensional image (Figure 3c). An example of TSFS map is shown in figure 1.18 for a water sample with high protein-like fluorescence intensity.

The last method for complex multi-compounds mixture detection is to record the fluorescence signal as excitation-emission matrices. EEMs represent fluorescence contour maps, in which repeated emission scans are collected at numerous excitation wavelengths providing highly detailed information (Coble, 1996; and references therein) (Figure 3d). Coble (1996) mentions that, once the EEMs have been fully corrected for instrumental configuration, data can be analyzed as excitation spectra, emission spectra or surface spectra, even though originally collected as emission scans (Figure 3d). The EEMs are very simple to analyse because the fluorescence intensity maximum are identified as $\lambda_{excitation}$ / $\lambda_{emission}$ pairs. Usually, the images are colour coded, the highest intensity being represented with red and the lowest with blue.

At a closer inspection of a water fluorescence spectrum, other maxima can be observed. These belong to the scattering of the incident light and are most intense when dealing with turbid solutions and solid opaque samples. Scattering can affect the fluorescence signal, therefore it is of utmost importance to check the absorbance measurements and correct the fluorescence response. Scattered light can be divided into Rayleigh scatter and Raman scatter, according to its nature. Rayleigh scatter is the scattering of light by particles and molecules smaller than the wavelength of the light. Rayleigh scattering represents so-called elastic scatter, meaning that no energy loss is involved, so that the wavelength of the scattered light is the same as that of the incident light. The Rayleigh scatter can be observed

as a diagonal line in fluorescence landscapes for excitation wavelengths equalling the emission wavelengths, as seen in figure 3d. Due to the construction of grating monochromators used for excitation in most spectrofluorometers, also some light at the double wavelength of the chosen excitation will pass through to the sample. For this reason an extra band of Rayleigh scatter, 2nd order Rayleigh, will typically appear in fluorescence measurement (Christensen, 2005).



a) fluorescence emission spectrum

b) synchronous fluorescence spectrum

c) total synchronous fluorescence map

d) excitation – emission matrix; the excitation wavelength domain on the ordinate and the emission on the abscissa

Fig. 3. Typically recorded fluorescence spectra for water samples

Raman scatter is an inelastic scatter, caused by the absorption and re-emission of light coupled with vibrational states. A constant energy loss will appear for Raman scatter, meaning that the scattered light will have a higher wavelength than the excitation light, with a constant difference in wavenumbers. In figure figure 3a, the Raman scattering can be seen as a diagonal line with a systematic, increasing deviation from the Rayleigh scatter line, since the axis is shown as wavelengths, which is not proportional to the energy of the light.
The Raman scatter line can be used to check for instrument stability and to quantify the degree of contamination from a water sample by using the normalised fluorescence intensity to the Raman peak. The advantages offered by the Raman line are: (a) the independence of the chemistry since it measures the properties of the solvent; (b) the ease of application and sensitivity; (c) versatility since it can be applied at any wavelength between 200 and 500 nm. In the case of water the Raman line offers the advantage that it is very stable, appearing in the spectrum at the same offset from the excitation wavelength.

3. Dissolved organic matter fluorescence

The dissolved organic matter (DOM), the ubiquitous fraction in soil and aquatic ecosystems, is a heterogeneous mixture of humic substances, fatty acids and phenolic compounds, amino acids, nucleic acids, carbohydrates, hydrocarbons and other compounds, being among the largest reservoirs of carbon on the planet (Spitzy and Leenheer, 1991; Thomas, 1997; Swietlik and Sikorska, 2004). The dynamics and characteristics of DOM strongly influence a number of key ecosystem processes, including the attenuation of solar radiation, control of nutrient availability, alteration of contaminant toxicity, material and energy cycling (Cammack 2002; PhD Thesis; and references therein). The composition of DOM differs depending on source: it is estimated to contain 0.5 mg/L dissolved organic carbon in alpine streams or 100 mg/L in wetland streams (Spitzy and Leenheer, 1991; Frimmel, 1998). Only 25 % of DOM is fully characterized. It is estimated that 40–70 % from aquatic dissolved organic matter is composed of humic substances (Thurman, 1985; Senesi, 1993).
By the type of production, DOM can be classified as natural or derived from human activity (human wastes, farm wastes, leachates, etc.), but by the origin, DOM can be either allochthonous or autochthonous. Allochthonous DOM, is the fraction that is formed outside the water system and transported inside through discharge, geological and land-use activities or dry and wet deposition (McDowell and Likens, 1988; Hudson et al., 2007). The composition and concentration of allochthonous DOM, in aquatic systems, is dependent mostly on the soil type, catchment, precipitation, vegetation, flow path of water through different soil horizons and other soil processes (Hope et al., 1997; Aitkenhead et al., 1999). Autochthonous DOM is formed within the water system, through derivation from polymerisation and degradation of existing DOM, release from living and dead organisms and through microbial syntheses within the body water (Thomas, 1997).
Within the complex heterogeneous mixture of DOM, only the following components are mostly studied by fluorescence: proteins and humic substances. The protein fluorescence is given by the amino acids tryptophan, tyrosine and phenylalanine, and is related to the activity of bacterial communities, as shown by Cammack et al. (2004) and Elliot et al. (2006a). The humic substances fluorescence indicates 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). Humic substances are divided into two major fractions, depending on the solubility at different pH values: humic

acids which are insoluble in aqueous solution at pH lower than 2, but soluble at higher pH and fulvic acids soluble in water under all pH conditions (Aiken et al., 1985). DOM fluorophores are schematically represented in figure 4, along with their corresponding excitation/emission wavelengths domains. Due to the difficulties associated with identifying of the 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 the standards of these materials (Hudson et al., 2007).

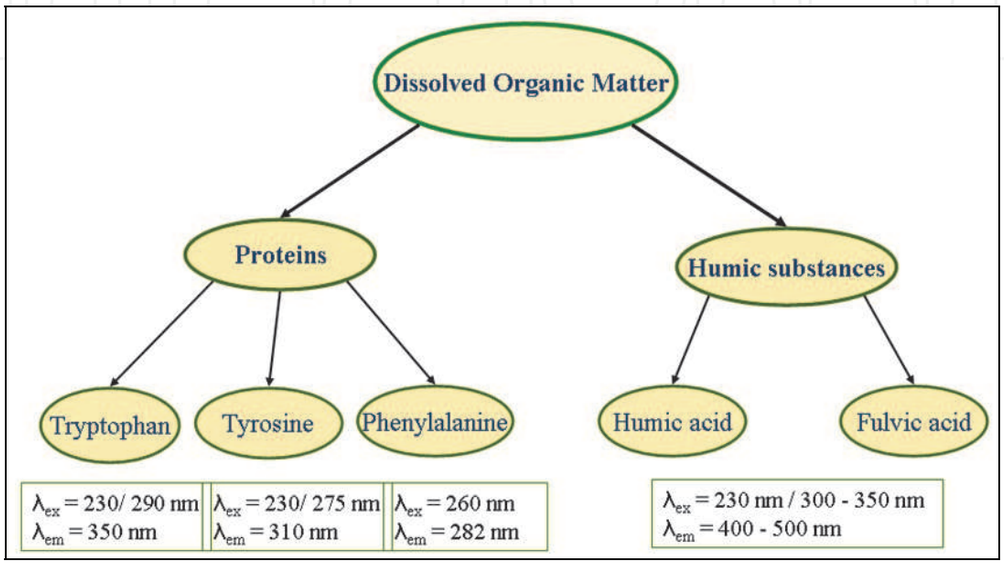


Fig. 4. Schematic representation of DOM fluorescent fractions with the specific excitation/emission wavelengths domains.

Beside standard nomenclature (e.g. humic-like), Coble (1996) defined the humic substances as peak **A** ($\lambda_{excitation}$ = 230 nm, $\lambda_{emission}$ = 400 – 500 nm) and peak **C** ($\lambda_{excitation}$ = 300 – 350 nm, $\lambda_{emission}$ = 400 – 500 nm), tryptophan as peak **T** and tyrosine as peak **B**. Tryptophan, also, presents two excitation wavelengths, therefore, $\mathbf{T_1}$ corresponds to the peak at $\lambda_{excitation}$ = 290 nm and $\mathbf{T_2}$ to the peak at $\lambda_{excitation}$ = 230 nm (Figure 5).

Certain types of contaminants and their relative impact on the system can only be determined by analysing the fluorescence intensity, excitation and emission wavelengths of the above mentioned fluorophores. In the past decades, numerous studies have shown that these fluorophores can provide more information about the characteristics of DOM and the aquatic system. According to some studies, peak C fluorescence intensity correlates with total organic carbon (Smart et al., 1976; Vodacek et al., 1995; Ferrari et al., 1996) and shows a linear relationship with aromaticity (McKnight et al., 2001). Fluorescence intensity of peak C also relates with the molecular weight of the organic fractions, showing lower values for smaller molecular weight fractions (Stewart and Wetzel, 1980). Peak C emission wavelength shows the degree of hydrophobicity, a higher emission wavelength corresponding to greater degree of hydrophobicity (Wu et al., 2003). Peak T presents a very strong correlation with the standard parameter biological oxygen demand (BOD). Some researchers (Reynolds and Ahmad, 1999; Hudson et al., 2008) even tested the possibility of using peak T fluorescence as a surrogate for the standard water quality parameter, BOD. The relationship between

biological activity of aquatic plankton, along with algae metabolism rates, and peak T fluorescence intensity for different DOM types has also been observed (Bieroza et al., 2009 and references therein).

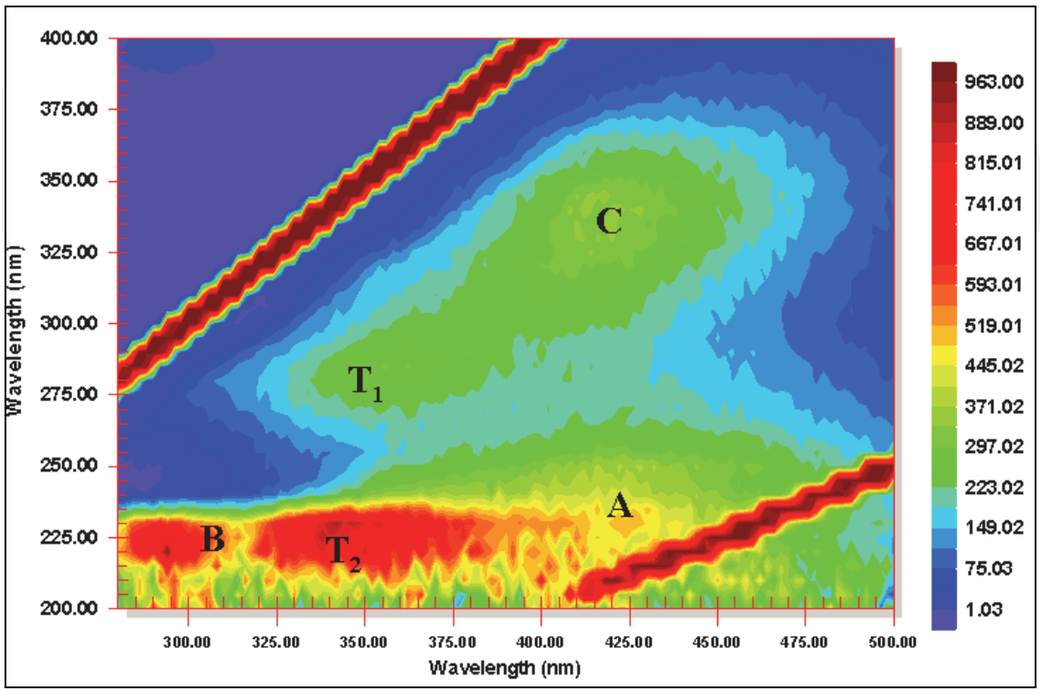


Fig. 5. Excitation – emission matrix presenting the fluorescence domains of the humic – like, peaks A and C, and protein-like fractions, peak B (tyrosine) and peaks T_1 and T_2 (tryptophan).

4. Fluorescence properties of common aquatic pollutants

Water systems naturally contain, as shown in previous sections, organic matter with two very important fluorescence components: humic substances and proteins, which differ in quantity depending on the water body. When high quantities of one component is produced or released into the water, then the balance of the ecosystem is disrupted with potentially long-term effects. Apart from humic substances and proteins, there are other compounds that can contaminate the water and can be detected with fluorescence spectroscopy: polycyclic aromatic hydrocarbons (PAHs), pesticides, environmental hormones. Pesticides and PAHs reach the aquatic environment through direct runoff, leaching, careless disposal of empty containers, equipment washing a.s.o. (Konstantinou et al., 2006). Due to their toxic nature, persistence in the environment and presence in any type of system (soil, surface water, groundwater) many studies concentrated on sensitive, selective and early detection of these pollutants (e.g. Ferrer et al., 1998; Jiji et al., 1999; Jiji et al., 2000; Selli et al., 2004; Deepa et al., 2008). The standard techniques for pesticides and PAHs detection are gas and liquid chromatography, which require tedious extraction or separations procedures and expensive equipments. Fluorescence spectroscopy is a rapid and cost effective alternative, since PAHs and many pesticides are naturally fluorescent (Jiji et al., 1999). When dealing with more components, the use of SFS or EEM techniques is recommended.

For instance, petroleum products contain a complex mixture of generically classified as: aromatic hydrocarbons and alyphatic hydrocarbons. Only aromatic hydrocarbons exhibit fluorescence and the emission wavelength is proportional to the number of aromatic ring one compound has. Therefore, monoaromatic compounds (benzene, toluene, xylene and phenols) emit fluorescence between 250 – 290 nm. Two aromatic ring compounds, like naphthalene, show a fluorescence peak at 310 – 330 nm, phenanthrenes (three aromatic rings) between 345 – 355 nm and so on (Pharr et al., 1992; Abbas et al., 2006). Pharr et al. (1992) has shown that, only by using standard fluorescence emission spectra, distinguishing between 2 brands of gasoline would be impossible.

Care should be taken when measuring petroleum products if sophisticated fluorescence spectra are recorded. Since they contain a large number of fluorophores, it is difficult to choose the proper excitation domain and if there is a high concentration of petroleum products in the sample the inner filter effect can interfere and change the real feature of fluorescence emission (Ryder, 2005). The phenomenon of concentration-dependent red-shift of fluorescence, observed in multifluorophoric systems at high concentrations, has been successfully used in the analytical fluorimetry for systems like petroleum derivatives, humic substances, biological fluids, etc. Divya and Mishra (2008) proposed a method to get the appropriate excitation wavelength by using derived absorption spectra for different concentration of petroleum products (Figure 2.6).

The spectral fingerprints for the major petroleum products that can be present as environmental pollutants (petrol, kerosene, Diesel and engine oil) have been acquired by Patra and Mishra (2002a), Cristescu et al. (2009) and Carstea et al. (2009a). The authors observed that heavy oil, Diesel and engine oil, show fluorescence maxima in the longer emission wavelength region (420 – 550 nm) while lighter oils, petrol and kerosene, present peaks mainly in an intermediate wavelength region (310 – 400 nm).

Patra and Mishra (2002b) also studied the effects of adulteration of petroleum products on the fluorescence signal. The authors found that the emission wavelength changes according to the concentration of adulterant into the solution. Similar effect has been observed at petrol adulterated with kerosene, in a more recent study performed by Divya and Mishra (2008).

Beside adulteration, the effects of age on petroleum products have been studied (Li et al., 2004; Deepa et al., 2006). It is well-known that the persistence of PAHs in the environment depends on numerous factors as: physical and chemical characteristics of PAHs and medium, concentration, dispersion and bioavailability of PAHs. Generally, high molecular weight PAHs (> 4 rings) present higher degree of toxicity and longer persistence in the environment compared to low molecular weight PAHs (< 3 rings). For example, the tricyclic phenantherene half-life ranges from 16 to 126 days in soil, whereas benzo[a]pyrene (5 rings) has a half-life ranging from 229 to 1,500 days (Chauhan et al., 2008). According to this fact, one can assume that the fluorescence spectrum could change if a petroleum product ages. Deepa et al. (2006) studied the fluorescence signal of transformer oil during its aging process, which was thermally induced at 100^{o}C for 31 days. They observed a sudden dramatic decrease in fluorescence intensity after only 17 days, for raw oil, followed by a slight increase until day 31. Also, the excitation and emission maxima increased starting with the 20th day. Deepa et al. (2006) explained that when transformer oil was degraded, its acidity increased, resulting an increase in C-O band and C=C double bands. During thermal decomposition, paraffinic compounds have dehydrogenated and formed hydroperoxides, resulting in, after oxidation, aldehydes and ketones by a free radical mechanism. The

authors explained that the sudden drop in fluorescence intensity, on the 17th day, could be caused by the presence of antioxidants in the oil sample which inhibited degradation until the antioxidants were consumed.

Li et al. (2004) evaluated the fluorescence signal of more aged products, but using a different method for adulteration, compared to Deepa et al. (2006). Weathering was induced after preserving the samples in a refrigerator for no more then 15 days. The researchers concluded that, because the weathering process is so complex and unpredictable, the attempts to both characterize the oil and the exact extent of weathering would be impossible. At one sample, which presented, before weathering 3 emission peaks at ~ 360, 375 and 415 nm, when excited with 254 nm, a decrease in fluorescence intensity was noticed.

PAHs can enter and pollute the environment not only by petroleum products, but also by car tyres, coal tar or creosote. Therefore the analysis of distinct PAHs is important, especially for the 16 PAHs included in the EPA list as being very toxic, mutagenic and carcinogenic. Giamarchi et al. (2000) recorded the fluorescence spectra for 5 PAHs, most commonly tested in drinking water: fluorene, naphthalene, phenantherene, benzo[a]pyrene, fluoranthene, with excitation wavelength at 263 nm, illustrated in figure 2.9. Also, Giamarchi et al. (2000) obtained almost the same emission peaks in a mixture of the 5 PAHs, both at high concentration and low concentration. Naphthalene fluorescence was overlapped by fluorene due to high intensity and one peak belonging to benzo[a]pyrene and phenanthrene was also overlapped. Ferrer et al. (1998) applied SFS to 10 PAHs, as pure samples and mixtures and obtained similar results. Hence, Giamarchi et al. (2000) recommend the separation of PAHs, from the mixture, in order to clearly identify each component. Similar study has shown that by using a mathematical model the compounds can be separated. Jiji et al. (1999) resolved the spectra of pyrene and chrysene, from a mixture, with 3 way-PARAFAC model. In the same way, Jiji et al. (1999) separated the spectra of pesticides which also contain a mixture of fluorophores which overlap. Some authors (Burel-Deschamps et al., 2006; Pascu et al., 2001) used absorption spectroscopy and laser induced fluorescence for pesticide monitoring in water. Organochlorurate pesticides in water, crude oil and oil components in water and soil with detection limits of 10^{-1}–10^{-2} ppm were obtained.

In conclusion, fluorescence spectroscopy can be used not only to detect protein-like and humic-like fractions, but also petroleum and pesticide pollution. It is highly important, when dealing with these pollutants, to identify the specific contaminant in order to act quickly for decontamination. But, fluorescence spectroscopy can only be applied for preliminary information about a contaminant, researchers recommending subsequent standard analyses.

5. Real-time monitoring of water quality

Fluorescence spectroscopy has been intensely used in recent decades for the analysis of DOM and organic pollutants in water. Additionally, fluorescence technique correlates with standard parameters, like biological oxygen demand or dissolved oxygen (Pfeiffer et al., 2007; Hudson et al., 2008). Some researchers have pointed out that it may be possible to use fluorescence spectroscopy for water quality monitoring purposes, in order to identify DOM characteristics at temporal scale, detect pollutants and be used as a surrogate for standard measurements (for example, Ahmad & Reynolds, 1999; Henderson et al., 2009; Carstea et al. 2010).

5.1 Fluorescence fingerprints of various water types

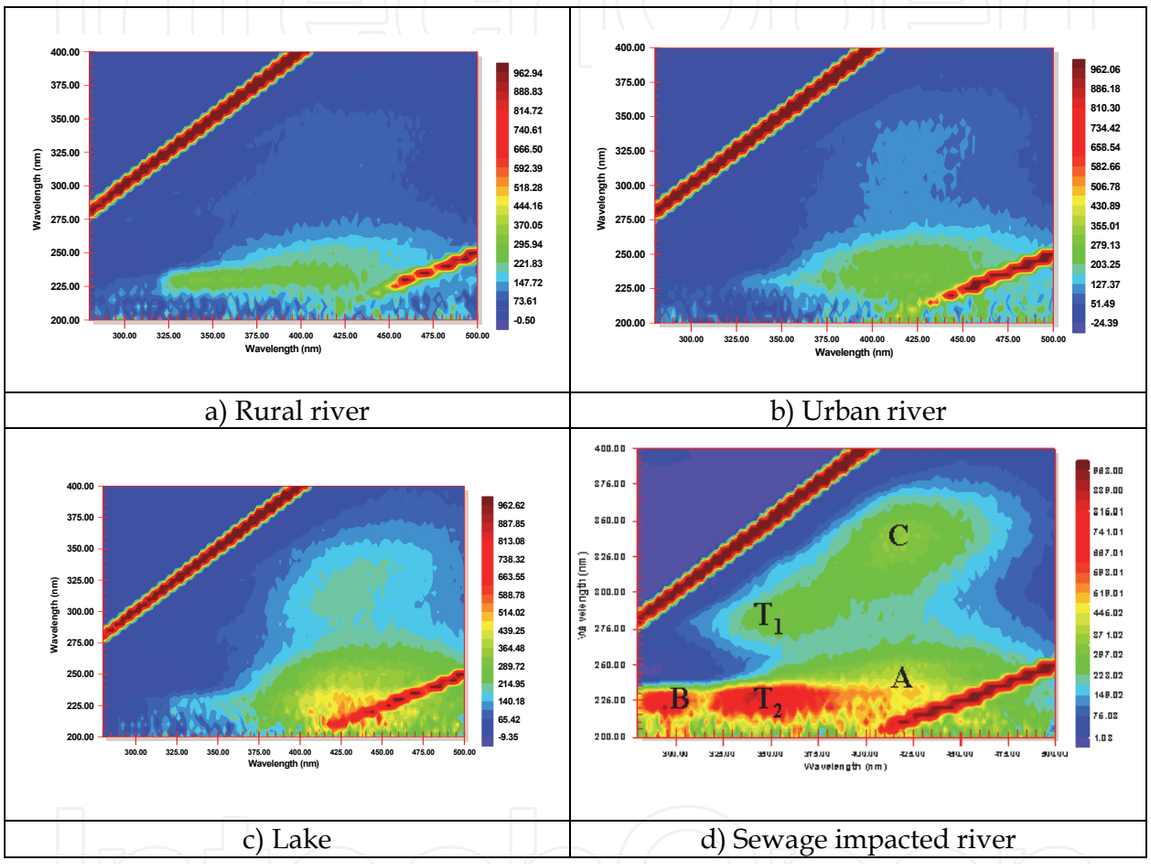
In order to identify the type of pollution in a water system, it is necessary to establish the natural characteristics of the water body, the quantity and quality of DOM and the relative proportions of terrestrially and microbially derived components. This can be obtained by determining the fluorescence fingerprint of a specific water body. Several studies had identified the specific fluorescence signature of different aquatic systems. Most of research concentrated on characterizing marine DOM properties, but few scientists attempted to analyse other water systems, like riverine, lentic systems, canals or surface runoff. Until approximately two decades ago, most researchers failed to distinguish between marine and freshwater DOM components, due to instruments limitations. Technological developments had a major contribution to fluorescence studies by allowing scientists to record complex two or three-dimensional spectra.

Coble (1990) and Coble et al. (1996) were the first ones to offer a comprehensive investigation of DOM fractions in marine and freshwaters. They found that the position of fluorescence peaks occur at shorter wavelengths for marine water than for freshwater. Also, a very intense peak B, corresponding to tyrosine, was seen at gulf and bay samples, which was not observed at riverine samples. Similarly, Kowalczuk et al. (2005) obtained different spectral characteristics at samples taken from estuarine, coastal and Gulf Stream waters. The researchers had found that the dominant component in estuarine spectra is the humic-like fluorescence and in the Gulf Stream is the protein-like. But, the coastal sample presented a mixture of these two fractions, due to significant microbial reprocessing and local production of DOM. Coble (1996) and Parlanti et al. (2000) had identified another peak, which can be seen only at marine samples, named as peak M, related to biological activity in areas of primary productivity, i.e. “fresh” humic material. Considering the variations in estuarine, coastal and marine water samples, Tedetti et al 2010 has evidenced that fluorescence spectroscopy is a very good tool for tracking anthropogenic inputs in the coastal waters.

Riverine samples have been analysed in few occasions, by scientists, in order to identify the peculiarities of a certain water body at temporal and spatial scale (Hudson et al., 2007). Most studies had shown that the humic substances largely dominate the fluorescence spectra, but the protein-like fraction can also be detected, depending on the type, location and inputs of the river (Baker et al., 2004; Carstea et al., 2009a; Carstea et al., 2009b). It has been shown that riverine DOM presents a seasonal variation (Baker et al., 2003; Carstea unpublished data), but also a subtle daily variation (Spencer et al., 2007). Riverine DOM varies greatly at spatial scale, as evidenced for example by Baker & Spencer (2004), who have undertaken a study on samples collected from several locations along a river, from source to its flow into the sea. Researchers have proved that fluorescence emission wavelengths of humic substances tend to decrease with distance from the source of the river, while the fluorescence intensity increased. Fluorescence intensity was highly influence by the type of surrounding (rural or urban) and activity (agriculture, airport etc.).

Even more sensitive to the surrounding environment are the lentic systems, as evidenced by Ghervase et al. (2011). The authors state that fluorescence spectra are dominated by the presence of the protein-like component of allochthonous origine, due to the high quantities of surface runoff or due to fauna and vegetation within the ecosystem. Borisover et al. (2009) has identified both DOM components in a lake with multiple river inputs and a seasonal variation at the surface of the lake.

Below, examples of fluorescence EEMs for a rural river, urban river, lake and a sewage-impacted river are presented in order to better illustrate the difference in fluorescence properties of DOM (Figure 6). Rural sample fluorescence spectrum shows both DOM fractions, originating from farm wastes, while the urban sample presents mostly the humic-like component. At the lake sample, high intensity humic-like component can be seen. The sample with sewage influence shows great impact from pollution by intense peaks corresponding to the protein-like fraction. More details regarding fluorescence fingerprints of various aquatic systems can be found in (Carstea et al., 2009b).



a) Rural river | b) Urban river

c) Lake | d) Sewage impacted river

Fig. 6. Fluorescence excitation –emission matrix for water samples.

5.2 Fluorescence monitoring of freshwater systems

So far, real-time fluorescence data have mostly been obtained for marine water with instruments specifically designed for this application (Chen, 1999; Barbini et al., 2003; Conmy et al., 2004; Drozdowska, 2007). One of the techniques used for real-time analyses is laser induced fluorescence (LIF). Chen (1999), Drozdowska (2007) and Bukin et al. (2007) have used LIF, at various wavelengths, to characterize DOM, arriving at the conclusion that with fluorescence rapid mapping of major processes can be made without sampling artifacts and measurement delays. Belzile et al. (2005) and Downing et al. (2009) have used a WebStar submersible fluorometer (λ_{ex}/ λ_{em} = 370 / 460 nm), while Del Castillo et al. (2001) and Conmy et al. (2004) used a SAFIre model (multiple excitation filters from 228 – 490 nm and emission filters at 228 – 810 nm). They reached at the conclusion that fluorescence

spectroscopy is highly capable to assess in real-time DOM characteristics and detect small differences in optical properties. However, the calibration of the system is time consuming and the excitation and emission domains are very limited.
Similar equipment has been used for freshwater studies in order to obtain continuous real-time data (Spencer et al., 2007; Downing et al., 2009). Spencer et al. (2007) has undertaken the first in situ monitoring of DOM properties, specifically the humic component, at temporal scale. The authors were able identify a diurnal variation of DOM using fluorescence spectroscopy, but recommended the analysis of multiple optical parameters in order to capture full characterization of DOM variability.
However, the in situ DOM monitoring was limited by the fixed excitation and emission wavelengths, allowing the measurement of only the terrestrial components. Another issue was the clogging of pre-filters, which required frequent filter replacement. Continuous monitoring has been performed by Carstea et al. (2009) who collected water samples at hourly scale, but the measurements were made within 24 h and not in real-time. This was the first research of continuous monitoring on fluorescence EEMs, allowing assessment of both DOM fractions. These studies revealed that dissolved organic matter varied at a daily timescale, depending on the river type, and was highly influenced by precipitation. As this study raised many topics for further research, Carstea et al. (2010) attempted to undertake the first study on real-time analysis of DOM using fluorescence EEMs. The experiment was made on a small urban catchment using a standard bench-top fluorometer connected to a fibre-optic probe. Researchers have proved that fluorescence technique is very reliable for real-time studies and that it can be applied in several systems for at least two week, without any cleaning procedure of the tubing system. Hourly pollution pulses were detected together with a significant contamination event with diesel oil. In conclusion, fluorescence spectroscopy is a very effective technique for in situ monitoring, offering many possibilities for further research for various applications.

5.3 Potential applications

It has been established, so far, that this technique can characterize natural organic matter (Baker and Spencer, 2004; Winter et al., 2007) and detect different types of aquatic pollutants, like sewage (Baker, 2001; Reynolds, 2002), oil (Budgen et al., 2008; Patra and Mishra, 2002; Carstea et al., 2010) or pesticides (Jiji et al., 2000). Furthermore, DOM fluorescence data correlate with water quality parameters such as: total organic carbon (Vodacek et al., 1995), aquatic plankton (Mopper and Schultz, 1993), faecal coliforms (Pfeiffer et al., 2008) and biological oxygen demand (Reynolds and Ahmad, 1997; Hudson et al., 2008).
Although the technique advantages have been thoroughly tested, in various conditions, there are still multiple potential applications, which have not been tested, yet. For instance, Ahmad and Reynolds (1999) have suggested this method for on-line process control in sewage treatment plants. Subsequently, other studies have implied the use of fluorescence spectroscopy as a potential monitoring tool for recycled water (Henderson et al.,2009), drinking water treatment processes (Cheng et al., 2004; Bieroza et al., 2009), urban watersheds with sewage effluents (Hur et al., 2008) and evaluation of DOM composition and concentration in relation to the production of disinfection by products during drinking water chlorination (Spencer et al., 2007 and references therein). However, many issues have to be clarified before using this method for the previously mentioned application: possibility for automated data analysis, optimized calibration procedure to ensure reliability and repeatability of results and optimum instrument configuration (Henderson et al., 2009).

6. Conclusions

The study has presented fluorescence spectroscopy ability to characterize in real-time and in situ DOM fractions properties. Fluorescence spectroscopy can be influenced by numerous factors, but these issues can be overcome by the use of a calibration curve.
Fluorescence fingerprints are very useful in establishing the natural characteristics of the water body, the quantity and quality of DOM and the relative proportions of terrestrially and microbially derived components. Based on the natural characteristics, researchers can easily identify a pollution event. Fluorescence spectroscopy presents several opportunities for future research with potential application in drinking water and waste water treatment monitoring.

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8. References

Abbas, O., Rebufa, C., Dupuya, N., Permanyer, A. & Kister, J. (2006). Assessing petroleum oils biodegradation by chemometric analysis of spectroscopic data. *Talanta*, Vol. 75, No. 4, pp. 857–871, ISSN 0039-9140.

Ahmad, S.R. & Reynolds, D.M. (1999). Monitoring of water quality using fluorescence technique: Prospect of on-line process control. *Water Research*, Vol. 33, No. 9, pp. 2069-2074, ISSN 0043-1354.

Aiken, G.R. (1985). Isolation and concentration techniques for aquatic humic substances, In: *Humic substances in soil, sediment and water: geochemistry and isolation*, Aiken, G.R., McKnight, D.M., Wershaw, R.L. & MacCarthy, P., pp. 363–385, Publisher Wiley-Interscience, ISBN 0471882747, New York.

Aitkenhead, J.A., Hope, D. & Billett, M.F. (1999). The relationship between dissolved organic carbon in stream water and soil organic carbon pools at different spatial scales. *Hydrological Processes*, Vol. 13, No. 8, pp. 1289-1302, ISSN 1099-1085.

Baker, A. (2001). Fluorescence excitation-emission matrix characterisation of some sewage impacted rivers. *Environmental Science & Technology*, Vol. 35, No. 5, pp. 948-953, ISSN 1520-5851.

Baker, A., Inverarity, R., Charlton, M. & Richmond, S. (2003). Detecting river pollution using fluorescence spectrophotometry: case studies from the Ouseburn, NE England. *Environmental Pollution*, Vol. 124, No. 1, pp. 57-70, ISSN 0269-7491.

Baker, A. & Curry, M. (2004). Fluorescence of leachates from three contrasting landfills. Water Research, Vol. 38, No. 10, pp. 2605-2613, ISSN 0043-1354.

Baker, A. & Spencer, R.G.M. (2004). Characterization of dissolved organic matter from source to sea using fluorescence and absorbance spectroscopy. *Science of the Total Environment*, Vol. 333, pp. 217-232, ISSN 0048-9697.

Baker, A., Ward, D., Lieten, S.H., Periera, R., Simpson, E.C. & Slater, M. (2004). Measurement of protein-like fluorescence in river and wastewater using a handheld spectrophotometer. *Water Research*, Vol. 38, No. 12, pp. 2934-2938, ISSN 0043-1354.

Baker, A. (2005). Thermal fluorescence quenching properties of dissolved organic matter. Water Research, Vol. 39, No. 18, pp 4405-4412, ISSN 0043-1354.

Baker, A., Elliott, S. & Lead, J.R. (2007). Effects of filtration and pH perturbation on organic matter fluorescence. Chemosphere, Vol. 67, No.10, pp. 2035-2043, ISSN 0045-6535.

Barbini, R., Colao, F., Fantoni, R., Ferrari, G.M., Lai, A. & Palucci, A. (2003). Application of a lidar fluorosensor system to the continuous and remote monitoring of the Southern Ocean and Antarctic Ross Sea: results collected during the XIII and XV Italian oceanographic campaigns. *International Journal of Remote Sensing*, Vol. 24, No. 16, pp. 3191–3204, ISSN 1366-5901.

Bieroza, M., Baker, A. & Bridgeman, J. (2009). Relating freshwater organic matter fluorescence to organic carbon removal efficiency in drinking water treatment. *Science of the Total Environment*, Vol. 407, No. 5, pp. 1765-1774, ISSN 0048-9697.

Budgen, J.B.C., Yeung, C.W., Kepkay, P.E. & Lee, K. (2008). Application of ultraviolet fluorometry and excitation–emission matrix spectroscopy (EEMS) to fingerprint oil and chemically dispersed oil in seawater. *Marine Pollution Bulletin*, Vol. 56, No. 4, pp. 677–685, ISSN 0025-326X.

Burel-Deschamps, L., Giamarchi, P., Stephan, L., Lijour, Y. & Le Bihan, A. (2006). Laser–Induced Fluorescence Detection of Carbamates Traces in Water. *Journal of Fluorescence*, Vol. 16, No. 2, pp. 177-183, ISSN 1573-4994.

Cammack, W.K.L. (2002). Dissolved organic matter fluorescence: Relationships with heterotrophic bacterial metabolism. PhD Thesis, Department of Biology, McGill University, Montréal, Québec, Canada.

Cammack, W.K.L., Kalf, J., Prairie, Y.T. & Smith, E.M. (2004). Fluorescent dissolved organic matter in lakes: Relationship with heterotrophic metabolism. *Limnology and Oceanography*, Vol. 49, No. 6, pp. 2034-2045, ISSN 1939-5590 .

Camobreco, V.J., Richards, B.K., Stenhuis, T., Peverly, J.H. & McBride, M.B. (1996). Movement of heavy metals through undisturbed and homogenized soil columns. *Soil Science*, Vol. 161, No. 11, pp. 740–750, ISSN 1538-9243.

Carstea, E.M., Baker, A., Boomer, I. & Pavelescu, G. (2009a). Continuous fluorescence assessment of organic matter variability on the Bournbrook River, Birmingham, UK. *Hydrological Processes*, Vol. 23, No. 13, pp. 1937–1946, ISSN: 1099-1085.

Carstea, E.M., Cristescu, L., Pavelescu, G. & Savastru D. (2009b). Assessment of the anthropogenic impact on water systems by fluorescence spectroscopy. *Environmental Engineering and Management Journal*, Vol. 8, No. 6, pp. 1321-1326, ISSN 1843-3707.

Carstea, E.M., Baker, A., Bieroza, M. & Reynolds D.M. (2010). Continuous fluorescence excitation emission matrix monitoring of river organic matter. *Water research*, Vol. 44, No. 18, pp. 5356-5366, ISSN 0043-1354.

Chauhan, A., Fazlurrahman, Oakeshott, J.G. & Jain, R.K. (2008). Bacterial metabolism of polycyclic aromatic hydrocarbons: strategies for bioremediation. *Indian Journal of Microbiology*, Vol. 48, No. 1, pp. 95–113, ISSN 0973-7715.

Chen, J., Gu, B.H., LeBoeuf, E.J., Pan, H.J. & Dai, S. (2002). Spectroscopic characterization of the structural and functional properties of natural organic matter fractions. *Chemosphere*, Vol. 48, No. 1, pp. 59-68, ISSN 0045-6535.

Chen, R.F. (1999). In situ fluorescence measurements in coastal waters. *Organic Geochemistry*, Vol. 30, No. 6, pp. 397-409, ISSN 0146-6380.

Cheng, W.P., Chi, F.H. & Yu, R.F. (2004). Evaluating the efficiency of coagulation in the removal of dissolved organic carbon from reservoir water using fluorescence and ultraviolet photometry. *Environmental Monitoring and Assessment*, Vol. 98, No. 1-3, pp. 421–431, ISSN 1573-2959.

Christensen, J. (2005). Autofluorescence of Intact Food - An Exploratory Multi-way Study. PhD thesis, Faculty of Life Sciences, University of Copenhagen, Denmark.

Coble, P.G., Green, S.A., Blough, N.V. & Gagosian, R.b. (1990). Characterization of dissolved organic matter in the Black Sea by fluorescence spectroscopy. *Nature*, Vol. 348, pp. 432 – 435.

Coble, P.G. (1996). Characterisation of marine and terrestrial dissolved organic matter in seawater using excitationemission matrix spectroscopy. *Marine Chemistry*, Vol. 51, No.4, pp. 325-346, ISSN 0304-4203.

Conmy, R.N., Coble, P.G. & Del Castillo, C.E. (2004). Calibration and performance of a new in situ multi-channel fluorometer for measurement of colored dissolved organic matter in the ocean. *Continental Shelf Research*, Vol. 24, No. 3, pp. 431–442, ISSN 0278-4343 .

Cristescu, L., Pavelescu, G., Carstea, E.M. & Savastru, D. (2009). Evaluation of petroleum contaminants in soil by fluorescence spectroscopy. *Environmental Engineering and Management Journal*, Vol. 8, No. 5, pp. 1269-1273, ISSN 1573-2959.

Deepa, S., Sarathi, R. & Mishra, A.K. (2006). Synchronous fluorescence and excitation emission characteristics of transformer oil ageing. *Talanta*, Vol. 70, No. 4, pp. 811-817, ISSN:0039-9140.

Deepa, S. & Mishra, A.K. (2006). Synchronous Fluorescence Spectroscopy and its Applications. *ISRAPS Bulletin*, Vol. 18, No. 1-2, pp. 4-8.

Del Castillo, C.E., Coble, P.G., Morell, J.M., Lopez, J.M. & Corredor, J.E. (1999). Analysis of the optical properties of the Orinoco River plume by absorption and fluorescence spectroscopy. *Marine Chemistry*, Vol. 66, No. 1-2, pp. 35-51, ISSN 0304-4203.

Divya, O. & Mishra, A.K. (2008). Understanding the concept of concentration-dependent red-shift in synchronous fluorescence spectra: Prediction of $\lambda_{SFS}^{\max}$ and optimization of $\Delta\lambda$ for synchronous fluorescence scan. *Analytica Chimica Acta*, Vol. 630, No. 1, pp. 47–56, ISSN:0003-2670.

Downing, B.D., Boss, E., Bergamaschi, B.A., Fleck, J.A., Lionberger, M.A., Ganju, N.K., Schoellhamer, D.H. & Fujii, R. (2009). Quantifying fluxes and characterizing compositional changes of dissolved organic matter in aquatic systems in situ using combined acoustic and optical measurements. *Limnology and Oceanography: Methods*, Vol. 7, pp. 119-131, ISSN 1939-5590.

Drozdowska, V. (2007). Seasonal and spatial variability of surface seawater fluorescence properties in the Baltic and Nordic Seas: results of lidar experiments. *Oceanologia*, Vol. 49, No. 1, pp. 59–69, ISSN 0078-3234.

Elkins, K.M. & Nelson, D.J. (2001). Fluorescence and FT-IR spectroscopic studies of Suwannee river fulvic acid complexation with aluminium, terbium and calcium. *Journal of Inorganic Biochemistry*, Vol. 87, No. 1-2, pp. 81-96, ISSN 0162-0134.

Elliott, S. (2006). Fluorescence signatures in natural aquatic systems. PhD thesis, University of Birmingham, UK.

Elliott, S., Lead, J.R. & Baker, A. (2006). Characterisation of the fluorescence from freshwater, planktonic bacteria. Water Research, Vol. 40, No. 10, pp. 2075-2083, ISSN 0043-1354.

Ferrari, G.M., Dowell, M.D., 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*, Vol. 55, No. 3-4, pp. 299-316, ISSN: 0304-4203.

Ferrer, R., Beltran, J.L. & Guiteras, J. (1998). Multivariate calibration applied to synchronous fluorescence spectrometry. Simultaneous determination of polycyclic aromatic hydrocarbons in water samples. *Talanta*, Vol. 45, No. 6, pp. 1073-1080, ISSN 0039-9140.

Frimmel, F.H. (1998). Characterization of natural organic matter as major constituents in aquatic systems. *Journal of Contaminant Hydrology*, Vol. 35, No. 1-3, pp. 201-216, ISSN 0169-7722.

Giamarchi, P., Stephan, L., Salomon, S. & Le Bihan, A. (2000). Multicomponent determination of a polyaromatic hydrocarbon mixture by direct fluorescence measurements. *Journal of Fluorescence*, Vol. 10, No. 4, pp. 393-402, ISSN 1573-4994.

Ghervase, L., Ioja, C., Carstea, E.M., Niculita, L., Savastru, D., Pavelescu, G. & Vanau G. (2011). Evaluation of lentic ecosystems from Bucharest City, *International Journal of Energy and Environment*, Vol. 5, No. 2, pp. 183-192, ISSN 1109-9577.

Ghosh, K. & Schnitzer, M. (1980). Macromolecular structures of humic substances. *Soil Science*, Vol. 129, pp. 266-276, ISSN 1538-9243.

Henderson, R.K., Baker, A., Murphy, K.R., Hambly, A., Stuetz, R.M. & Khan, S.J. (2009). Fluorescence as a potential monitoring tool for recycled water systems: A review. *Water Research*, Vol. 43, No. 4, pp. 863-881, ISSN 0043-1354.

Hope, D., Billett, M.F., Milne, R. & Brown, T.A.W. (1997). Exports of organic carbon in British rivers. *Hydrological Processes*, Vol. 11, No. 3, pp. 325-344, ISSN 1099-1085.

Hudson, N., Baker, A. & Reynolds, D. (2007). Fluorescence analysis of dissolved organic matter in natural, waste and polluted waters—a review. *River Research and Applications*, Vol. 23, No. 6, pp. 631–649, ISSN 1535-1467.

Hudson, N., Baker, A., Ward, D., Reynolds, D.M., Brunsdon, C., Carliell-Marquet, C. & Browning, S. (2008). 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. *Science of the Total Environment*, Vol. 391, No. 1, pp. 149–158, ISSN 0048-9697.

Hur, J., Hwang, S.J. & Shin, J.K. (2008). Using synchronous fluorescence technique as a water quality monitoring tool for an urban river. *Water, Air and Soil Pollution*, Vol. 191, No. 1-4, pp. 231–243, ISSN 1573-2932.

JiJi, R.D., Cooper, G.A. & Booksh, K.S. (1999). Excitation-emission matrix fluorescence based determination of carbamate pesticides and polycyclic aromatic hydrocarbons. *Analytica Chimica Acta*, Vol. 397, No. 1-3, pp. 61–72, ISSN: 0003-2670.

JiJi, R.D., Andersson, G.G. & Booksh, K.S. (2000). Application of PARAFAC for calibration with excitation–emission matrix fluorescence spectra of three classes of environmental pollutants. *Journal of Chemometrics*, Vol. 14, No. 3, pp. 171–185, ISSN 1099-128X.

Kelton, N., Molot, L.A. & Dillon, P.J. (2007). Spectrofluorometric properties of dissolved organic matter from Central and Southern Ontario streams and the influence of iron and irradiation. *Water Research*, Vol. 41, No. 3, pp. 638 – 646, ISSN 0043-1354.

Konstantinou, I.K., Hela, D.G. & Albanis, T.A. (2006). The status of pesticide pollution in surface waters (rivers and lakes) of Greece. Part I. Review on occurrence and levels. *Environmental Pollution*, Vol. 141, No. 3, pp. 555-570, ISSN 0269-7491.

Kubista, M., Sjoback, R., Eriksson, S. & Albinsson, B. (1994). Experimental correction for the inner-filter effect in fluorescence spectra. *The Analyst*, Vol. 119, No. 3, pp. 417–419.

Lakowicz, J.R. (2006). Principles of Fluorescence Spectroscopy. Third edition, Publisher Springer New York.

Li, J., Fuller, S., Cattle, J., Way, C.P. & Hibbert, D.B. (2004). Matching fluorescence spectra of oil spills with spectra from suspect sources. *Analytica Chimica Acta*, Vol. 514, No. 1, pp. 51–56, ISSN 0003-2670.

McDowell, W.H. & Likens, G.E. (1988). Origin, composition and flux of dissolved organic carbon in the Hubbard Brook Valley. *Ecological Monographs*, Vol. 58, pp. 177-195, ISSN 0012-9615.

McKnight, D.M., Boyer, E.W., Doran, P., Westerhoff, P.K., Kulbe, T. & Andersen, D.T. (2001). Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. *Limnology and Oceanography*, Vol. 46, No. 1, pp. 38-48, ISSN 1939-5590.

Mopper, K. & Schultz, C.A. (1993). Fluorescence as a possible tool for studying the nature and water column distribution of DOC components. *Marine Chemistry*, Vol. 41, No. 1-3, pp. 229-238, ISSN 0304-4203.

Ohno, T. (2002). Fluorescence inner-filtering correction for determining the humification index of dissolved organic matter. *Environmental Science and Technology*, Vol. 36, No. 4, pp. 742-746, ISSN 1520-5851.

Osburn, C.L., Morris, D.P., Thorn, K.A. & Moeller, R.E. (2001). Chemical and optical changes in freshwater dissolved organic matter exposed to solar radiation. *Biogeochemistry*, Vol. 54, pp. 251-278, ISSN 1573-515X.

Pagano, T.E. & Kenny, J.E. (1999). Assessment of Inner Filter Effects in Fluorescence Spectroscopy using the Dual- Pathlength Method– a Study of Jet Fuel JP-4. *Proceedings of SPIE*, Vol. 3856, pp. 289-297.

Patel-Sorrentino, N., Mounier, S., Lucas, Y. & Benaim, J.Y. (2004). Effects of UV-visible irradiation on natural organic matter from the Amazon basin. *Science of the Total Environment*, Vol. 321, No. 1–3, pp. 231-239, ISSN 0048-9697.

Patra, D. & Mishra, A.K. (2002a). Total synchronous fluorescence scan spectra of petroleum products. Analytical and Bioanalytical Chemistry, Vol. 373, No. 4-5, pp. 304-309, ISSN 1618-2650.

Patra, D. & Mishra, A.K. (2002b). Recent developments in multicomponent synchronous fluorescence scan analysis. *Trends in Analytical Chemistry*, Vol. 21, No. 12, pp. 787 – 798, ISSN 0165-9936.

Parker, C.A. & Barnes, W.J. (1957). Some experiments with spectrofluorimeters and filter fluorimeters. *The Analyst*, Vol. 82, No. 978, pp. 606–618.

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*, Vol. 31, No. 12, pp. 1765-1781, ISSN 0146-6380.

Pascu, M.L, Cristu, D., Vasile, A. & Moise, N. (1995). Pesticide monitoring in water using laser-induced fluorescence and absorption techniques. *Proceedings of SPIE*, Vol. 2461, pp. 648-652.

Pharr, D.Y., McKenzie, J.K. & Hickman, A.B. (1992). Fingerprinting Petroleum Contamination Using Synchronous Scanning Fluorescence Spectroscopy. *Groundwater*, Vol. 30, No. 4, pp. 484–489, ISSN 1745-6584.

Pfeiffer, E., Pavelescu, G., Baker, A., Roman, C., Ioja, C. & Savastru, D. (2008). Pollution analysis on the Arges River using fluorescence spectroscopy. *Journal of Optoelectronics and Advance Materials*, Vol. 10, No. 6, pp. 1489 – 1494, ISSN 1841 - 7132.

Reynolds, D. & Ahmad, S.R. (1995). Effect of metal ions on the fluorescence of sewage wastewater. *Water Research*, Vol. 29, No. 9, pp. 2214-2216, ISSN 0043-1354.

Reynolds, D.M. & Ahmad, S.R. (1997). Rapid and direct determination of wastewater BOD values using a fluorescence technique. *Water Research*, Vol. 31, No. 8, pp. 2012-2018, ISSN 0043-1354.

Reynolds, D.M. (2003). Rapid and direct determination of tryptophan in water using synchronous fluorescence spectroscopy. *Water Research*, Vol. 37, No. 13, pp. 3055-3060, ISSN 0043-1354.

Ryder, A. (2005). Analysis of Crude Petroleum Oils Using Fluorescence Spectroscopy, In: *Reviews in Fluorescence*, Eds. Geddes, C.D. , Lakowicz, J.R., Springer US, pp. 169-198.

Selli, E., Zaccaria, C., Sena, F., Tomasi, G. & Bidoglio, G. (2004). Application of multi-waymodels to the time-resolved fluorescence of polycyclic aromatic hydrocarbons mixtures in water. *Water Research*, Vol. 38, No. 9, pp. 2269–2276, ISSN 0043-1354.

Senesi, N. (1993). Nature of interactions between organic chemicals and dissolved humic substances and the influence of environmental factors, In: *Organic substances in soil and water: Natural constituents and their influences on contaminant behaviour*, Eds. Beck, A.J., Jones, K.C., Hayes ,M.H.B., Minglegrin, U., Publisher Royal Society of Chemistry, Cambridge.

Smart, P.L., Finlayson, B.L., Rylands, B.L. & Ball, C.M. (1976). The relation of fluorescence to dissolved organic carbon in surface waters. *Water Research*, Vol. 10, No. 9, pp. 805-811, ISSN 0043-1354.

Spencer, R.G.M., Pellerin, B.A., Bergamaschi, B.A., Downing, B.D., Kraus, T.E.C., Smart, D.R., Dahlgren, R.A. & Hernes, P.J. (2007). Diurnal variability in riverine dissolved organic matter composition determined by in situ optical measurement in the San Joaquin River (California, USA). *Hydrological Processes*, Vol. 21, No. 23, pp. 3181-3189, ISSN 1099-1085.

Spitzy, A. & Leenheer, J.A. (1991). Dissolved organic carbon in rivers, In: *Biogeochemistry of major world rivers*, Eds. Degens, E.T., Kempe, S., Richey, J.E., Publisher Wiley, New York, pp. 213-232.

Stedmon, C.A., Markager, S. & Bro, R. (1993). Tracing dissolved organic matter in aquatic environments using a new approach to fluorescence spectroscopy. *Marine Chemistry*, Vol. 82, No. 3-4, pp. 239-254, ISSN 0304-4203.

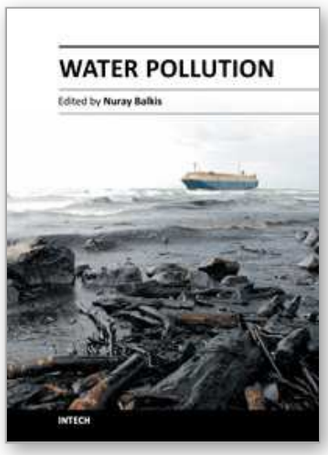
Stewart, A.J. & Wetzel, R.G. (1980). Asymmetrical relationships between absorbance, fluorescence, and dissolved organic carbon. *Limnology and Oceanography*, Vol. 26, No. 3, pp. 590-597, ISSN 1939-5590.

Swietlik, J. & Sikorska, E. (2004). Application of fluorescence spectroscopy in the studies of natural organic matter fractions reactivity with chlorine dioxide and ozone. *Water Research*, Vol. 38, No. 17, pp. 3791-3799, ISSN 0043-1354.

Thomas, J.D. (1997). The role of dissolved organic matter, particularly free amino acids and humic substances, in freshwater ecosystems. *Freshwater Biology*, Vol. 38, No. 1, pp. 1-25, ISSN 1365-2427.

Thurman, E.M. (1985). Organic geochemistry of natural waters, In: *Developments in biogeochemistry*, Eds. Niyhoff, M., Junk W., Publisher Springer Science & Business, Dordrecht, The Netherlands.

Valeur, B. (2001). Molecular Fluorescence: Principles and Applications, Publisher Wiley, Weinheim.



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Water pollution is a major global problem that requires ongoing evaluation and revision of water resource policy at all levels (from international down to individual aquifers and wells). It has been suggested that it is the leading worldwide cause of deaths and diseases, and that it accounts for the deaths of more than 14,000 people daily. In addition to the acute problems of water pollution in developing countries, industrialized countries continue to struggle with pollution problems as well. Water is typically referred to as polluted when it is impaired by anthropogenic contaminants and either does not support a human use, such as drinking water, and/or undergoes a marked shift in its ability to support its constituent biotic communities, such as fish. Natural phenomena such as volcanoes, algae blooms, storms, and earthquakes also cause major changes in water quality and the ecological status of water. Most water pollutants are eventually carried by rivers into the oceans.

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Phone: +86-21-62489820
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