Chapter 13
Characterization of Soil Organic Substances by UV-Vis Spectrophotometry in Some Soils of Hungary

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Abstract UV-Vis spectrometry methods are widely used to characterize soil organic matter, especially humic substances from different extracts of soils. Our aims were to investigate organic characteristics of soils, as part of a project focusing on relationships between solute mineral components and organic matter quality. The study was performed using UV-Vis spectrophotometry on six Hungarian soils. For the characterization of humic substances we used several visible and UV methods. Humic materials were extracted from soils using a variety of extractants. We found that a decrease in pH and an increase in dissolved electrolyte concentration supported coagulation in preferance to dissolution in almost every case. The highest absorbance values was given by extractants which contained sodium-pyrophosphate. In our experience double extraction methods (E_{NaF}/E_{NaOH} according to Hargitai, A talaj szerves anyagának meghatározása és jellemzése. In: Buzás I (ed) Talaj- és agrokémiai vizsgálati módszerkönyv II. Mezőgazdasági Kiadó, Budapest, 1988, $E400_{NaOH+Na4P2O7}/E400_{PBS}$) and E4/E6 are suitable for analysing humic substances. Comparing these three visible methods there are only slight differences between them. The results do not show strong correlation between visible and UV methods as these techniques analyse different properties of soil humic substances.

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Introduction

UV-Vis spectrometric methods are widely used to characterize soil organic matter, especially humic substances from different extracts of soils, because UV-Vis spectrophotometry is a rapid and – compared with isolation of different fractions – relatively simple technique to estimate humic properties (Hautala et al. 2000; Spiegel et al. 2007).

The dissolution of humic substances is mainly governed by their particle size (or molecular weight) (Chen et al. 1977), acidity, aromaticity or aliphatic character and is influenced by some external conditions. The absorbance in the UV-Vis range is influenced by some properties of organic compounds, e.g. absorbance increases with total C-content, molecular weight and quantity of aromatic rings within the molecules (Chen et al. 1977). Absorbance might also change due to vibration and rotation of the molecules and intermolecular interactions as well (Korshin et al. 1997).

When applying spectrophotometrical techniques several methodological problems and external conditions have to be taken into account. The main problem is that spectrometry is non-specific for analysing humic substances and does not give a band that is very characteristic of humic compounds (Schnitzer and Kahn 1972). In the UV-Vis range of the spectrum, broad absorption bands can be observed and this smoothness implies that different components (organic and inorganic components as well) might be present in the samples (Pansu and Gautheyrou 2006). The spectra are influenced by chromophore groups of humic substances, ions dissolving out from the soil (e.g. Fe^{3+}) and also by the presence of solid particles.

Our aim was to investigate the organic characteristics of soils, as part of a project focusing on the relationship between solute mineral components and organic matter quality. The study was performed by UV-Vis spectrophotometry on the humic horizon of six Hungarian soils.

Methods

Soil samples were extracted in solutions which had different pH and ionic strength (0.05 M $NaHCO_3$, pH = 8.0 (Chen et al. 1977); 1 % NaF; 0.5 % NaOH (Hargitai 1988), pH = 13.1; and 0.5 M NaOH, pH = 13.7) in 1:10 m:V ratio. We used buffer solutions (Fujitake et al. 1999) (e.g. 0.05 M phosphate BS, pH = 4.0; 0.05 M NaOH with 0.05 M $Na_4P_2O_7$, pH = 12.1) (after Kumada 1985) to enhance stability. However, use of buffers poses a potential disadvantage, namely that sodium-pyrophosphate solution selectively extracts Fe- and Al-ions complexed to organic matter (Blakemore et al. 1987) and consequently the chemical properties of the soil surface could change significantly (Tombácz 2003).

Extraction of the organic compounds involved 16 h of shaking, followed by 12 h of settling and 20 min centrifuging at 5,000 rpm.

Absorbance measurements were made on a double-beam Shimadzu 3600 UV-Vis-NIR spectrophotometer in quartz cuvettes. Path length (1-cm or 2-mm) of the cuvettes depended on the concentration of the soil extracts. As a blank we used the solution in which the samples were solved. For every sample continuous spectra (shift: 1 nm) were scanned from 800 to 180 nm.

For the characterization of humic substances we applied the following methods:

For UV methods we used 0.05 M $NaHCO_3$ to extract humic substances from the soils.

- The proportion of the specific UV absorbance value on 254 nm and the SOC content (E254/SOC) is in strong correlation with the number of aromatic groups. The absorbance at 272 and 280 nm (Chin et al. 1994) refer to the aromaticity and molecular weight, respectively.
- The ratio of the absorption value at two different wavelengths is a widely used parameter to determine the aromatic characteristic of humic substances. In this work we used and compared two methods: the E2/E3 (E250/E365) and URI (E210/E254) (Her et al. 2008).

In visible range the E4/E6 ratio (the absorbance at 465 nm divided by the absorbance at 665 nm) was used. In this case the visible spectrum is measured in 0.05 M $NaHCO_3$ solution (after Kumada 1985 and Chen et al. 1977). It shows the degree of humification. The lower E4/E6 values refer to a higher degree of condensation within the molecules and low aliphatic chain content. Ratios lower than 5 are characteristic of humic acids (3–5), while fulvic acids have higher ratios (higher than 7 and 5 according to Chen et al. 1977 and Kononova 1966, respectively).

Besides this we applied visible methods in which we used two types of solutions for the extractions.

- According to a Hungarian method called Hargitai (1988) the absorbance of the solution extracted by 1 % NaF divided by that extracted with 0.5 % NaOH at different wavelengths (this is called Q-value) provides qualitative information of humic and fulvic acids (Hargitai 1988). The humic substances are bound to clay and carbonate minerals through Ca^{2+} and Al^{3+} ions. NaF can abrupt these strong complexes. NaOH splits these organomineral complexes by alkaline hydrolysis. NaF dissolves well-decomposed humic fractions with higher molecular size while raw humic materials are better dissolved by NaOH.
- The proportion of the absorbance for the solutions extracted by 0.05 M NaOH – 0.05 M $Na_4P_2O_7$ (pH 12.1) and 0.05 M phosphate buffer solution (pH 4.0), respectively, was calculated at 400 nm wavelength. In our experience this ratio gives information about the relative amount of humic and fulvic acid material too.

Materials

We measured the upper horizon for each soil described below. Depth and selected soil chemical properties of the horizons are presented in Table 13.1. Short descriptions of each soil is shown below.

- Calcic Mollic Gleysol (Humic, Arenic) (CMG):

The soil was developed from a marshland Histosol near Gerje stream (geographic coordinates: WGS84 N47°12′31.8″ E019°40′42.5″). The main properties are the results of hydromorphic features including dark colour, Fe-, Mn- and carbonate concretions and gleyic colour pattern.

- Calcic Phaeozem (Arenic) (CP):

This soil profile was exposed on a former floodplain near Gerje stream (N47°12′29.7″ E019°40′42.6″). The site is on a plateau. The soil is a weekly developed Calcic Phaeozem as it has only a thin B horizon developed in carbonatic sand.

- Rendzic Leptosol (humic) (RL):

Samples of RL originate from a soil profile, located on a karstic plateau in Gerecse Mts (N47°42′15.0″ E018°30′31.6″). The vegetation of the area is dominated by closer forest. The Munsell color of the soil shows that the soil was developed from the thin red clay that covers limestone. Clay minerals occurring in the soil can be bound with humic materials and produce organomineral complexes.

- Haplic Luvisol (HAL):

The soil profile was excavated in a closed deciduous forest on a 3° slope in Sopron Mts (N47°40′14.8″ E016°33′53.1″). The parent material is carbonatic Pleistocene loess, but there is no carbonate in the solum, the pH is strongly acidic. In the analysed A1 horizon located at 5–20 cm depth the soil particles are coated by humic concretions.

Table 13.1 Depth and soil chemical properties of the investigated soil horizons

Sample	Measured horizon	Depth (cm)	Munsell color	$pH_{(H2O)}$	$CaCO_3$ %(m/m)	SOC %(m/m)	C/N
CMG	A_h	0–30	2.5Y 2.5/1	7.18	51.3	5.53	41.85
CP	A_l	0–10	2.5Y 2.5/1	7.17	5.88	1.61	19.51
RL	AC	0–16	5YR 4/3	6.6	0.82	8.35	123.74
HAL	A_l	5–20	10YR 5/3	5.6	0.00	1.07	50.99
CC	A_p	0–20	10YR 3/1	8.6	5.41	2.22	45.80
PS		230–300	10YR 3/3	7.0	0.42	1.12	48.58

Legend: *CMG* Calcic Mollic Gleysol, *CP* Calcic Phaeozem, *RL* Rendzic Leptosol, *HAL* Haplic Luvisol, *CC* Calcic Chernozem, *PS* Paleosol

– Calcic Chernozem (Mollic) (CC):

Samples of CC came from a typical Pleistocene loess covered area near Martonvásár (N47°20′17.7″ E018°49′04.2″). The soil is in an arable field on a plateau. The soil organic substances are bound to carbonate minerals through Ca^{2+} ions forming Ca-humate complexes.

– Paleosol (PS):

It is a well-developed doline filling soil in Börzsöny Mts (N47°49′04.0″ E019°04′13.7″), presumably originating from the last interglacial (according to luminescent data). It has sharply separated genetic horizons. Micromorphology shows it has moderately developed texture and the soil aggregates can be separated. The analyzed horizon contains significant amount of organic matter that appears as humic film or as crack fillings.

Results and Discussion

Various quality and quantity of humic materials were diluted by different extraction methods (Fig 13.1a). It depended on several external conditions, firstly the pH. When pH decreased the amount of extracted humic compounds decreased in parallel. The quantity of the extracts is influenced by the ionic strength of dilution and its ion content. The highest amount of humic material is mobilized not by NaOH alone but by a solution made of $Na_4P_2O_7$ or $Na_4P_2O_7$ with NaOH. Similar results were reported by Kumada (1985).

Organic compounds of the analysed six soils are very different (Fig. 13.2b, c). When the humic matter was dissolved in 0.05 M NaOH along with 0.05 M $Na_4P_2O_7$ (pH = 12.1), the CMG and RL samples had the highest absorbance value on a specific wavelength ($E400_{NaOH+Na4P2O7}$). Using pH = 4 PBS buffer, the RL and HAL (which had the lower intensity by extraction of NaOH-$Na_4P_2O_7$) had the highest intensity values. Soil types such as chernozem had low extinction values (CC, PS). The $E400_{NaOH+Na4P2O7}/E400_{PBS}$ ratio of the intensities measured at 400 nm well featured the characteristic of the soils: we identified rather acidic, low molecular weight humic substances in the HAL, RL and CP samples, which dissolved relatively well in the acidic range (so they are predominantly fulvic acids). Samples which have high carbonate or iron content are probably more abundant in humates that form weak complexes with Ca- and Fe-ions and do not dissolve in acidic pH. The high ratio of the PS may caused by not only organic complexes but also inorganic components that are dispersed by sodium-pyrophosphate.

According to the E4/E6 ratio (Table 13.2) we classified the soil samples into three groups. Samples with highest rate – that means more abundant fulvic acids – are HAL (7.65) and RL (6.47), which have low pH and base saturation. The CC (3.85), CMG (4.08) and PS (4.05) basically consist of humic acids according to E4/E6 rate, while the weakly developed soil CP (4.72) is composed of circa similar amount of humic and fulvic acids.

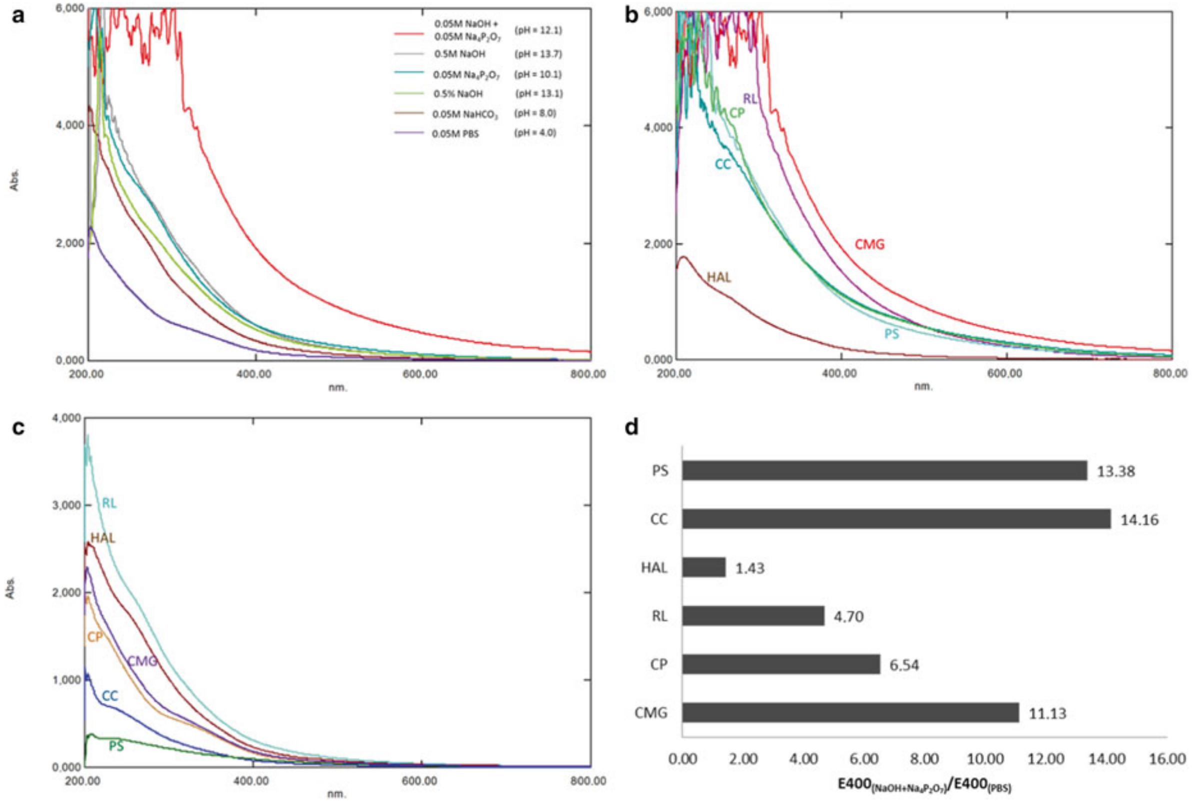

Fig. 13.1 (**a**) UV-Vis spectra of CMG diluted by different extraction methods; (**b**) Spectra of soils extracted by 0.05 M NaOH and 0.05 M $Na_4P_2O_7$ (pH 12.1); (**c**) Spectra of soils extracted by 0.05 M Phosphate buffer solution (PBS) (pH 4.0); (**d**) $E400_{NaOH+Na4P2O7}/E400_{PBS}$: ratio of the absorbance on 400 nm for the solutions extracted by 0.05 M NaOH – 0.05 M $Na_4P_2O_7$ and 0.05 M PBS

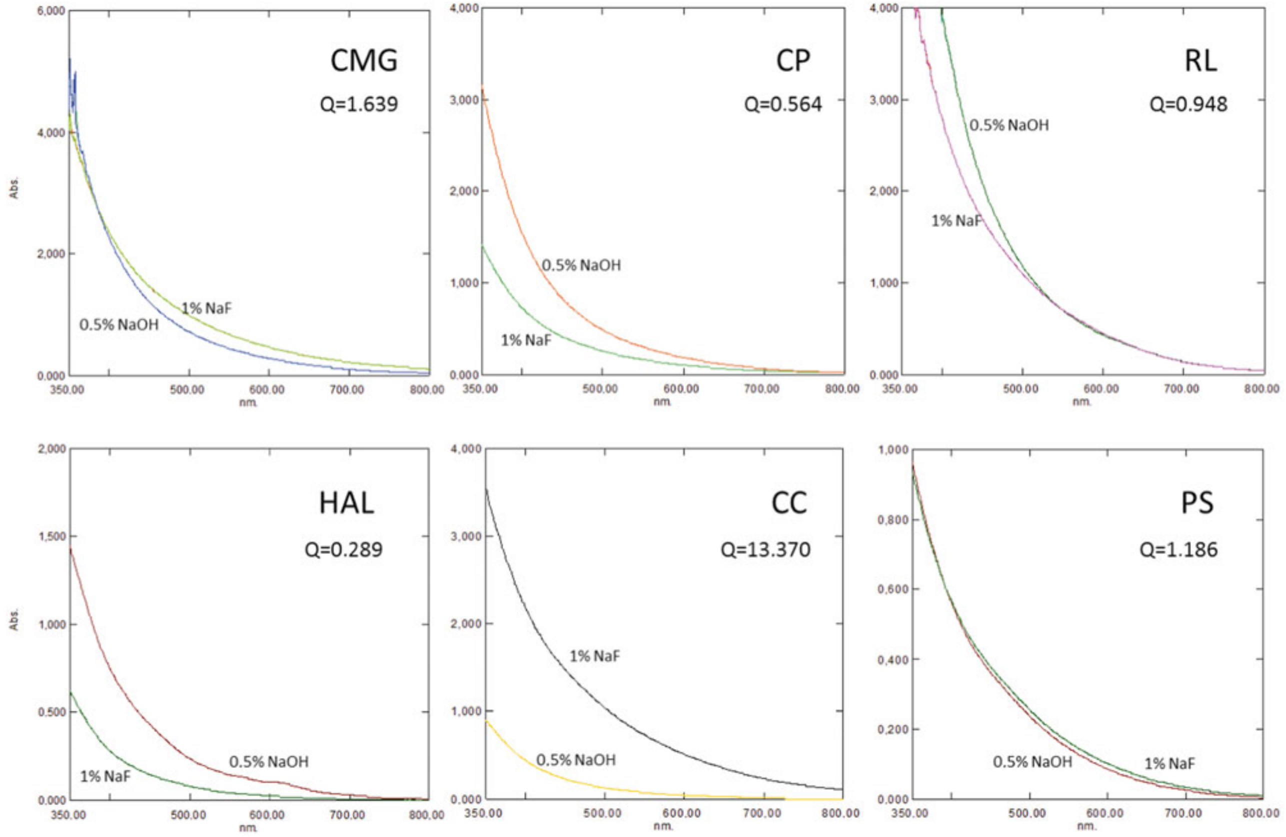

Fig. 13.2 Vis spectra and Q value (E_{NaF}/E_{NaOH}) of soils extracted by 1 % NaF and 0.05 % NaOH

Table 13.2 Specific ratios from UV-Vis spectra

Sample	E4/E6	Q (E_{NaF}/E_{NaOH})	$E400_{NaOH+Na4P2O7}/E400_{PBS}$	E2/E3	E210/E254	E254/SOC
CMG	4.08	1.64	11.13	4.27	1.63	1.322
CP	4.72	0.56	6.54	3.99	1.61	1.544
RL	6.48	0.95	4.70	4.35	1.74	1.661
HAL	7.65	0.29	1.43	4.52	1.54	1.449
CC	3.85	13.37	14.16	3.91	1.66	1.335
PS	4.05	1.19	13.38	3.19	0.75	1.55

According to the Q value of Hargitai method CC is distinct among the soils we examined, as it has a higher degree of humification than the others. Besides CMG also has a humic stability number higher than 1. We interpret that in these two soils, well-decomposed, higher molecular size humic materials (they are probably bound to Ca-ions) dominate. Q-value for HAL and CP is much less than 1, which shows that in these soils the acidic, less polycondensated and low molecular-weighted humic substances prevail. The Q-value of PS and RL is around 1. It suggests that in the case of these paleo- and fossil soils there was enough time for humic material with larger humic stability number to develop.

Soils with higher SUVA/TOC content have higher aromaticity. In our case the aromaticity decreased in the following order: CC > CP > PS > CMG > HAL > RL.

The lower E2/E3 ratio of the PS, CC and CP soils means higher aromaticity. According to Takács and Füleky (2010) the aromatic features decrease in a Soil HA > Soil FA > NOM (natural organic matter) order. Based on this it is not surprising that the humic acid rich chernozem-type soils and a well-developed, several times (probably quarternary) re-humified PS have higher aromaticity. HAL has got less aromatic features. It is an acidic soil which has <50 % base saturation and mainly contains fulvic acids.

The low E210/E254 ratio refers to a higher content of aromatic groups and lower content of non-aromatic function groups (Her et al. 2008). According to this, the order of decreasing aromaticity corresponds with increasing the amount of functional groups (e.g. carboxylic, phenolic -OH groups) was the following: PS > CMG > CC > HAL > CP > RL.

The results do not show strong correlation between visible and UV methods as these techniques analyse different properties of the soil humic substances. Relationships exist only in a narrower range between the Hargitai and E2/E3 methods (r = 0,949). It is an expected result that the E254, E272 and E280 data and E2/E3 ratio are in good correlation (e.g. E254 – E2/E3: r = 0,908), however, we could not show relationship between E2/E3 and E210/E254.

Conclusion

Humic materials from the soils were extracted using different methods. We observed that a decrease in pH and an increase in dissolved electrolyte concentration supported coagulation as opposed to dissolution almost in every case.

The highest absorbance values was given by extractants which contained Sodium-pyrophosphate, but a part of the intensity was likely augmented by inorganic components (e.g. Fe^{3+}, Al^{3+}, clay minerals) in these cases.

Our results show that double extraction methods (E_{NaF}/E_{NaOH} according to Hargitai 1988, $E400_{NaOH+Na4P2O7}/E400_{PBS}$) and E4/E6 are suitable for analysing humic substances. Comparing these three visible methods there are only slight differences between them. We observed that CC and CMG soils contain higher molecular-weighted humic materials (mainly humic acids) according to the three applied visible methods. Acidic, less polycondensated and low molecular-weighted humic substances (mainly fulvic acids) dominate in HAL and CP. We obtained similar results for PS and RL according to E4/E6 and $E400_{NaOH+Na4P2O7}/E400_{PBS}$ methods, but we did not get unambiguous results for humic stability by the Hargitai method.

The results do not show strong correlation between visible and UV methods as these techniques analyse different properties of soil humic substances.

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