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Application News

No. SCA_105_010

Fluorescence Spectroscopy

Dissolved organic matter analysis (DOM) And its appearance under different environmental Conditions – fluorescence EEM matrices of different sources

▪ DOM in Water

As described DOM is the short form for dissolved organic compounds in water. Such materials can be very complex and quantities of them in water are depending on the „lifestyle“ of it. Three of such expected components are L-Tyrosine which is fluorescence active, Tryptophan and humic acid. [1] Humic acid is result of natural decomposition of plants and leaves. In water humic acid will acidify and though will be responsible when pH-value of the water is shifting to < 7, where 7 is neutral. For comparison a tonic water has pH level of approx. 3. But on the other hand humic acid is found as being an ion exchanger.
Samples for testing were from different sources. Sources were natural water, beverage and for comparison biological dead, distilled water for analytical purposes. From all were done fluorescence measurements.

❑ Natural Fresh water

Fresh water standing in a tube under pressure of approx. 1.5 to 2 bar and this under condition of 15 up to 40 degree Celsius.
The sample was pumped up from 10 to 15 m under NN and had a temperature of approx. 15 degree Celsius. Such fresh water was transferred under pressure of 1.5 to 2 bar
The samples fresh 1 and fresh 2 were kept in the refrigerator under 8 degree Celsius.

❑ Waste water – natural environment

Were collected from park ponds. First sample was picked from a pond without connection to fresh water flow and second a pond with fresh water flowing in very smoothly. These ponds have their biosphere and are used from water related animals, birds, insects and fishes.
In case of pond source 1 (waste 1) only birds, insects and source 2 (waste 2) all natural creatures are possibly using it.

❑ Mineral water

A bottle of still mineral water was fallen into focus because of not absolutely clear and colourless. The subjective judgement caused the test of the bottled water. Mineral water naturel a beverage from a source 300 m under NN.

Fig.1. Water in natural environment, pond/sea style and little river flowing thru slowly (source of sample waste 2)

❑ Bi-distilled water

Bi distilled water was taken as reference. Such water was used to prepare standards from the pure DOM materials standards which were used for comparison.

- **Sample preparation**

From all samples was taken some milliliter to fill a 10 mm non-fluorescent quartz cuvette (Hellma).

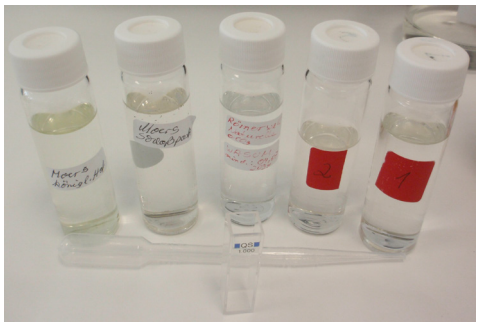

Fig. 2. bottled Water and environmental samples, fluorescence cuvette and pipette

With a disposal pipette the liquid was transferred. The liquids had all room temperature when measurement started. The „waste" water 2 was filtrated because the waste inside were little earth clods and insect larvae.

- **LabSolutions RF and EEM Matrix**

The analysis was done with Shimadzu Fluorescence spectrophotometer RF-6000 under control of LabSolutions RF software. One benefits of the software is the application method EEM matrix – short 3D Spectrum. EEM is the short for Excitation-Emission-Matrix. Such matrix is like a finger print describing a material. It is very useful for screening of mixtures. In this case the EEM was prepared under condition of high sensitivity, higher response time and specific high resolved mapping interval.

Mapping parameters as fixed in [1]:
EX wavelength interval: 2 nm
EM wavelength interval: 1 nm
Scan speed: 2000 nm/min
Bandwidth: 3 nm for EX/EM slit
Wavelength range: EX- 250 – 400 nm,
EM 250 – 600 nm

- **Analysis Result**

EEM Matrices from different waters in a range of 0 to 100 intensity units are presented in figure 3 to 8.

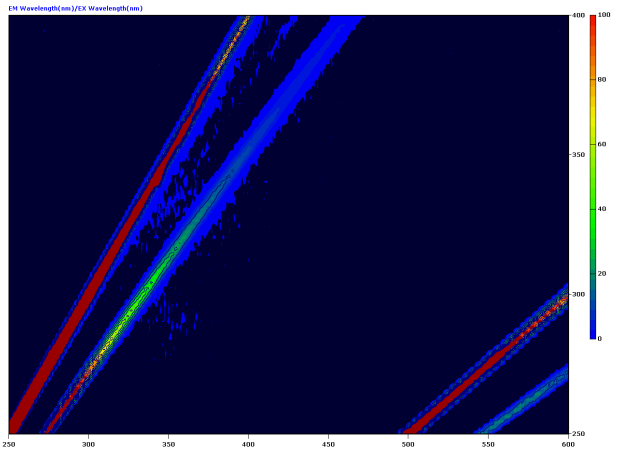

Fig.3. EEM spectra from bidistilled water – intensity range 0 to 100 Intensity Units, A, B and C could not be detected

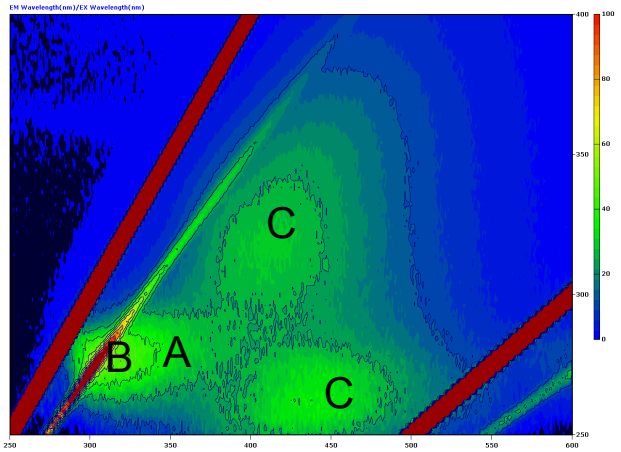

Fig 4. fresh water source 1 contains proteins like A, B and C

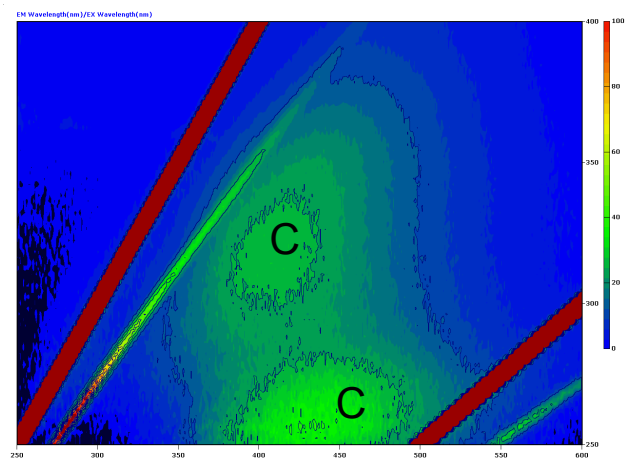

Fig 5. fresh water source 2 contains proteins like C

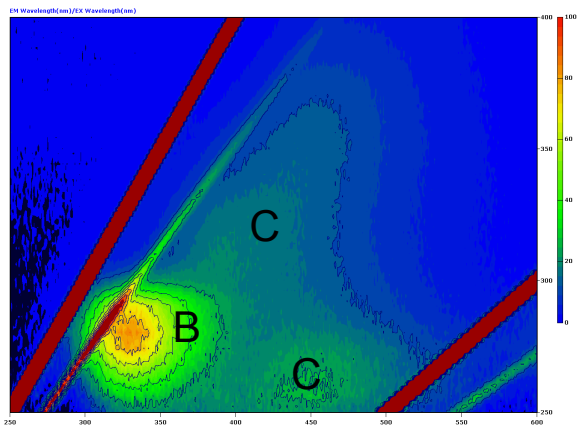

Fig 6. waste water source 1

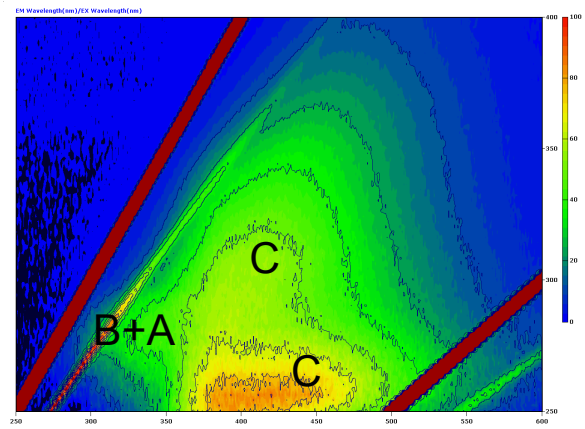

Fig 7: waste water source 2

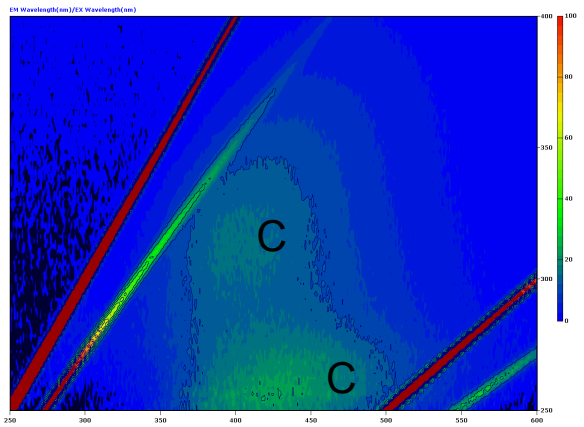

Fig 8: mineral water still, intensity from 0 to 50

All 6 figures with DOM EEM matrices are presented in a scale of 0 to 100 intensity units.

Dependent on age, temperature and pressure the distribution of DOM varied.
Even though from same source (fresh water) and from same water hose the water showed that it is a matter of time finding the three different proteins in traces with fluorescence spectroscopy.
For further discussion lets name tryptophan like protein A, tyrosine like protein B and humic acid like C.
In figure 3 the ultimative result for pure water proved on the three proteins. It shows no content.
In figure 4 you can see a triple spot view. These spots are standing for A, B and C1+C2 like proteins. It is the so called fresh water (fresh 1) which was standing in a water hose, was heated under full sun exposure. Under this condition the water (fresh 2) changed from a mix containing mostly C (fig. 5) to a mixture of A, B and C (fig. 4). Figure 6 and 7 show a shift of the substance composition from mostly C humic acid like (fig. 7) to mainly tyrosine for the standing water (fig. 6).

The mineral water contains mostly the component C humic acid like in very low concentrations.

Table 1 shows an overview between the laboratory samples and the real life samples. It shows the maximal intensity in the three ranges of DOM proteins [1].
These are:
A: EX = 275 nm, EM = 340-381 nm
B: EX = 275 nm, EM = 310 -320 nm
C2: EX = 300-370 nm, EM = 400-500 nm
C1: EX = 240-260 nm, EM = 450-500 nm
Whereas EX is Excitation, EM is Emission, and [nm] the unit for the wavenumber scale.

As approximation in a single point linear calibration the mineral water had a content of 5 to 7 mg/L humic acid like protein. This generated a colouring which the sensitive human eye can already detect. The emission signal range of humic acid of the life material is shifted to shorter wavelengths. The pH-value was checked and found with 5 to 7 for the Life samples, the standard was stabilized by pH-value of 8 to 9. It is known that the fluorophores react on the pH-value but this aspect was not theme of this application.

Fluorophore	Samples								
	Humic acid - 20 mg/L	Tyrosine 1 mg/L	Tryptophane 1 mg/L	Bi-distilled	Mineral water	Fresh 1	Fresh 2	Waste 1	Waste 2
A	0	0	976	0	0	34.0	0	86.1	38.6
B	0	583	0	0	0	44.5	0	45.7	32.0
C1	100	0	0	0	27	34.9	36.6	24.9	82.6
C2	51	0	0	0	17	30.0	26.5	17.3	40.0

Table 1: Intensities extracted from the EEM matrices of fresh, waste and other water samples and fluorphore samples.

▪ Calibration

For comparison three solutions were prepared.
1. L-Tyrosin 1 mg/L in pure water
2. Tryptophan 1 mg/L in pure water
3. Huminic acid 20 mg/L in pH 8 to 9 stabilized pure water NaOH solution [1]. The EEM matrices shown [Fig. 9] are the result of the stepwise movement of the Excitation grating in 1nm steps recording the Emission spectra fitting to the Excitation wavelength. The fingers moving over the screen are the excitation light energies and Raman signals of water from 1st and 2nd order light.

▪ Literature

[1] Shimadzu Asia Pacific, Application News, No. AD-0133, 2016.

▪ Instrumentation

The application was done with the Shimadzu RF-6000 Fluorescence spectrophotometer. In Combination with LabSolutionsRF software 3D Spectrum measurements were performed.
The 3D Spectrum method generated EEM spectra views.

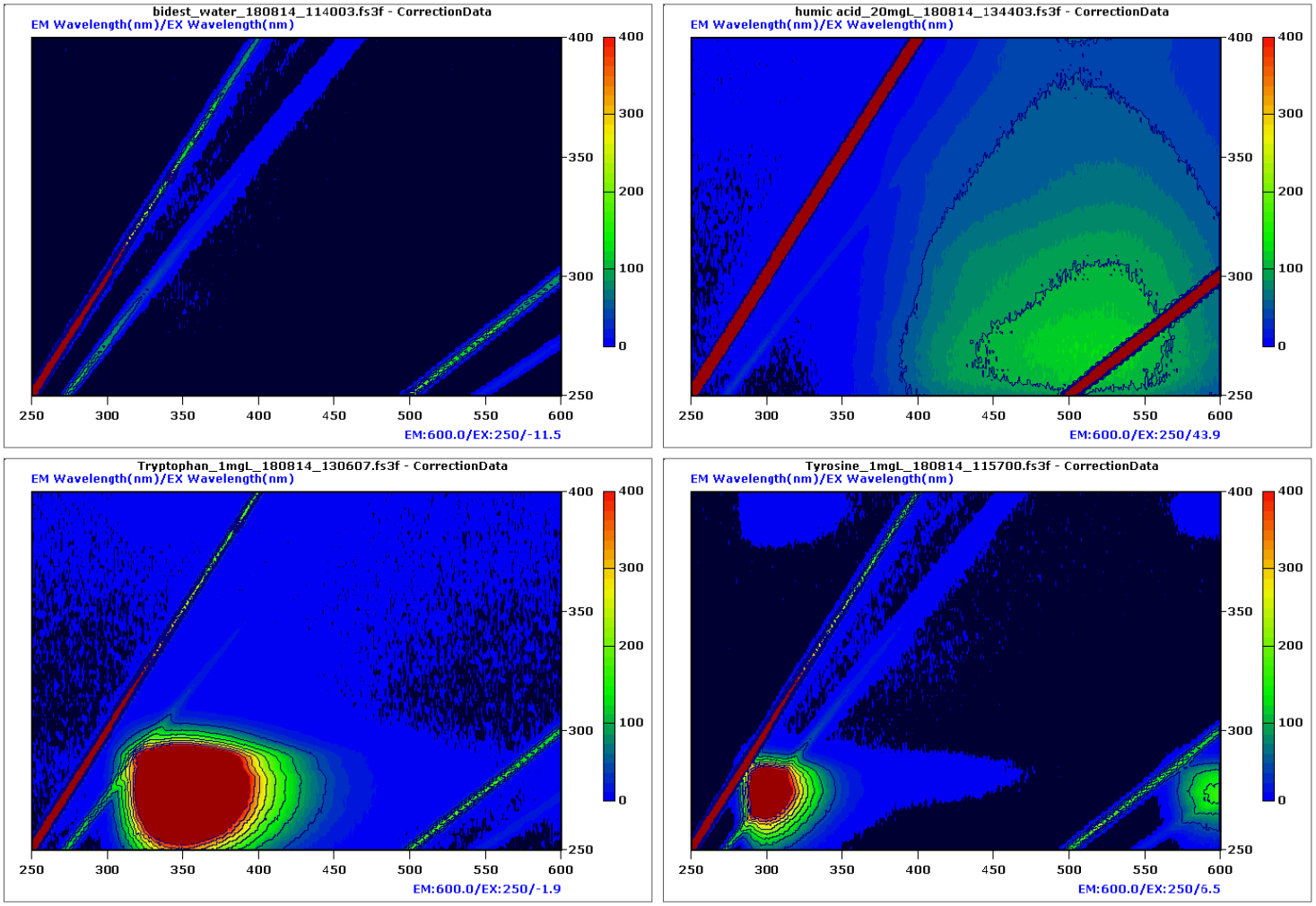

Fig 9. View on 4 EEM-Matrices which are top left bi-distilled water, top right humic acid, lower left tryptophane and lower right tyrosine standards in same bi-distilled water.in a scale of 0 to 400 intensity units, concentrations see under calibration

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