

3rd Student Workshop on Ecology and Optics of Coastal Zones

10 - 13 July 2017

Museum of the World Ocean, Kaliningrad, Russia

54°44'N 20°29'E

**IDENTIFICATION OF FLUOROPHORES
IN AQUATIC NATURAL DISSOLVED
ORGANIC MATTER**

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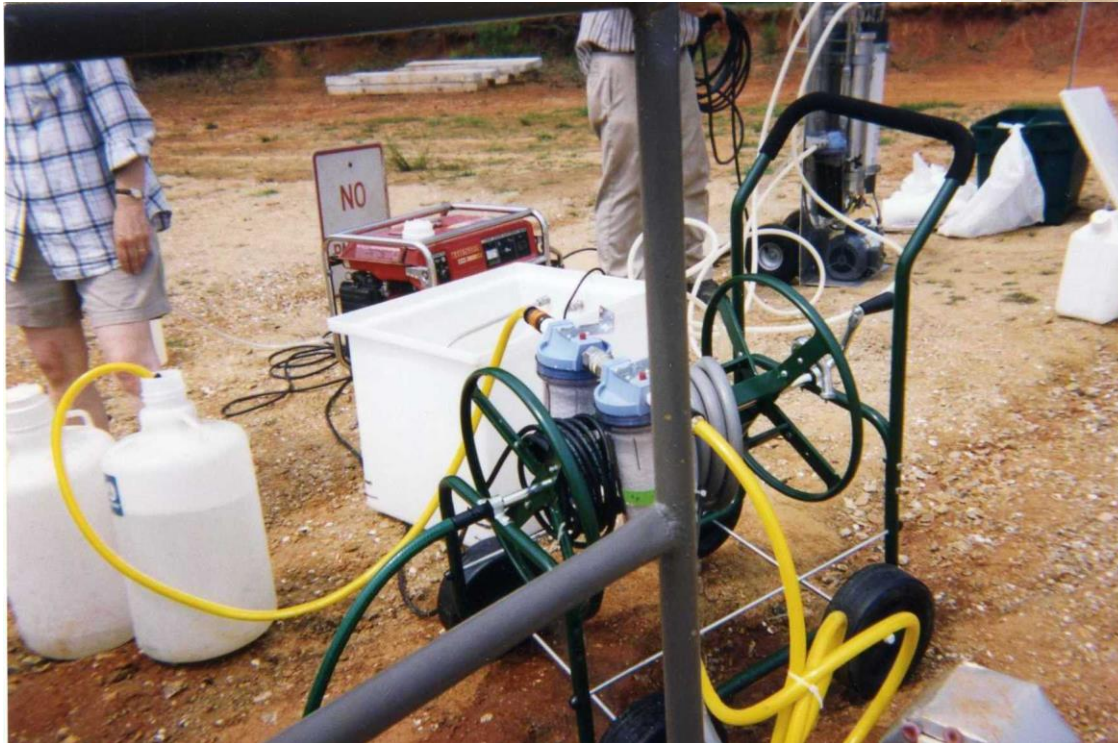
Suwannee River, Georgia, USA



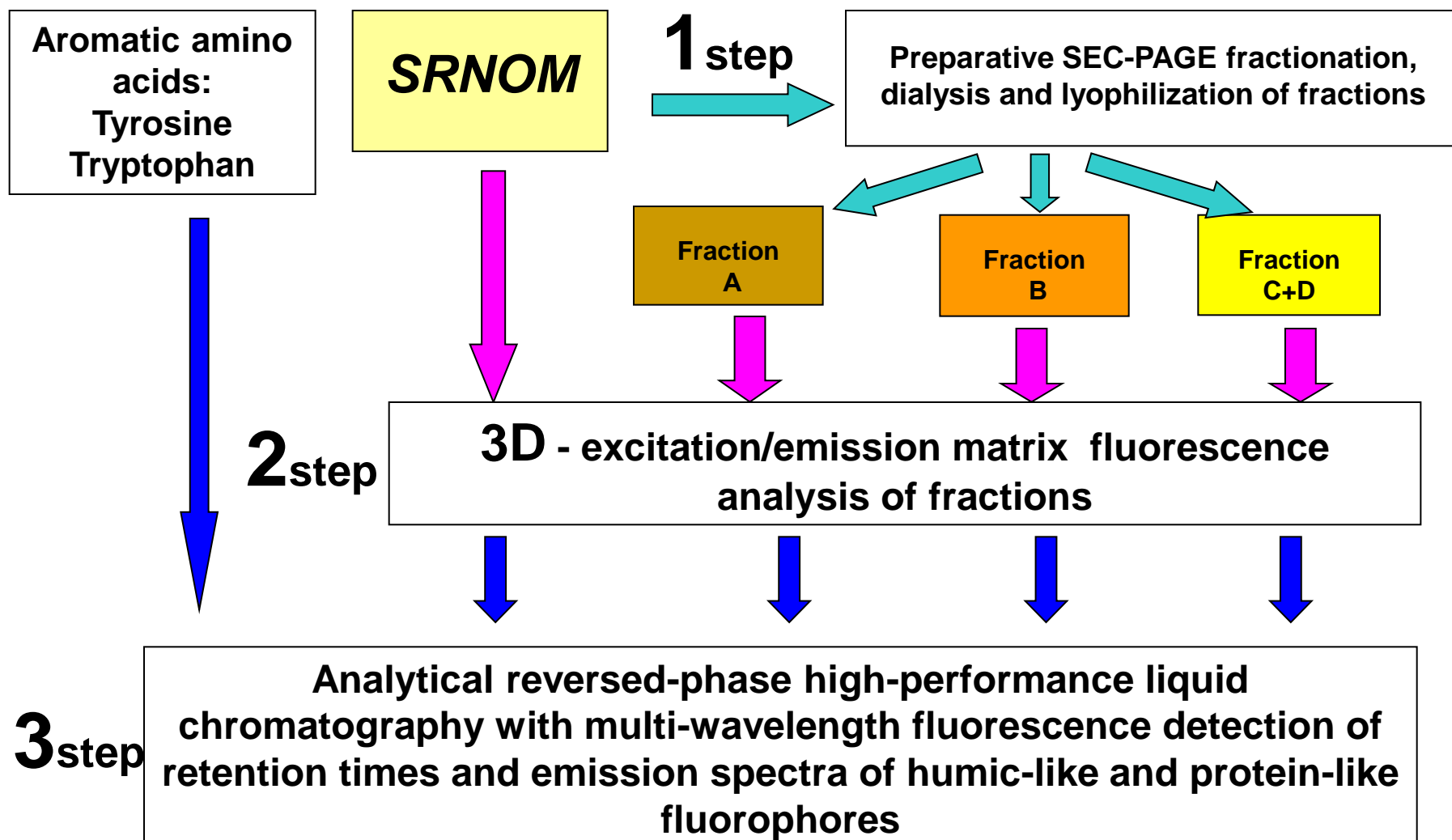
SRNOM

international
standard 1R101N
C_{SRNOM}=40 mg/L

SRNOM isolation by reverse osmosis

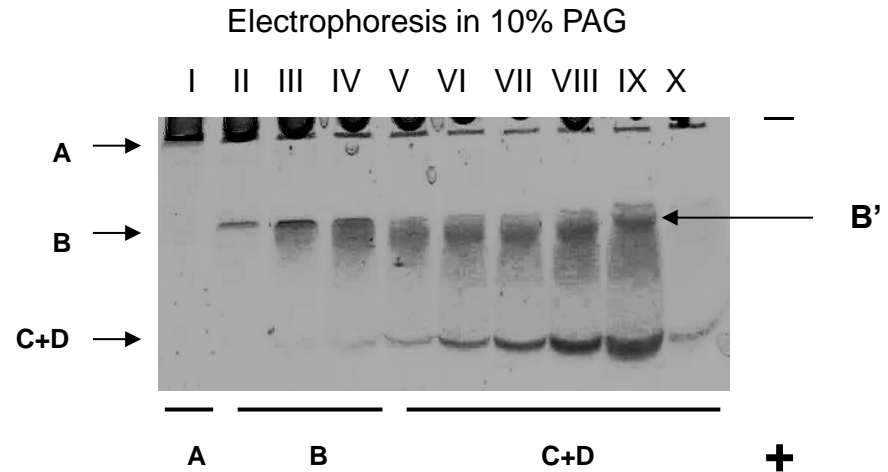
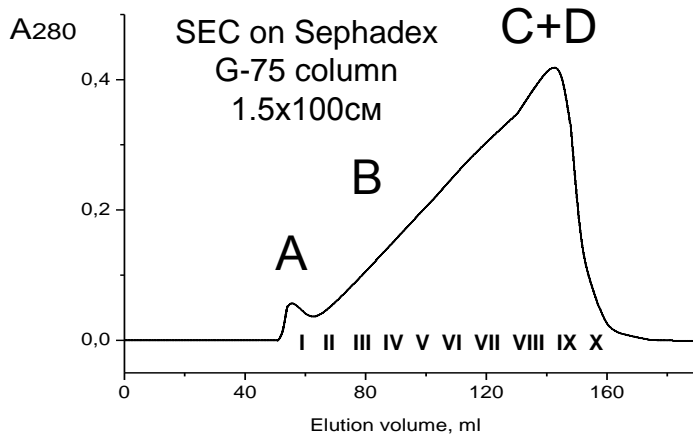


Scheme of experiment

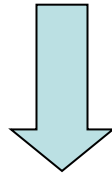


1.

Preparative SEC fractionation in 7M urea and analytical electrophoresis in 10% PAG as testing system



Dialysis through 10 kDa cellulose tubes and lyophilization of fractions



$MS_A > MS_B > MS_{C+D} > 10\text{kDa}$

Fraction A - 4%
 Fraction B - 10%
 Fraction C+D - 35%

Organic matter with $MS < 10\text{kDa}$ - 51%

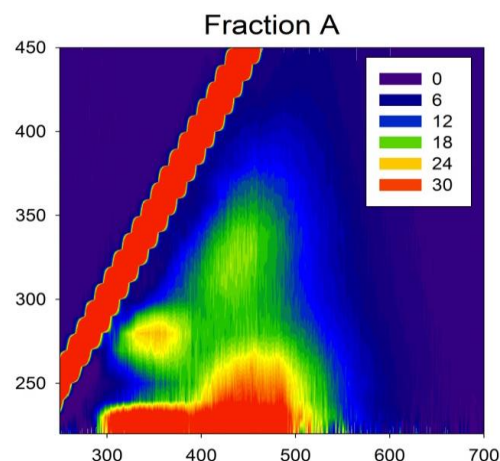
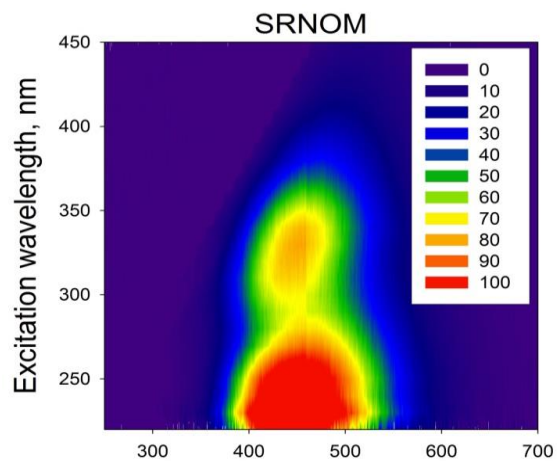
Трубецкой О.А., Трубецкая О.Е., Ришар К. 2009. Водные ресурсы 36:543-550

Trubetskaya O.E., Richard C., Trubetskoj O.A. 2015. Environmental Science and Pollution Res. 14:495-500

Trubetskaya O.E., Richard C., Voyard G., Marchenkov V.V., Trubetskoj O.A. 2016. Desal. Water Treatm. 57:5358-5364

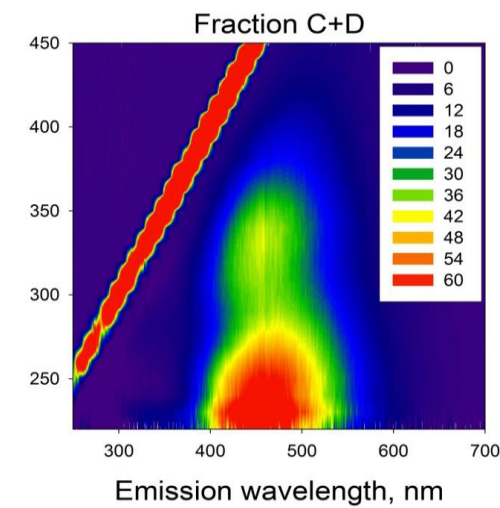
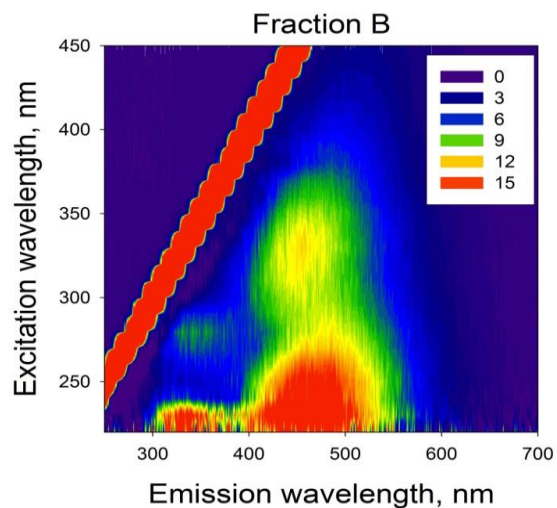
2.

3D - excitation/emission matrix fluorescence analysis of fractions A, B and C+D



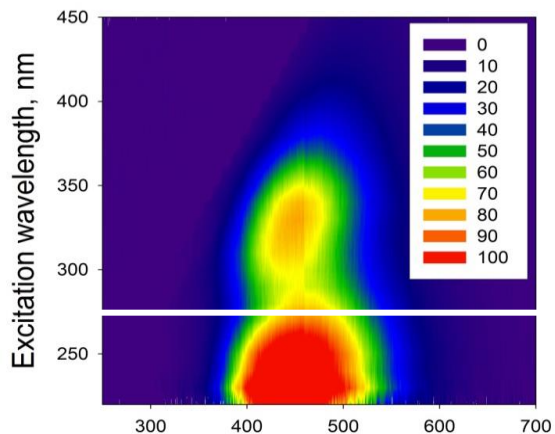
$MS_A > MS_B > MS_{C+D} > 10 \text{ kDa}$

$A_{270} = 0.05 \text{ a.u.}$

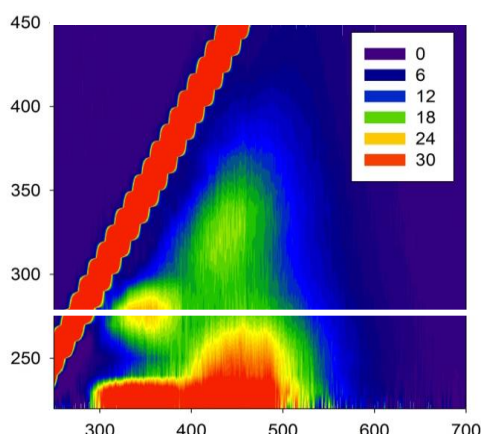


3D- и 2D- fluorescence analysis of fractions A, B and C+D and selection of optimal conditions for RP-HPLC

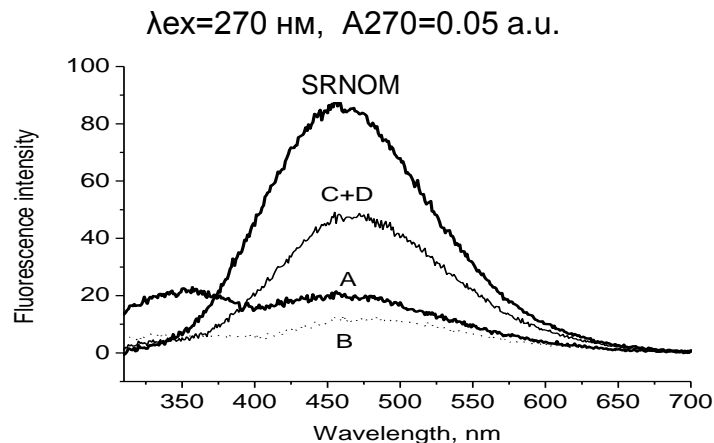
SRNOM



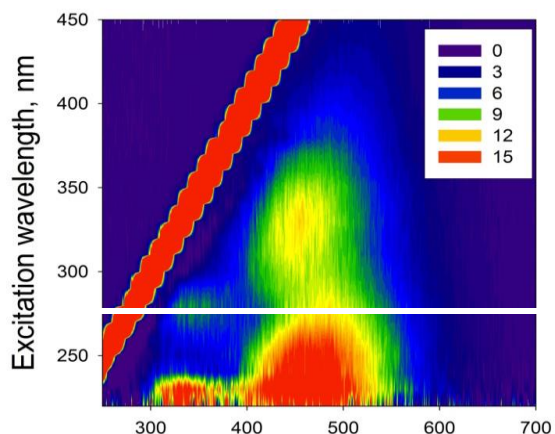
Fraction A



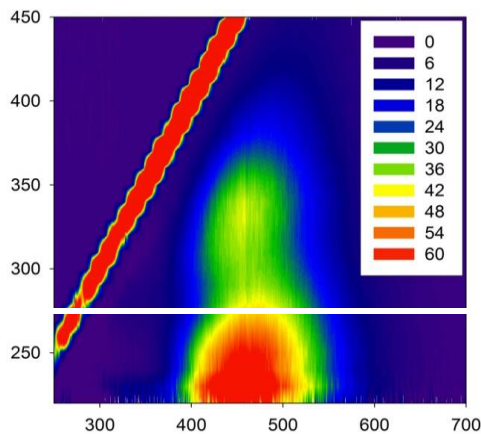
2D-fluorescence spectra



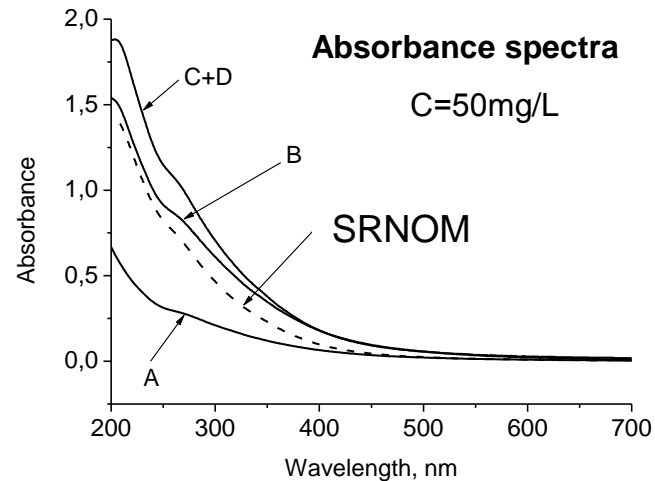
Fraction B



Fraction C+D



Absorbance spectra



Emission wavelength, nm

Emission wavelength, nm

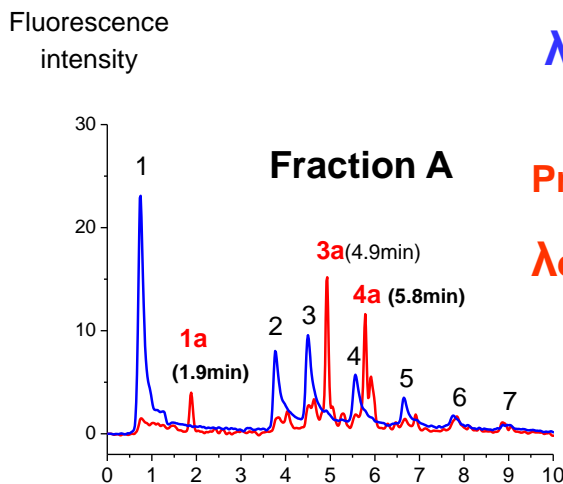
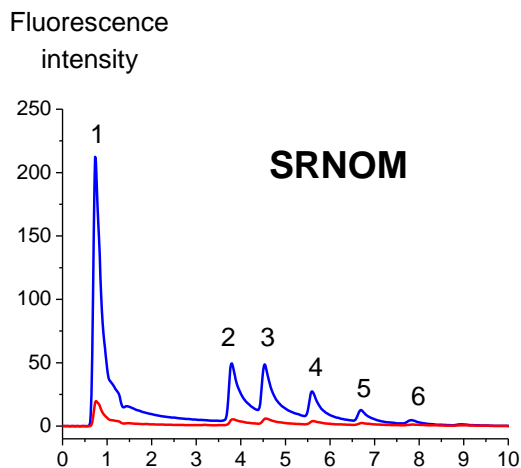
Wavelength, nm

3.

Analytical reversed-phase high-performance liquid chromatography with multi-wavelength fluorescence detection

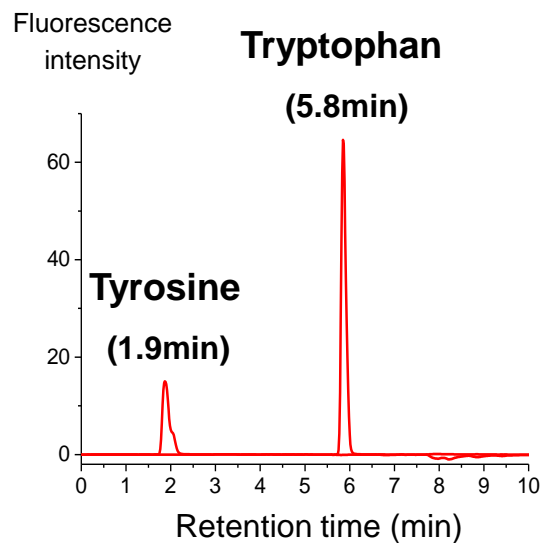
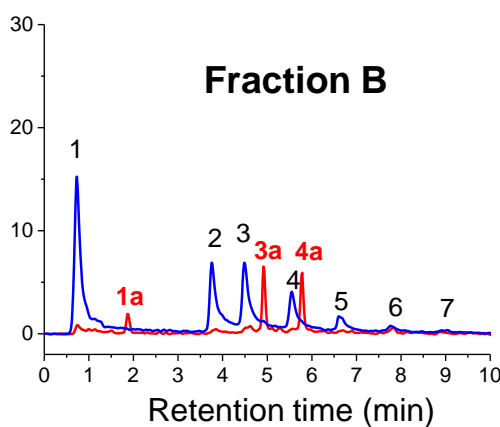
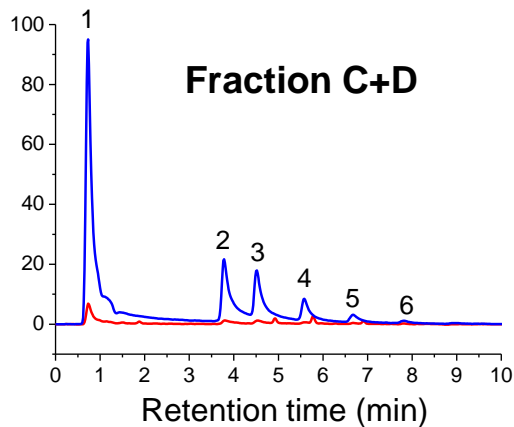
Humic-like fluorophores detection

$\lambda_{ex}/\lambda_{em} = 270\text{nm}/450\text{nm}$

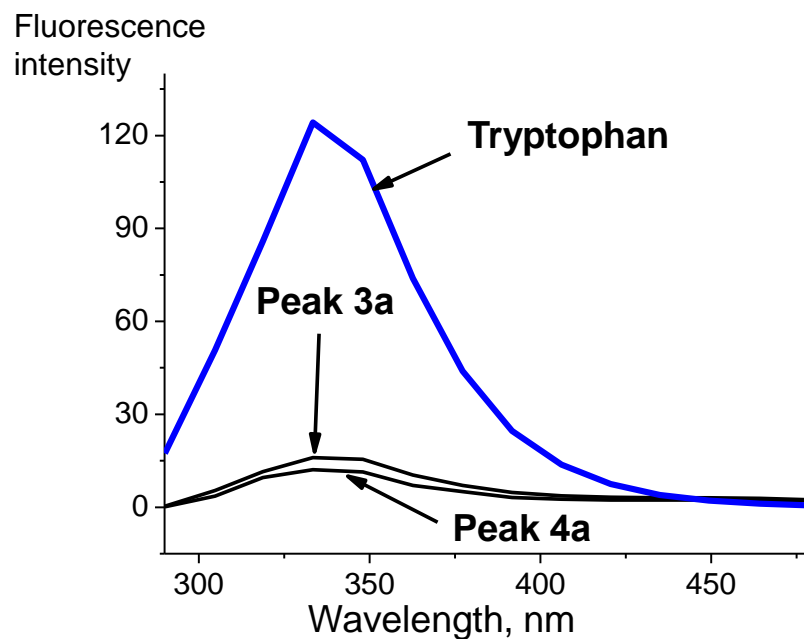
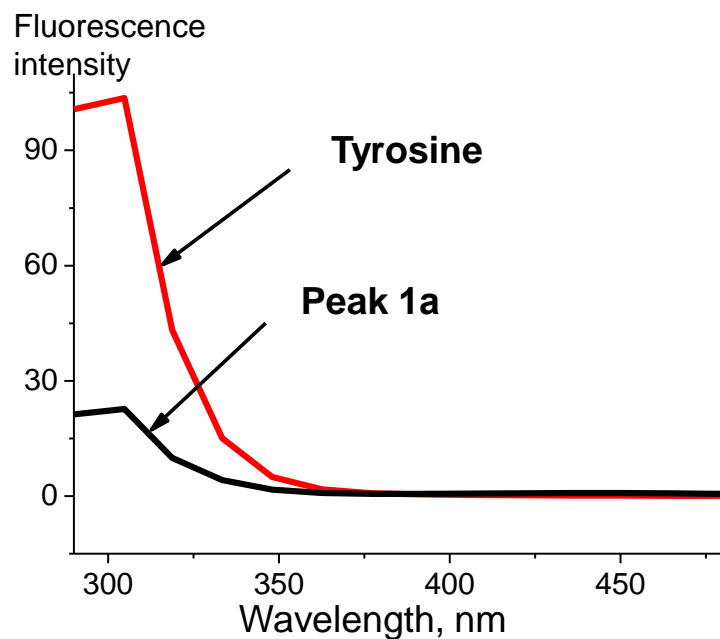


Protein-like fluorophores detection

$\lambda_{ex}/\lambda_{em} = 270\text{nm}/330\text{nm}$



Fluorescence spectra of chromatographic peaks 1a, 3a, 4a and amino acids tyrosine and tryptophan from the data of multi-wavelength fluorescence detector at $\lambda_{ex} = 270$ nm, $\lambda_{em} = 290-580$ nm



Retention time :

Tyrosine - 1.9 min

Peak 1a - 1.9 min

Tryptophan - 5.8 min

Peak 4a - 5.8 min

Peak 3a - 4.9 min

CONCLUSIONS

- Himic-like fluorescence of SRNOM is caused by the sum of several fluorophores having different emission maxima - hydrophilic with $\lambda = 435$ nm and several hydrophobic ones with $\lambda = 450-465$ nm
- About 50% of the protein-like fluorescence of SRNOM is due to the presence of free amino acids of tyrosine and tryptophan in the fractions of the largest and average molecular size
- The detection of free amino acids in the aquatic NOM is extremely important for understanding the role of DOM as a natural archive of amino acids and potential source of structural components for protein synthesis as the basis of life.

Sandro Botticelli
"The Birth of Venus" 1482-1486



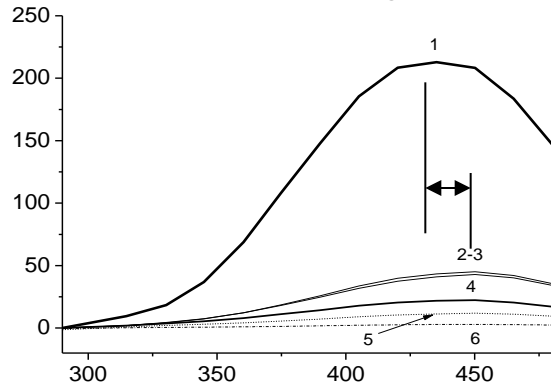
The work has been supported by:

- **COBASE, USA**
- **Russian Foundation for Basic Research 13-05-00241 and 15-04-00525**
- **International project №12 between CNRS (France) – RAS(Russia)**

Спектры флуоресценции хроматографических пиков по данным мультиволнового детектора флуоресценции, настроенного на $\lambda_{воз} = 270\text{нм}$, $\lambda_{исп} = 290-580\text{нм}$

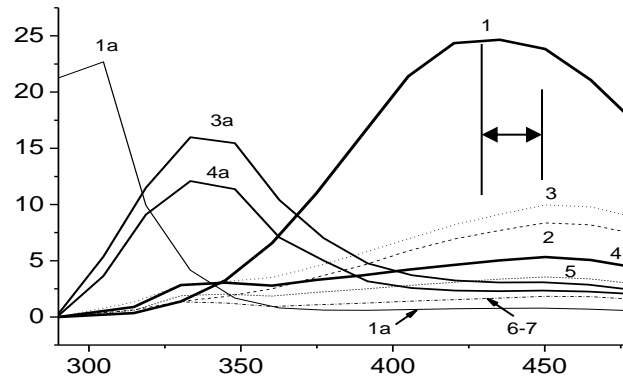
Интенсивность флуоресценции

СуРОВ



Интенсивность флуоресценции

Фракция А

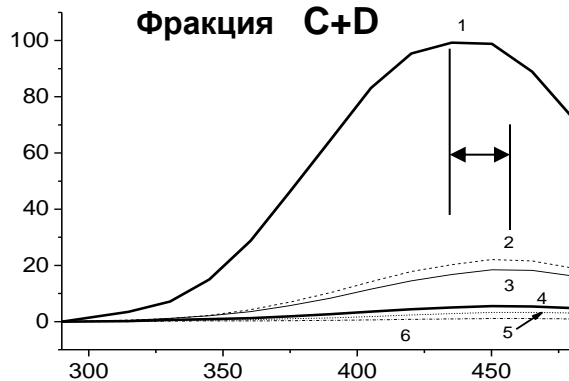


1. Гуминоподобный гидрофильный флуорофор (пик 1) – $\lambda_{макс} = 435\text{нм}$

2. Гуминоподобные гидрофобные флуорофоры (пики 2-7) $\lambda_{макс} = 450-465\text{нм}$

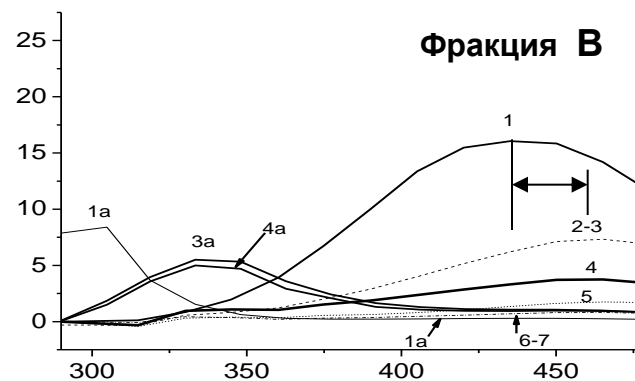
3. Белковоподобные флуорофоры (пики 2-7) $\lambda_{макс} = 350\text{нм}$

Фракция C+D



Длина волны (нм)

Фракция В



Длина волны (нм)

4. Свободные аминокислоты

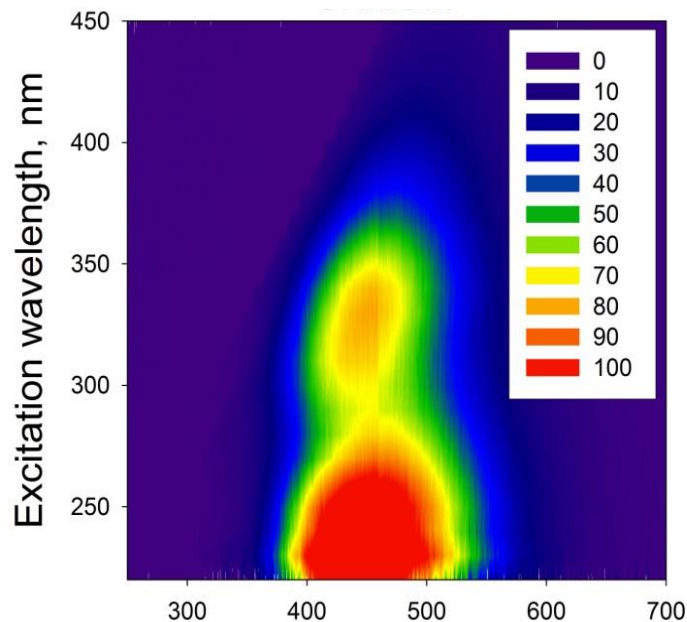
тирозин (пик 1a) с $\lambda_{макс} = 300\text{нм}$, время выхода с колонки – 1.9мин

триптофан (пик 4a) $\lambda_{макс} = 350\text{нм}$, время выхода с колонки – 5.8мин

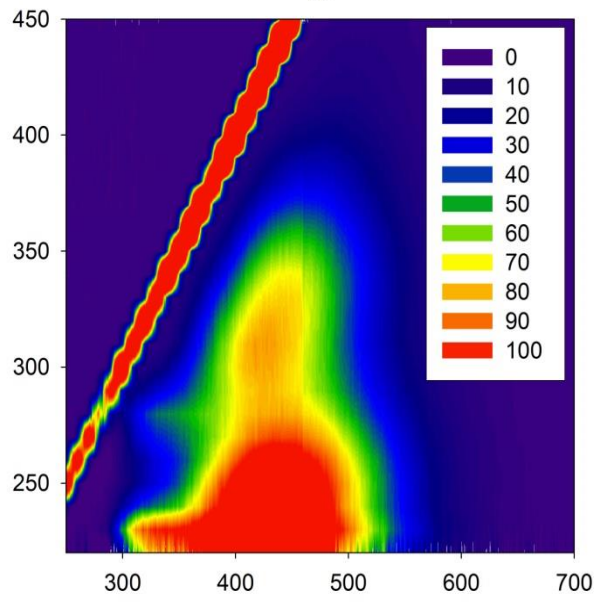
3D-флуоресцентные диаграммы РОВ трех водных источников различного генезиса и географического положения

$A_{270}=0.05$ о.е.

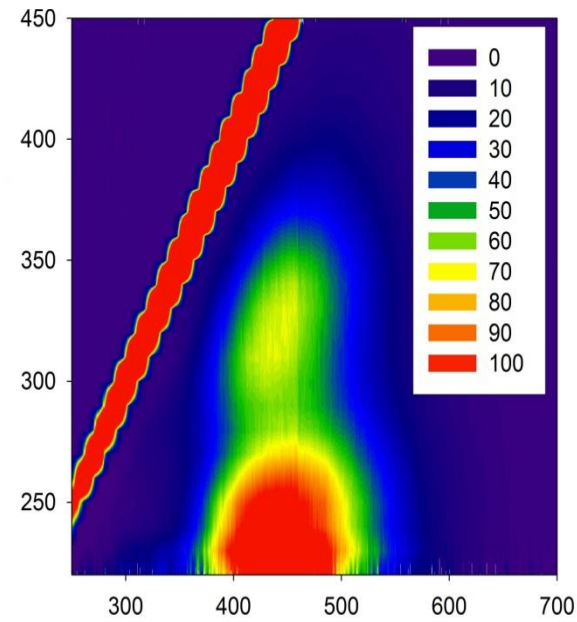
Река Сувани
Джорджия, США



Онежское озеро
Карелия, Россия



Водопроводное озеро
Карелия, Россия



Emission wavelength, nm

Emission wavelength, nm

Emission wavelength, nm

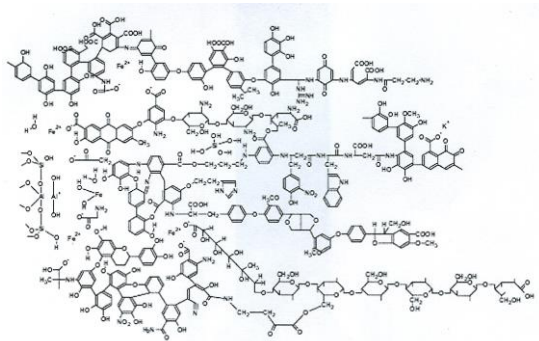
- Важнейшим свойством и отличительной чертой класса ГВ от биологических молекул является их устойчивость к разложению микроорганизмами и другими абиотическими факторами
- ГВ проявляют ярко выраженные поверхностно-активные свойства
- ГВ образуют коллоидные растворы со средним минимальным диаметром частиц от 90 до 200 Å

Концентрация кислород-содержащих функциональных групп в составе средней ГВ

Функциональные группы	Мг-экв/грамм
COOH	4,5
Фенольные OH	2,1
Спиртовые OH	2,8
Хиноидные C=O	2,5
Кетонные C=O	1,9
ОСН ₃	0,3

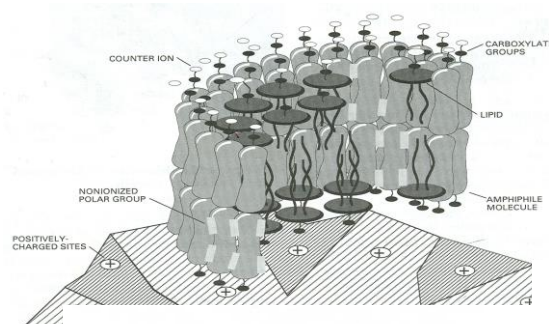
Гипотетические модели строения гуминовых веществ

Макромолекулярная модель

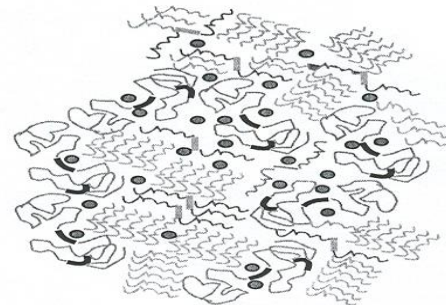


Kleinhempel, 1970

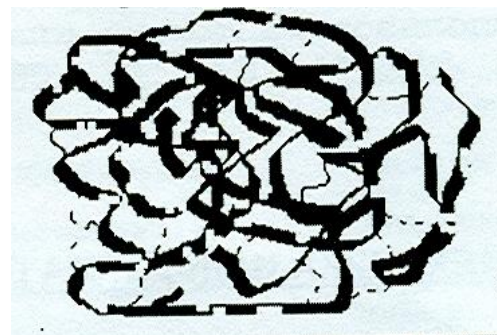
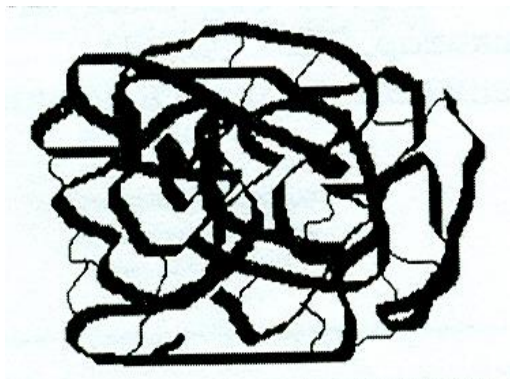
Супрамолекулярная модель



Wershaw, 1986



Piccolo, 1997



Hutta et al., J.Chromatography, 2011

Твердофазный ^{13}C -ЯМР ГК чернозема и фракций А, В и С+D

