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Excitation-Emission Matrix (EEM) Fluorescence Spectroscopy for Analysis of Dissolved Organic Matter (DOM) in Natural Water and Wastewaters

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Introduction

Decomposing plant materials, soil, living organisms and human activity cause dissolved organic matter (DOM) to be present in water. DOM is an important indicator for water quality, as it reflects the microbial activities, human activities and geological conditions around the area of water bodies.

Excitation-emission matrix (EEM) fluorescence spectroscopy, also referred to as FEEM, has been used to characterize the DOM and identify fluorescence emitting organic substances in natural water [1-2]. Here, we describe analysis examples of DOM in several types of water samples using the 3-dimensional (3D) EEM measurement on the RF-6000 Spectrofluorophotometer.

Experimental

Selected organic compounds standards are used for EEM measurement as representatives of major classes of DOM components. The compounds, including tryptophan, tyrosine and humic acid, were purchased from Sigma Aldrich, USA. Type E-1 ultra pure water with resistivity of 18 MΩ was used to prepare standards. Tryptophan, tyrosine and humic acid are used due to their fluorescent properties and are related to proteins and peptides.

The tryptophan and tyrosine were dissolved with ultra pure water to a concentration of 1 mg/L. The humic acid was dissolved with ultra pure water to a concentration of 20 mg/L and adjusted to a pH of 8 to 9 using sodium hydroxide solution. The samples used in this study were surface water from a local pond and wastewater samples from two different industrial sources. The samples were filtered through a 0.2 µm nylon filter prior to measurement.

EEM spectra of the samples were measured using the 3D spectrum mode in LabSolutions RF workstation on RF-6000. The measurement conditions are shown in Table 1.

Parameter	Value
Spectrofluorophotometer	Shimadzu RF-6000
Spectrum Type	3D Spectrum
Wavelength Range	Excitation (Ex) 250 nm to 400 nm, Emission (Em) 250 nm to 400 nm
Wavelength Interval	Ex 2.0 nm, Em 1.0 nm
Scan Speed	2000 nm/min
Bandwidth	Ex 3 nm, Em 3 nm

Table I: Instrument conditions

Experimental

The analysis of the dissolved organic matter was performed using a Shimadzu RF-6000 Fluorospectrophotometer. Individual emission and excitation spectra can be extracted from the 3D FEEM measurement. (Figure 4 b,c,e,f)

50 mL of water samples were collected from two lakes in polypropylene centrifuge tubes. Two samples were collected at each location and measured in triplicates. The samples were allowed to settle before analysis; pH was not adjusted.

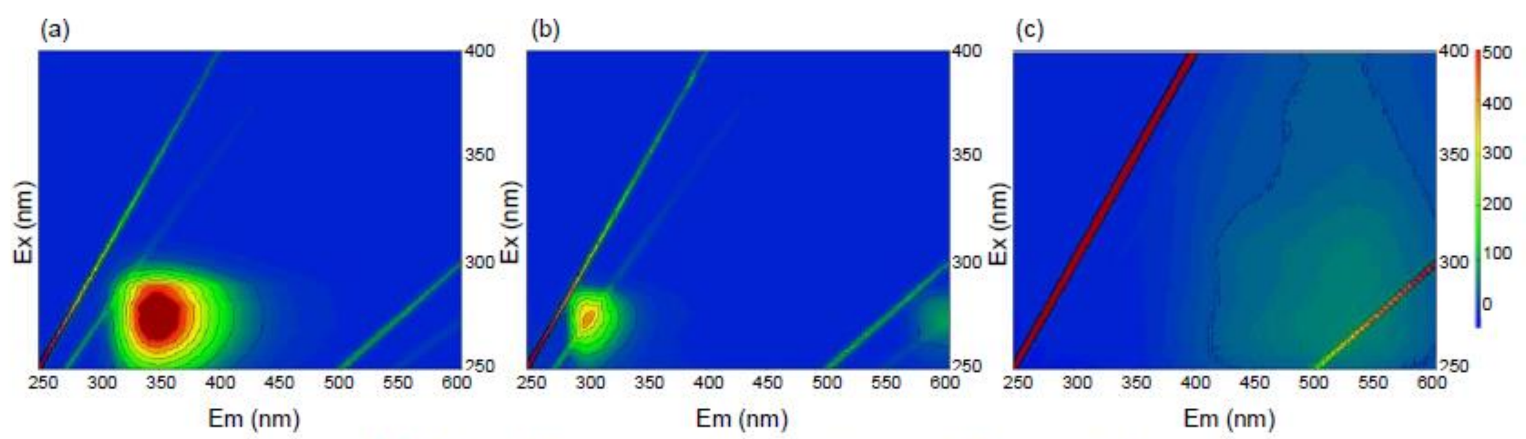

Figure 1. EEM 3D spectra of (a) tryptophan, (b) tyrosine, (c) humic acid in pure water

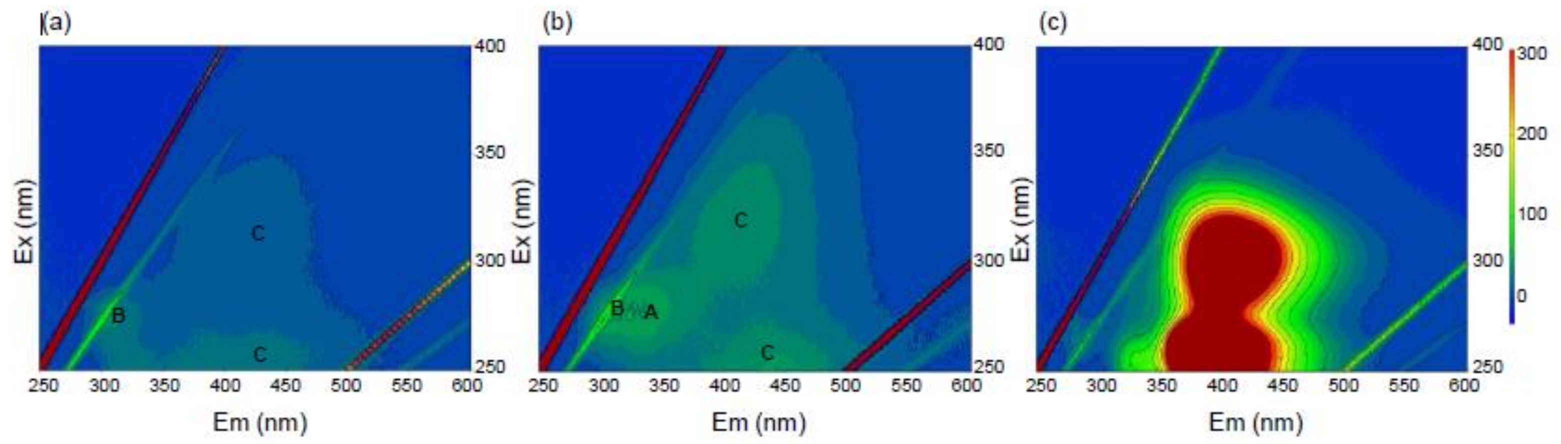

Figure 2. EEM spectra of (a) pond surface water, (b) wastewater from source X, (c) wastewater from source Y

	Fluorophore	Excitation (nm)	Emission (nm)
A	Tryptophan-like, protein-like	225 - 237	340 - 381
		275	340
B	Tyrosine-like, protein-like	225 - 237	309 - 321
		275	310
C	Humic-like	237 -260	400 - 500
		300 - 370	400 - 500
	Marine humic-like	312	380 - 420

Table 2. Excitation emission spectra table for the common fluorophores.


Figure 3. Sampling Sites Lake #1 (left) Lake #2 (right).

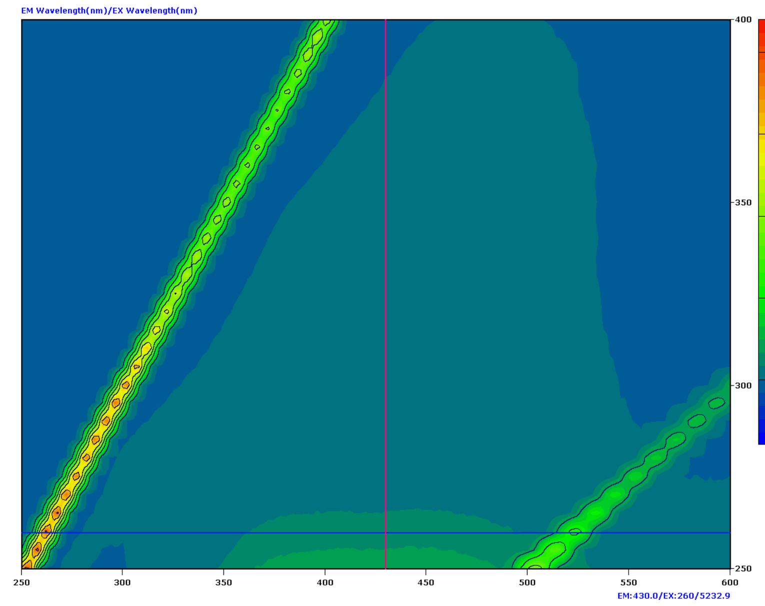
Figure 4a. EEM Spectrum of Lake #1

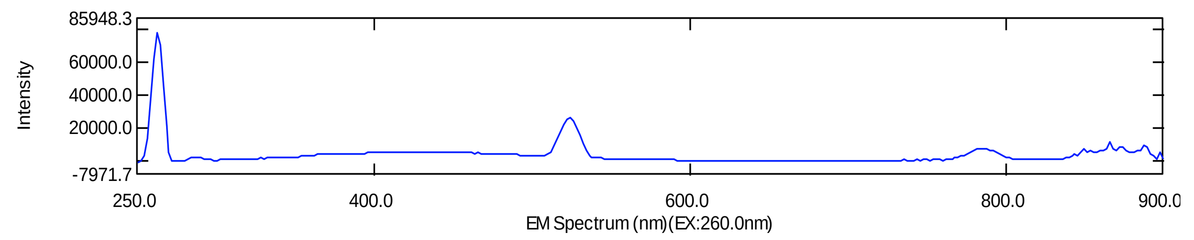

Figure 4b. Emission spectrum corresponding to cursor in Figure 4a.

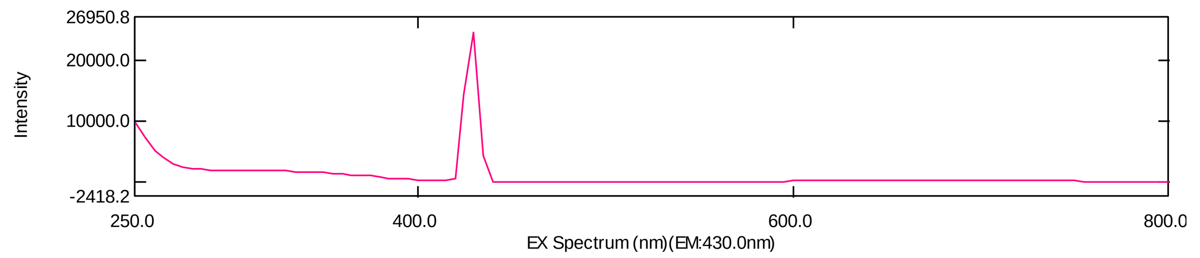

Figure 4c. Excitation spectrum corresponding to cursor in Figure 4a.

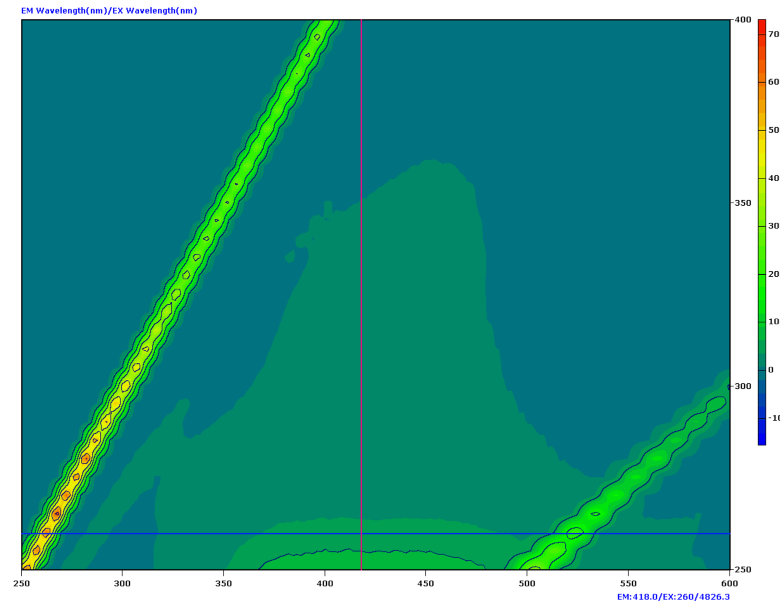
Figure 4d. EEM Spectrum of Lake #2

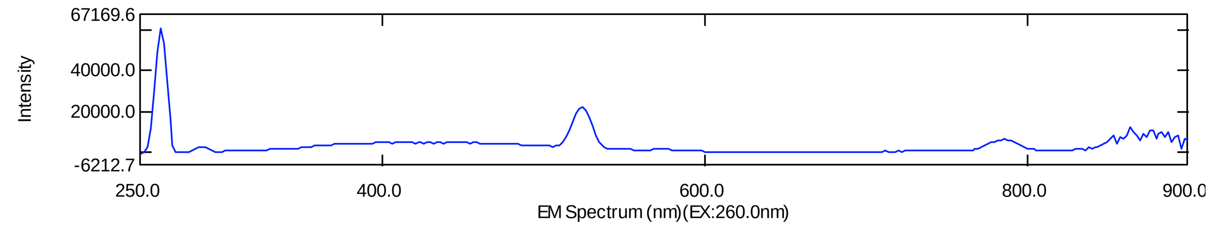

Figure 4e. Emission spectrum corresponding to cursor in Figure 4d.

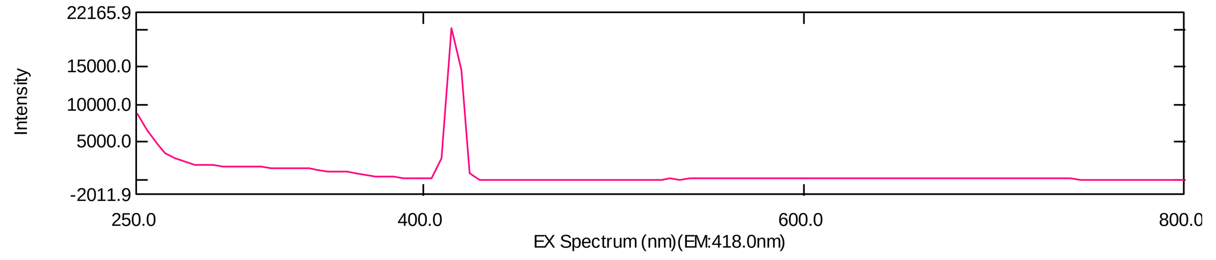

Figure 4f. Excitation spectrum corresponding to cursor in Figure 4d.

Figure 4(a-f). Samples taken from local lake in Columbia, MD. Two separate locations of the lake were measured separately. The Emission spectra for the possible humic acid (blue) with the excitation spectra (red).

Results & Discussion

EEM spectra of standards:

Organic acids such as humic and fulvic acids, along with amino acids of proteins are the indicative components of DOM which can be characterized by the EEM of aquatic fluorophores.

Tryptophan, tyrosine and phenylalanine have fluorescence properties due to the presence of anindole group; these compounds indicate the presence of protein and peptides. The EEM 3D spectra of Tryptophan and tyrosine standards are shown in Figure 1a and 1b, respectively.

The EEM spectrum of humic acid in water is less defined, as shown in Figure 1c. Humic acids have multi-chromophoric groups and its fluorescence behavior differs from a single chromophore molecule. Humic acid fluorescence differs from its different sources due to the small differences in composition. It has a broad range with Em of 400 ~ 600 nm at Ex of 250 ~ 370 nm [1]. Within the spectrum characteristic of humic-like DOM, a narrower is characteristic of humic-like substances of marine origin.

EEM analysis of natural water and wastewaters:

The EEM spectrum of the pond surface water (Figure.2a) shows tyrosine-like fluorescence as well as a broad Em 350 ~ 500 nm with Ex 250 ~ 370 nm which could be attributed to the presence of humic materials. The EEM spectrum of the lake surface water (Figures 4a, 4d) shows consistency with the presence of humic acid which is indicative of the presence of DOM in natural waters. The FEEM signatures for tryptophan and tyrosine however could not detected in these samples.

The two wastewater samples have different EEM spectra, indicating that the DOM content has different composition. The wastewater from source X show tryptophan-like (A), tyrosine-like (B) and humic-like fluorescence (C) (Figure2b). The wastewater from source Y shows the presence of a strong fluorophore of Em 350 ~ 480 nm at Ex 250 ~ 280 nm and 280 ~ 350 nm (Figure. 2c). These results suggest that specific composition and/or amount of DOM was different between those samples.

Conclusion

The excitation-emission matrix (EEM) fluorescence spectroscopy can be used as a rapid analytical tool in the characterization of DOM in natural water and wastewater. Measuring additional DOMs using the EEM method with the RF-6000 could create a database of DOM materials for measuring the water quality and could be potentially used to assess water quality, wastewater treatment process and monitor pollution of natural water.

References

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