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Study of humic substances by fluorescence spectroscopy

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Abstract

The purpose of this study is to determine main fluorophores of soil humic substances using 2D and 3D synchronous fluorescence spectroscopy (SFS). The measured synchronous spectra were compared with standards IHSS. Differences between humic and fulvic acids as well as our and IHSS samples are discussed.

Keywords: Humic substances, synchronous fluorescence spectroscopy.

Introduction

Athough the optical properties of humic substances (HS) have been investigated by many authors, the structural basis of these properties remains unclear. HS contain conjugated olefinic, aromatic, phenolic–semiquinone–quinone structures with functional groups and chromophores, which have been utilized in their characterization by fluorescence spectroscopy. Obtained results provide strong evidence that the absorption and emission spectra must arrise from a very large number of absorbing and emitting species or states (Boyle et al. 2009; Del Vecchio and Blough 2004). Total luminiscence spectra showed that the fluorescence spectra may be used to disriminate between soil-derived and aquatic derived IHSS humic substances and between humic and fulvic acids isolated from the same source (Mobed et al. 1996). Other authors utilized fluorescence spectroscopy for determination of humification degree of soil and compost humic acids (Milori et al. 2002; Fuentes et al. 2006) or characterization of HS isolated from different soil types (Barančíková et al. 1997). The effect of preparation procedure on the structural integrity of humic acids was investigated by fluorescence spectroscopy using pyrene as a hydrophobic probe. Changes in native fluorescence as well as pseudo-micelle formation were deduced on the basis of measured spectra (Engebretson and Wandruszka 1999).

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Extending the 2D SFS to 3D SFS in this work gives the total synchronous characteristics of multifluorophoric sample of HS at various possible wavelength intervals, which could help to characterize better multifluorophoric systems (as humic substances).

Materials and methods

HA and FA extracted from cambisol, cambisol leach (0,1 M sodium pyrophosphate and 0,1 M NaOH) and standard IHSS Elliot HA and FA were used in this work. Details of HA and FA extraction as well as used measurement technique are described in (Patra and Mishra 2002).

2D SFS spectra were measured within range 200 nm and 600 nm by Spectrofluorimeter AMINCO Bowman Serie 2, at fixed wavelength interval ($\Delta\lambda$ = 40 nm) between excitation and emissionmonochromators. 3D SFS spectra were scaned from 200 to 600 nm with gradual increase of $\Delta\lambda$ from 30 to 150 nm with step 5 nm. This allows detailed characterization present fluorophores and resolution peaks, that we cannot see by measure 2D synchronous scan with one $\Delta\lambda$ value.

Results and Discussion

The 2D spectra in Fig. 1 showed IHSS Elliot FA and cambisol FA have the same course with maximum at emission wavelength 399 nm, which is lower wavelength than that obtained for IHSS Elliot HA and cambisol HA (468 and 507 nm, respectively). The cambisol leach had two dominant peaks at 507 nm, which signifies presence of HA and at 379 nm, which signifies presence of FA, because the leach contain both types of HS.

3D SFS spectrum of cambisol HA is obtained by plotting fluorescence intensity as a combined function of the emission wavelength and of the difference between emission and excitation wavelenght $\Delta\lambda$ [2]. Two main fluorophores groups were found in cambisol HA sample: $\lambda_{exc.}$= 458 nm (for $\lambda_{em.}$= 468 nm) and $\lambda_{exc.}$= 396 nm (for $\lambda_{em.}$= 501 nm), where the first one is identical in the emission maximum with emission maximum for 2D spectrum.

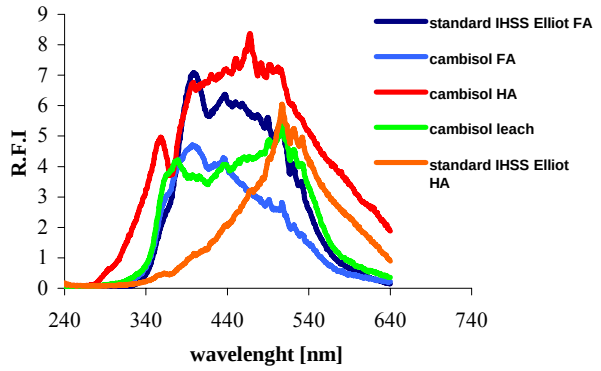

Figure 1: 2D synchronous spectrum with fixed wavelength (Δλ= 40 nm)

Conclusion

Differences in intensity and location of main peaks were observed for HA and FA. Whereas relatively simple aromatic/phenolic compounds were observed in FA spectra, extensively conjugated phenolics and quinones were identified for HA samples. Method of 3D SFS allowed characterization of main fluorophores, while method of 2D SFS is able to scan by only one fixed wavelength.

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