The Fluorescence Characterization of Organic Matter Extracted from Humic Acids isolated from South-Bohemian Peat

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An appreciable part of peat is formed by humic substances (traditionally divided into humic acids, fulvic acids and humin) of which humic acids predominate. Humic acids are naturally occurring biomolecules ubiquitous, not only in kaustobiolithe (lignite, leonardite, peat) but also in water in soil, and are the subject of intense research. Several fluorescence techniques have been used to the study and characterization of HA-peat organic extracts: from commonly emission spectrometry (emission scan), synchronous fluorescence spectrometry (synchronous scan) and total luminescence spectrometry (or excitation-emission matrix/contour map) to more recent and comprehensive methods such as Time-resolved fluorescence spectrometry (Time-Correlated Single Photon Counting – TCSPC) and Fluorescence correlation spectroscopy (FCS). In this work, excitation–emission matrix (EEM) spectrometry was used for the first time to extensively study various organic matter extracted from humic acids isolated from South-Bohemian peat.

Humic acids were isolated from South-Bohemian peat (locality Třeboň, Czech Republic). Humic acids were extracted using a procedure recommended by the International Humic Substances Society (IHSS). The peat humic acid (SBPHA) was placed in a cellulose thimble and Soxhlet gradually extracted for 12–50 h using trichloromethane, ethyl acetate, acetonitrile, acetone, n-propanol and methanol as a solvent. The organic matter fractions isolated from the peat humic acids were collected and concentrated by a rotary evaporator. Steady-state fluorescence spectra were recorded in aqueous solutions of 10 $mg \cdot L^{-1}$ organic matter extracts after overnight equilibration at room temperature, using FluoroLog luminescence spectrophotometer. The pH-values of the samples were adjusted to seven using a standard phosphate buffer. Total luminescence spectra (TLS) were obtained in the form of excitation/emission matrix (EEM) by scanning the wavelength emission over the range of 300–600 nm, also the excitation wavelength was in 5 nm steps from 240 to 550 nm. 1st and 2nd inner filter effects were corrected. The fluorescence intensity values of samples (in counts per second, CPS) were corrected using the method devised by Lakowicz (Lakowicz 2006).

The excitation-emission matrix spectra (EEM) of organic matter fractions isolated from South-Bohemian peat HAs are shown in Figure 1. The maxima are mainly located at excitation wavelengths in the ultraviolet region (around 250–260 nm). In the visible region (at an excitation wavelength 430 nm) only one maximum was observed out of our whole sample set (EMeOH sample). The fluorescence EEM spectra of organic matter fractions (without sample extracted using $CHCl_3$) were characterized by unique fluorophore centered at an excitation/emission wavelength pair (EEWP) of 250–260/415–505 nm (fluorophore type A-fulvic-like respective sign α'). A large bathochromic shift (red shift) of peak A was observed

depending on the polarity solvent. The fluorescence maximum observed in the humic-like region (300/430 nm) in extract of acetonitrile has is not seen in the other EEM spectra. The characteristic domain (fluorophore type C-humic-like respective sign α) for humic substances originating from terrestrial origins with fluorescence maxima corresponding to excitation/emission wavelength pairs of 300–380/400–500 nm was observed in the organic matter fraction extracted with acetonitrile. Furthermore, fluorescence EEM spectrum of fraction extracted with trichloromethane was characterized by one fluorescence peak located at EEWP of 270/305 nm (fluorophore type B-tyrosin-like respective sign γ). The maximum at 255/335 nm for extract of trichlormethane represent essentially an isolated fluorophore in the ultraviolet region; therefore, we denote the maximum of this fluorescent domain as peak A/T and/or α'/δ. Fluorophore A/T, be found only in $CHCl_3$ extract. The maximum position is probably related to single aromatic systems and O-containing substituents. The shorter emission wavelengths measured in the EEM spectra can be associated with simple aromatic structures (one- to three-ring) and low molecular weight components. In contrast, the longer emission wavelengths can indicate the presence of condensed aromatic rings and electron-withdrawing groups such as carbonyl-containing substituents, and hydroxyl and alkoxyl groups.

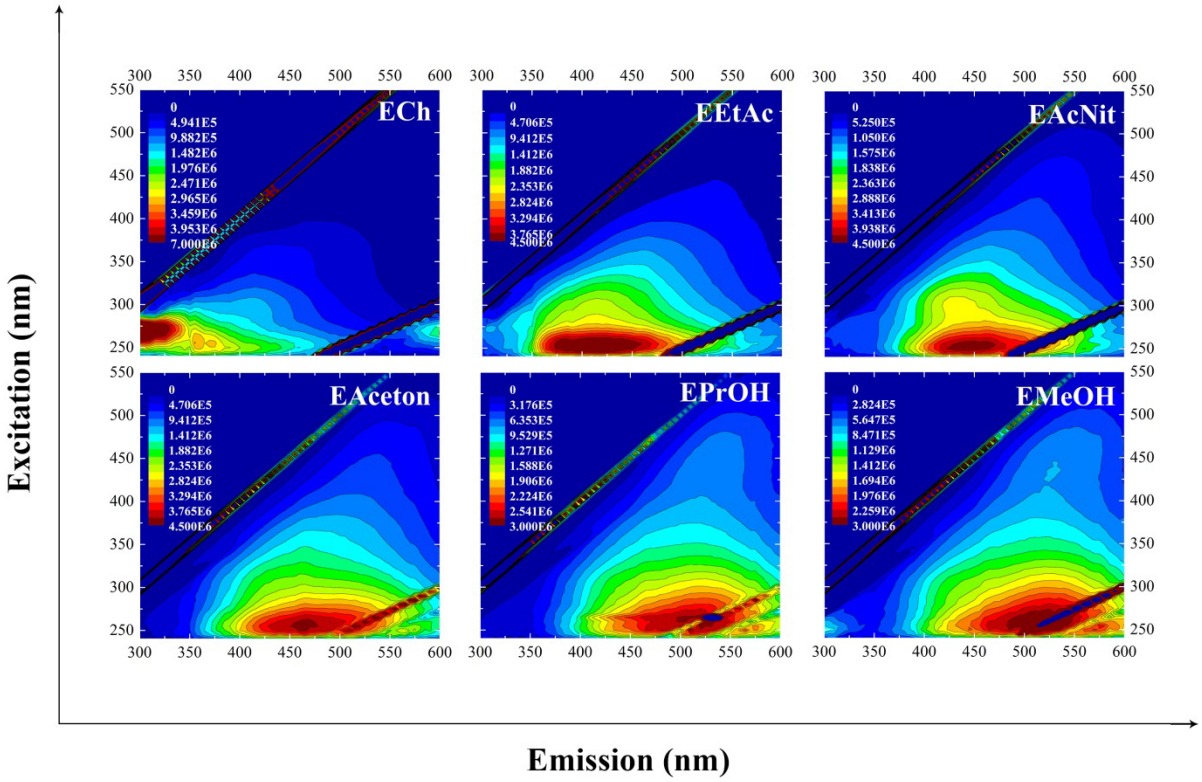

Figure 1: EEM spectra of organic matter extracted from humic acids isolated from South-Bohemian Peat

References:

Lakowicz J. R., Principles of Fluorescence Spectroscopy. 3rd Ed. New York: Springer, (2006) pp. 954.