



# EXCITATION EMISSION MATRIX STUDY OF DISSOLVED ORGANIC MATTER BY FLUORESCENCE SPECTROPHOTOMETER

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## Abstract

Using total luminescence excitation emission matrix (EEM) study, it is possible to identify the unknown structure present in the dissolved organic matter (DOM) of water or wastewater. This paper describes the development of EEM of sewage and dairy wastewater sample. Identification of unknown DOM structure was carried out by comparing respective EEM of sewage and dairy wastewater with external standards of simple aromatic acid and their derivatives e.g. salicylic acid and tryptophan & tyrosine. This method is very beneficial for characterization of unknown DOM to find out its functional group. This technique will prove a very useful tool for the real-time, non-invasive monitoring the level of carbon load in wastewater treatment plants and tracing diverse sources of dissolved organic matter in natural water bodies.

**Keywords:** DOM; fluorescence spectrophotometer; salicylic acid; tyrosine; tryptophan.

## Biographical notes:

### 1. Introduction

Quality of water has long been defined in terms of load of dissolved organic matter (DOM). DOM is a ubiquitous constituent of natural water bodies; which defines its quality and gross discharges of untreated effluent, landfill leachate, agriculture runoff, and wide variety of industrial sources are common forms of surface water pollution (Ellis, 1989). DOM concentration and composition both directly and indirectly influence the ecosystem. To understand the dynamics of DOM in aquatic ecosystem, it is essential to trace the different

fractions of DOM pool through the ecosystem. However, due to its complexity, the chemical characterization of DOM is a highly complicated process.

In pollution abatement work, assessment of carbon load in natural water bodies is necessary to control the level of pollution. Standard methods for the determination of levels of organic load in water and wastewater are chemical oxygen demand (COD), biochemical oxygen demand (BOD) and total organic carbon (TOC) analysis. These methods are slow and tedious. The use of these tests severely limits the number of samples which can be analyzed (Smart et al, 1976).

One of the most powerful methods for studying the DOM in water bodies is molecular fluorescence spectroscopy. Fluorescence spectroscopy is having a potential of high sensitivity and selectivity and is a zero background technique with error percentage as low as  $\pm 0.2\%$ . The fluorescence of the molecule is dependant on the structure of that molecule and upon the environment in which spectrum is measured. Therefore, mere fact that a molecule fluoresce can itself provide significant information regarding its structural features. DOM is a complex mixture of aromatic and aliphatic hydrocarbon structures that have attached functional group. It is envisaged that the fluorophores from DOM viz. humic, fulvic and protein like may act as a surrogate to give fluorescence and comprise approximately 60 -70% of the total organic carbon in soils and 40-60% of dissolved organic carbon in natural waters (Senesi 1993). Identification and differentiation of DOM from

the various sources of water is necessary to control the level of pollution load in terms of dissolved carbon. Using total luminescence excitation emission matrix (EEM) study, it is possible to identify the unknown structure present in the DOM of water or wastewater. Understanding the unknown structures is a key to better treatment of drinking water.

Large number of research bodies has been found in literature exploring the significant property of DOM matrix giving fluorescence. Recent documentation are made by the various authors on the fluorescence characteristics of river water (Yan, Myrick, 2000), ground water (Baker and Genry, 1999), sewage and sewage impacted river water (Reynolds and Ahmad, 1997; Baker and Lamont – Black, 2001; Baker 2002).

The objective of this study is to examine the range of environmental variability in fluorescence properties of naturally occurring DOM and to determine the extent to which DOM from different sources can be distinguished using fluorescence spectroscopy. Present study tried to develop three dimensional (3D) EEM spectra of DOM originated from four types of water-bodies viz. sewage water, lake water and tertiary treated wastewaters from dairy and ayurvedic herbal pharmaceutical industry. Study of EEM of external standards of simple aromatic acid and their derivatives e.g. salicylic acid and tryptophan & tyrosine was also carried out to find the functional group present in the DOM responsible for the fluorescence.

## 2. Experimental

### 2.1 Source of sample

Four different group of water samples were collected for the fluorescence analysis from various sources in and around Nagpur city (India). The first sample was a wastewater sample from a settled domestic sewage. Second one was from the natural source of Ambasary Lake. Third and fourth samples were tertiary treated wastewater effluents from dairy industry and baidhyanath ayurvedic medicine industry as an herbal pharmaceutical wastewater respectively.

Gross sampling was done weekly for a period of six months. Samples were collected using high density polyethylene bottles and were stored

below 4°C in the dark prior to analysis. All samples were allowed to reach at room temperature before analysis. Analysis of the filtered samples was done within 24 hr.

### 2.2 Instrumental

Fluorescence measurements were made on the Hitachi; F-4500 fluorescence spectrophotometer. The intense 150 watt xenon source provides maximum light energy over the entire 200-900 nm wavelength range of the spectrophotometer. The cuvette used for the spectral analysis was of high grade quartz composition with a 10 mm path length. The spectra (excitation & emission) were corrected for instrument biases. A rhodamine B quantum counter is used to check the spectral accuracy on excitation side and emission correction spectrum derived using ground quartz diffuser (as recommended by manufacturer). Comparison of the integrated Raman spectra of Milli-Q water over excitation wavelength revealed no change in lamp intensity during the measurement period.

COD test was performed by using a close digestion microwave method, details of procedure has been published elsewhere (Dharmadhikari, Vanerkar and Barhate 2005) and BOD by three day test with azide modification. TOC measurement was made on automatic carbon analyzer (Termo Electron Corporation TOC 1200) as per standard method (Clesceri, Greenberg, and Eaton, 1998). Optical density was measured on Perkin-Elmer Lambda - 900 ultraviolet/visible spectrophotometer 900 with 1.0 x 1.0-cm quartz cells. High density and colored samples were diluted with 0.01M KCl solution. pH were measured with a cyber scan 500 pH meter.

### 2.3 Analysis

EEM study of the standards viz. salicylic acid, tyrosine and tryptophan were carried out according to the conditions mentioned in the literature (Dean 1995). All the reagents used for the study were of AR grade. Characterization of DOM of the water bodies was carried out by measuring the fluorescence intensity and also by conventional parameters TOC, COD and BOD. Results are shown in the table 1. All the samples were studied in normal condition of pH and temperature. As the pH range of all the water-bodies was in the range of 5-8 and fluorescence intensity remain unaffected in this

range (Dharmadhikari, Barhate, 2003). DOM of all water bodies under study on Identification of unknown fluorophore was fluorescence spectrophotometer. carried out by developing 3D EEM spectra of Table1. Main characteristics of Group Samples (n =25)

Source of sample	pH	T0C mg/l	COD mg/l	BOD mg/l	Fluorescenc e Intensity
<b>Domestic Sewage</b>					
Mean	7.5	50.02	232.12	110.25	1141.75
Standard Deviation	0.18	33.16	74.85	47.14	451.74
Range	7.3 – 7.8	37 – 95	123 - 376	76-173	543 – 1866
<b>Lake water</b>					
Mean	7.4	47.84	126.018	60.92	86.32
Standard Deviation	0.15	17.75	43.24162	24.83778	56.25697
Range	7.1-7.8	19 –72	56.76 - 162	29 – 96	15-165
<b>Dairy Wastewater</b>					
Mean	7.6	106.05	254.322	120.68	4172.333
Standard Deviation	0.14	49.47	102.1586	45.64	2916.45
Range	7.4-7.9	37 - 167	122 - 417	46 - 162	1034 – 9123
<b>HerbalPharm. wastewater</b>					
Mean	8.6	499.79	4679.834	2050.3	439.8
Standard Deviation	0.28	135.49	1144.65	413.67	360.68
Range	7.9 – 9.8	327 - 68	3421 - 4256	1632- 2875	206 – 1300

### 3. Results and Discussion:

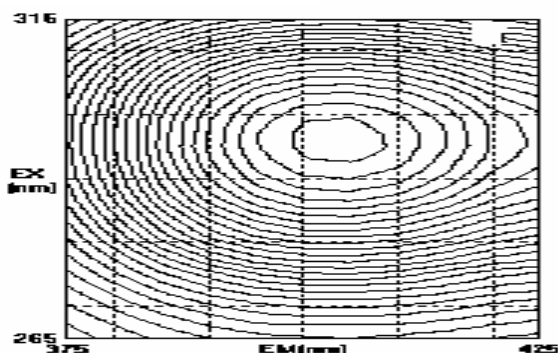
Fluorescence properties of natural waters were reported as early as 1910 by Dienart, however, very less information is available on fluorescence analysis of DOM in general bulk water matrices (e.g. lake, stream) & treated wastewater effluents particularly (Cabaniss & Shuman, 1987; Donahue et al, 1998; Smith & Kramer, 1998; McKnight et al 2001). Present work discussed the fluorescence study of lake and treated wastewater and can be useful to differentiate the source of surface water pollution.

### 3.1 EEM Study of Standards

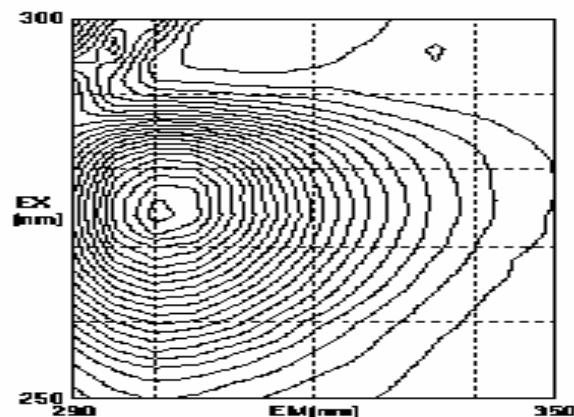
The usage of external standards of simple aromatic acid and there derivatives (e.g. Salicylic acid, benzoic acid) appears justified because the substance can be consider as precursors in the generation processes or alternatively, as degradation products in the braking down of humic substance. It seems reasonable to assume these entities in humic substance as significant contributors in humic substance fluorescence and use as reference fluorophores seems to be clearly justified (Frimmel, Abbt-Braun, Heumann, 2002). The use of standards contributes to the

understanding of the effect of environmental parameters on the spectral shape, even if its behavior is not always representative of natural sample (Seritti et al, 1998).

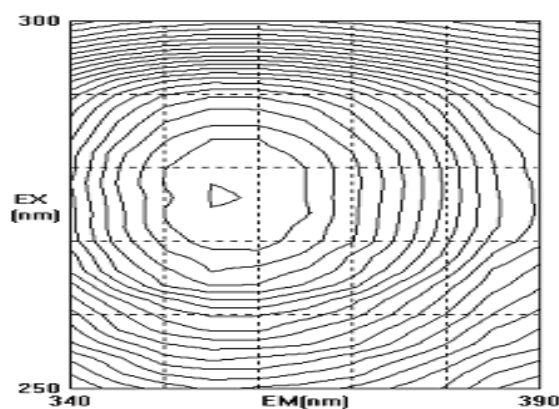
Excitation maximum (Ex max.) & emission maximum (Em max.) are not sufficient to get detailed structural information of the fluorophore present in the particular DOM. Total luminescence EEM spectroscopy provides a complete representation of the spectral features of a compound, in which fluorescence intensity is presented as a function of excitation wavelength on one axis and emission wavelength on other axis. Fluorescence contouring, in which repeated emission scans are collected at numerous excitation wavelengths results in EEM which provide highly detailed information which can be used to identify fluorescent compounds present in complex mixtures (Chritian, Callis, Davidson, 1981; Lochmuller & Saavedra, 1986, Liener & wolfbeis, 1988). 3D EEM spectra of standards (Figure No. 1, 2 & 3) shows rounded counters that may results from the single fluorophore (Coble et al, 1990). Fluorophore from natural water bodies or from treated effluent plants have elongated contours. The two major DOM components that have been found to fluoresce are humic substance (salicylic acid, blue fluorescence) and protein fractions tyrosin, tryptophan, ( a UV fluorescence Mopper & Schuitz, 1993). These counter configuration of standards may help to differentiate the fluorescent signature of DOM of various water bodies depending on there sources. This will help water quality manager for assessment of organic load.



**Figure 1** Three dimensional EEM spectra of standard Salicylic acid



**Figure 2.** Three dimensional D EEM spectra of standard Tyrosine



**Figure 3.** Three dimensional EEM spectra of standard Tryptophan

### 3.2 EEM Study of Samples

Fluorescence of the complex DOM in water-bodies is dependent on its type of fluorophore and inorganic salt matrix associated. Composition of fluorescent DOM depends on the rate of the oxidation and remineralization of settling organic particles. (Chen, Bada, 1992) Environmental parameters such as pH, metal ions etc. also plays major role in the total alteration of fluorescence measurement of natural water bodies. Metal affects only fluorescence intensity whereas pH alters both intensity and spectral shape (Cabaniss, Shuman 1987).

Study of fluorescence measurement for all the samples was carried out at optimized instrumental parameters which was kept constant throughout the experiment. Optimized instrumental parameters are given in the table 2.

**Table 2.** Optimized Instrumental Parameters of Sample

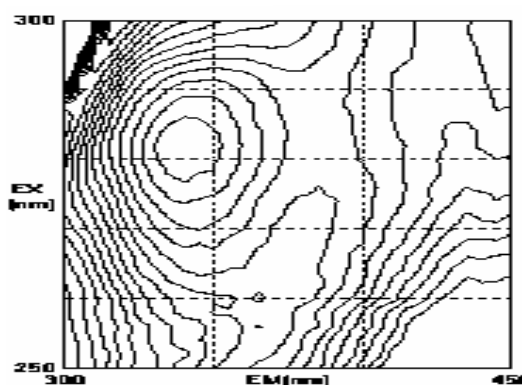
Source of sample	Excitation wavelength(Ex.)	Emission wavelength (Em.)	Ex. /Em. Slit width (nm)	PMT Voltage (V)
Domestic Sewage	290	350	5.0/10.0	700
Lake water	304	405	2.5/5.0	700
Dairy Wastewater	280	338	5.0 / 10.0	950
Herbal Pharma. wastewater	373	480	2.5/5.0	700

Response – Auto, Scan Speed (nm/min) - 240, were kept constant for all samples

### 3.2.1 EEM Study of Sewage Sample

For sewage sample major fluorescence signals was obtained at about 350nm when excited at 280nm. Results obtained are well comparable with the findings of previous workers (Reynolds & Ahmad, 1997). Sewage is a sink of organic compounds which are surrogate of giving intense fluorescence. 3D EEM spectra (Figure 4) of sewage can be compare with the standard 3D EEM spectra of salicylic acid, as humic and lignin substances are main component of sewage sample & salicylic acid is one of the precursor in degradation product of humic acid (Waggot & Butcher 1976).EEM spectra of sewage shows rounded counters. From the EEM spectra it is observed that salicylic acid is the major component present in the DOM of sewage. Study shows that fluorescence EEM spectra can easily provide information of the unknown structure present in the DOM of sewage.

The presence of dissolved oxygen is known to quench some fluorescing species. However, previous unpublished research has shown that sewage fluorescence is not quenched by the presence of dissolved oxygen and will not be affected by aeration (Reynolds & Ahmad, 1997).



**Figure 4.** Three dimensional EEM Spectra of Sewage

### 3.2.2 EEM Study of Lake Water

A normalized fluorescence spectrum of lake water was obtained at 405nm. 3D EEM spectra of lake water (Figure 5) shows elongated counters. EEM spectra of the DOM from lake water are unable to give any idea about the type of fluorophore present in DOM responsible for the fluorescence. For lake water we are getting weak fluorescence, because aquatic DOM in lakes and rivers is often quite dilute and present with an inorganic salt matrix. (Westerhoff, Chen, & Esparza, 2001). Weak EEM spectra obtained from the lake DOM shows that pollution loads of lake water is under control and not required any water treatment.

No interference will be obtained from chlorophyll or any other common natural pigment, chlorophyll fluoresces strongly in the far red part of the spectrum with maximum at 665 nm. (Yentsch and Menzel, 1963). Measurement of DOM from natural water bodies are of interest as interaction of humic substances with some charged pesticides,

herbicides, xenobiotics and some metal ions in order to predict rate and transport of these chemicals (Frimmel, 2002). In remote sensing it is important due to their effect on light propagation in natural waters.

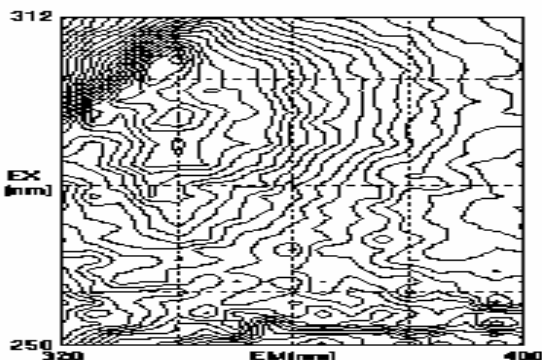


Figure 5. Three dimensional EEM Spectra of Lake Water

### 3.2.3 EEM Study of Dairy Wastewater

Dairy wastewater comprises of fluorophores of protein fractions of amino acid which are responsible for fluorescence at 338 nm with excitation at 280nm. 3D EEM study of dairy wastewater (Figure 6) is closely related with the 3D EEM spectra of tyrosine & tryptophan.

A liquid chromatographic study with fluorescence detection showed that the two fluorescent amino acids tyrosine and tryptophan are present in the soluble fraction of milk (Pulgarin, Molina, & Pardo, 2005). Hence, tyrosine and tryptophan are the only fluorophore responsible for the intrinsic fluorescence of the wastewater from the dairy industry. Standard tyrosine and tryptophan are highly fluorescent compounds, which in turn responsible for intense fluorescence obtained from dairy wastewater. Characterization of the DOM generated from dairy products can be possible and gives good EEM spectra giving idea about the functional group present in it.

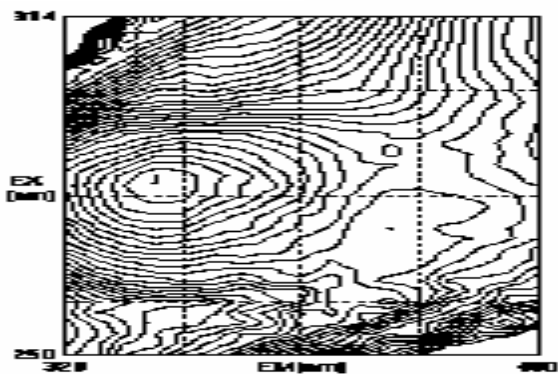


Figure 6. Three dimensional EEM Spectra of Dairy Wastewater

### 3.2.4 EEM Study of Herbal Pharmaceutical wastewater

EEM study of herbal pharmaceutical wastewater needs extra care to take. The brownish yellow color of wastewater is a hindrance for analysis by fluorescence spectrophotometer. Colour is due to the different additives used for various druge preparations High density and colored samples of herbal pharmaceutical wastewater were diluted by using 0.01M KCl solution in order to nullify inner filter effect due to absorbance at lower wavelength region and also help to maintain the pH of the samples. Ionic strength in the range of 0-1M KCl and humic acid concentration in the range of 5-100 mg/L had little effect on fluorescence spectral characteristics. (Jarafshan, et al, 1996). Dilution factor was considered at the time of fluorescence intensity measurements.

Fluorescence study of the herbal pharmaceutical wastewater shows weak fluorescence. A 3D EEM spectrum is shown in figure 7. Excitation wavelength was found in the range of 300 - 380 nm & emission wavelength was found in the range of 350 - 480nm. These figures agree closely with the emission maxima found for the wide range of colored samples in the United States reported by Ghassemi and Christman (1968). EEM study of the sample does not provide any idea of type of fluorophore present in the DOM. This weak fluorescence indicates the presence of high inorganic salt matrix associated with the DOM of water sample.

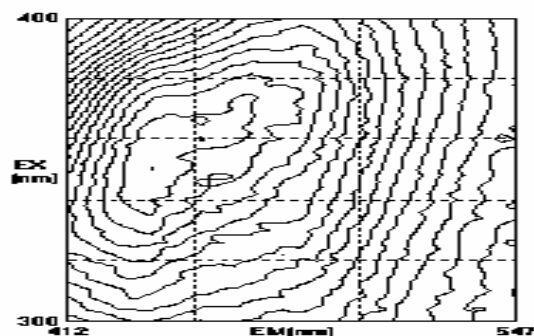


Figure 7. Three dimensional EEM Spectra of Herbal pharmaceutical Wastewater

## 4. Conclusion

From the study it was observed that this technique will prove a very useful tool for the real-time, non-invasive monitoring the level of carbon load in wastewater treatment plants and

tracing diverse sources of dissolved organic matter in natural water bodies. EEM spectra of the DOM provide the knowledge of the unknown structure of the fluorophore and ultimately helped for the better treatment for water and wastewater. It was observed that salicylic acid is the major component responsible for the fluorescence of the sewage. However, fluorescence obtained from the dairy wastewater is mainly due to the amino acid viz. tyrosin and tryptophan. An EEM spectrum's developed for lake water and herbal pharmaceutical wastewater does not provide any information of the factional group present in the respective DOM. Fluorescence intensity of the DOM is also depends on the type of fluorophore present it. Tyrosine and tryptophan are more fluorescent than salicylic acid; hence intense fluorescence is obtained from the dairy wastewater than that of sewage water. EEM study of lake water and herbal pharmaceutical wastewater shows that identification of the unknown fluorophore is only possible when the DOM is not embedded with inorganic salt matrix

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