SPECTROSCOPIC CHARACTERISTICS OF HUMATES ISOLATED FROM DIFFERENT SOIL TYPES

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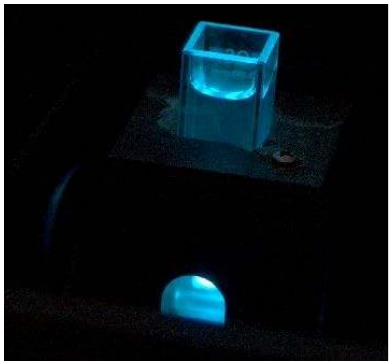
Aims of this project

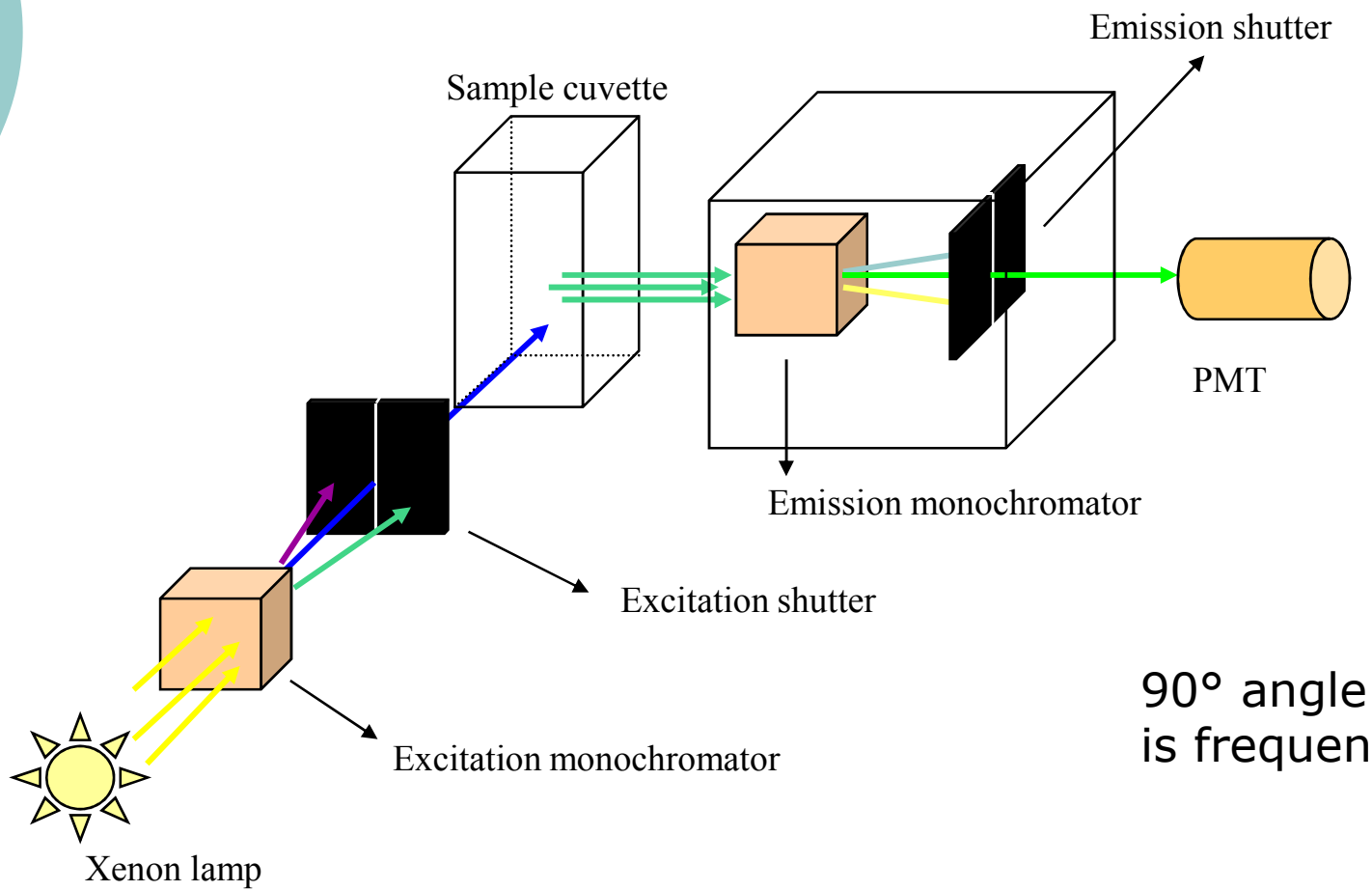
- The aim of our work was to compare spectroscopic properties of soil humates isolated from different soil matrices (Haplic Cambisol, Leptic Cambisol, Eutric Cambisol, Haplic Chernozem). The optical methods (UV-VIS and SFS) in this project were used.
- Then we compared the type of land use (arable soil x grassland) in Eutric Cambisol samples.

Fluorescence of humic substances

- State transition of electrons from excited singlet state to basic level of singlet (light emission). Transition is permitted. (~ns)
- Photoluminescence:for the excitation Xe lamp or laser are used.
- Excitation and emission wavelength can be chosen and set by monochromators. Excitation wavelength is lower than emisssion wavelength, emission wavelength corresponds to lower energy.
- Measurement of liquid or solid samples. (HK a FK in solution)
- Fluorescent molecules (with conjugated double bond system, aromatic substances)
- Humic substances (phenolic compounds, aromatic rings with amino groups, hydroxyl groups)-mixture of these substances.
- Comparing fluorescence peaks with peaks of pure substances or with standards (IHSS)



Spectrofluorimeter scheme



90° angle geometry
is frequently used

Materials and methods:

Czech soil samples, 4 subtypes of Cambisols, 1 Chernozem

Type of land use: arable soil x grassland Eutric Cambisol

- Localities:
  - Leptic Cambisol=Ocmanice
  - Haplic Cambisol=Náměšť
  - Eutric Cambisol 1, 2=Vatín
  - Haplic Chernozem=Praha, Ruzyně

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Materials and methods

- Isolation of HA was made according to the IHSS method (0.5 M NaOH)
- Sodium humates were prepared from HA and titration to pH=7 (dialyzation MWCO 3500, lyophilization at -50°C)

- Samples for UV-Vis: HS extracts were made in the mixture of 0.1 M $Na_4P_2O_7$ and 0.1M NaOH
- Samples for SFS: dissolving of humates in Mili-Q water, c=50 mg/l
- Elemental analysis (C, H, N, O contents) of isolated HA was kindly made in Engineering Test Institute Brno.
- We determined total carbon content, fractional composition, and humification degree of soil humates.

Materials and methods

- **UV-VIS**-spectrometerVarian Cary 50 Probe, glass fiber, scan range 300 - 700 nm, (MUAF).
- **SFS**-Spectrofluorimeter Aminco Bowman Series 2, scan range 200-600 nm, $\Delta\lambda = (\lambda_{em.} - \lambda_{ex.}) = 55$ nm, emission mode, 90° angle geometry, temperature 20 °C, bandpass: 4 nm, voltage: 1040 V , (BUT FCH)

Total organic carbon, humification degree

- TOC= was determined by short oxidimetric titration method by Nelson and Sommers (1982).
- Humification degree [HD] was calculated by using this equation:

$$HD = \frac{\sum HA * 100}{TOC}[\%]$$

by Orlov (1985)

where: ΣHA=humic acid content, TOC=total organic carbon content

Colour and fluorescence indexes

- *Colour index:*

Q4/6= A(465)/A(665) nm,

by Orlov (1985), Podlešáková (1992)

- *Fluorescence index:*

F=RFI(468)/RFI(522) nm ,

as a ratio of secondary and main peak

Table1 Fractional composition of soil samples, values of colour indexes and fluorescence indexes

Soil types	TOC [%]	HS [mg/kg]	HA [mg/kg]	FA [mg/kg]	HA/FA	HD [%]	$Q_{4/6}$	F
Leptic Cambisol	1.32	4.14	1.44	2.7	0.4	9.0	**9.1**	0.64
Haplic Cambisol	1.62	4.65	1.6	3.0	0.54	12.1	5.7	0.72
Eutric Cambisol 1-arable	1.76	6.8	1.8	**5.0**	0.5	10.1	8.1	0.57
Eutric Cambisol 2-grassland	**2.1**	7.6	**2.9**	**4.7**	0.6	13.8	8.3	**0.94**
Haplic Chernozem	1.88	5.3	**2.9**	2.4	**1.21**	**15.4**	5.2	0.58

Results

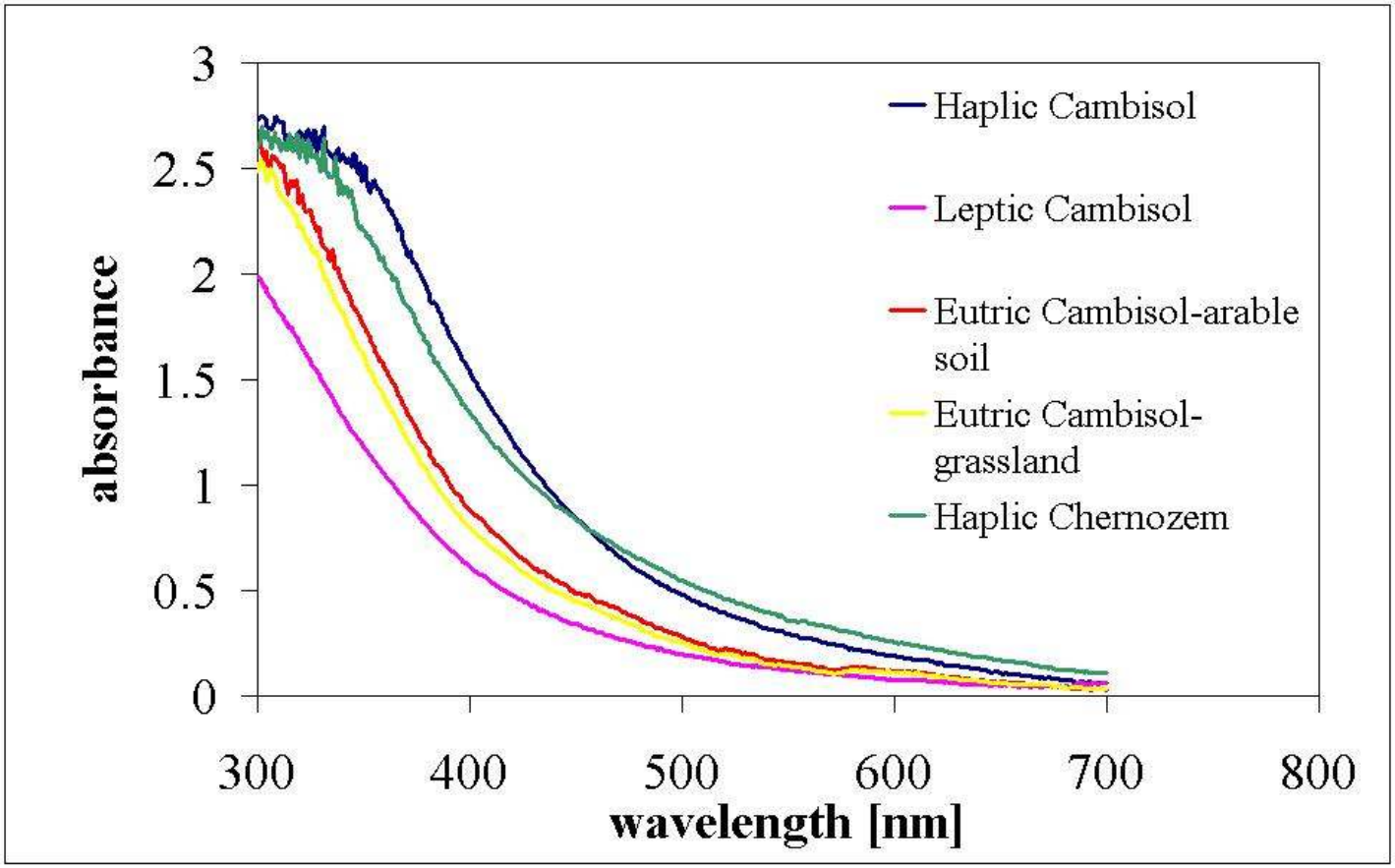
- The highest TOC content had Eutric Cambisol 2 (grassland)
- The highest HA content had Eutric Cambisol 2 and Haplic Chernozem
- The highest of HD values had Haplic Chernozem, the lowest HD values had Leptic Cambisol
- HS quality in grassland soil sample was higher than in arable soil (E. Cambisol)

Table 2 Ash free elemental analysis of soil humic acids (in atomic %).

Sample	C [%]	H [%]	N [%]	O [%]	Ash [%]
Leptic Cambisol	33.45	**47.44**	**3.07**	16.05	1.7
Haplic Cambisol	34.20	46.16	3.05	16.59	4.1
Eutric Cambisol 1(arable)	32.73	46.48	2.52	18.27	6.2
Eutric Cambisol 2 (grassy)	**35.59**	45.89	2.64	15.88	**8.4**
Haplic Chernozem	35.35	40.44	2.45	**21.76**	1.31

Fig. 1. UV-VIS spectra

$Q_{4/6}$ index (ratio A_{465}/A_{665} nm)

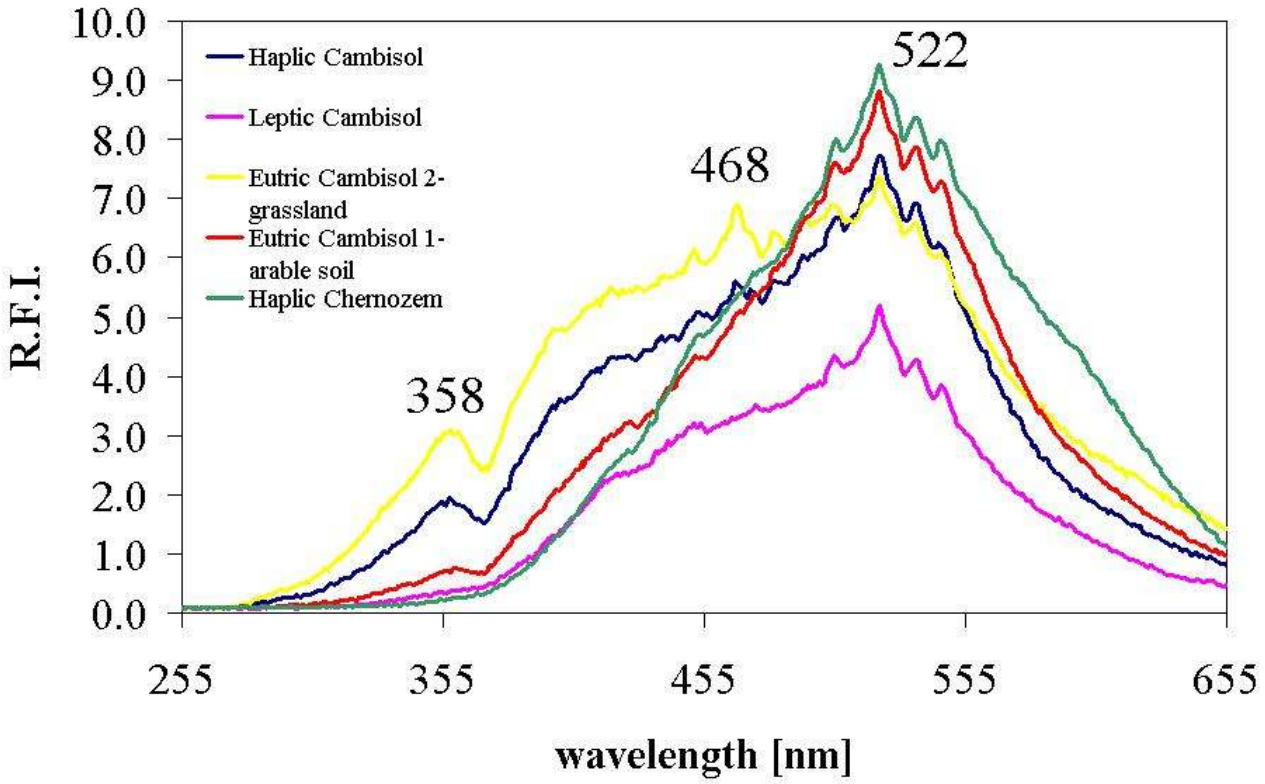


Samples: humic substances extracts were prepared in mixture 0.1M sodium pyrophosphate and 0.1M NaOH

Fig.2. SFS spectra at Δλ=55 nm

Fluorescence index F

F=RFI (468/522) nm



Samples: humates were dissolved in MILI Q water,

c= 50 mg/l

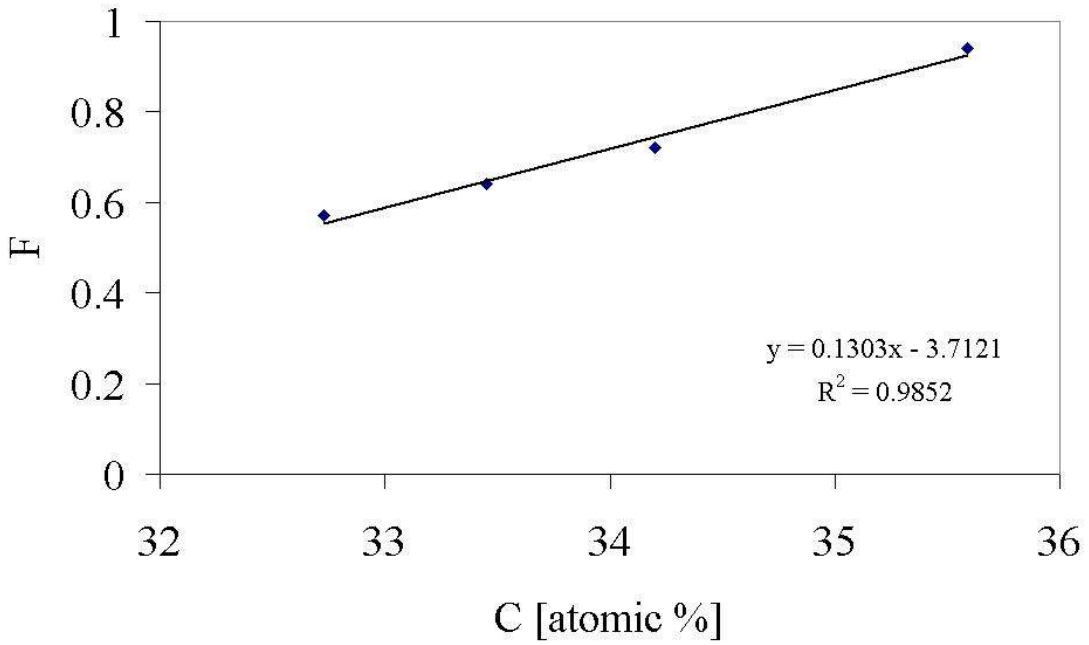
{522-55=467 nm}

Em. Exc.

Results

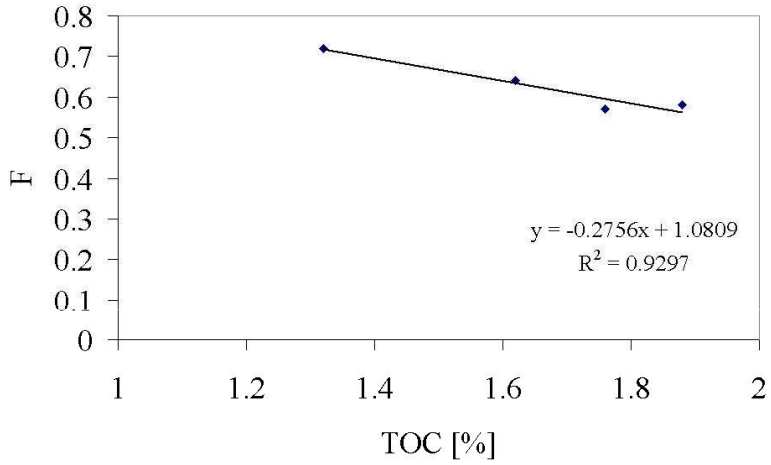
- All samples had main peak at emission 522 nm ($\Delta\lambda$=55 nm)
- Highest rel. intensity of fluorescence (RFI) at 522 nm had sample Haplic Chernozem
- Lowest rel. intensity of fluorescence (RFI) at 522 nm had sample Leptic Cambisol
- Arable soil humate (E. Cambisol 1) had higher RFI at higher wavelengths than grassland soil humate (E. Cambisol 2)

Results and disscussion

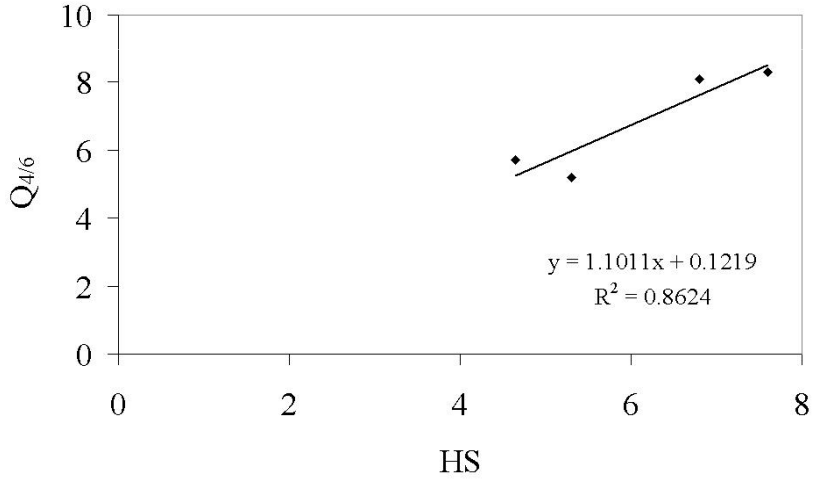


- Fig. 4 The dependence of fluorescence index on carbon content of soil humates (4 samples), R=0.99

Correlation



- Fig.3 Correlation between fluorescence index and total organic carbon content of soil humates (4 samples), R=0.96



- Fig. 5 The dependence colour index on humus content of soil humates (4 samples), R=0.93

Conclusion

- Optical properties are influenced by soil type and humus fractional composition. In this study colour indexes and fluorescence indexes by UV-Vis and SFS methods were determined. The highest quality and humification degree had Haplic Chernozem. The lowest quality had Eutric Cambisol (arable).

- From SFS measurement was evident that all samples had the same main fluorophore at excitation 467 nm a emission 522 nm ($\Delta\lambda$=55 nm). The difference between the type of land use (arable, grassland) in the shape of the spectra was found. Linear correlations between TOC and F, between F and C content and between colour index Q4/6 and humus content were determined.

Acknowledgement

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Thank you for your attention!