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# PREDICTION OF PROPERTIES OF SOIL HUMIC SUBSTANCES FROM FTIR SPECTRA USING PARTIAL LEAST SQUARES REGRESSION

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Humic substances (HS) play a key role in aquatic and terrestrial ecosystems. The understanding of the ecological functionalities of HS is based on the analysis of their properties, which is normally a very time-consuming procedure. Therefore we tested the possibility to apply the partial least squares regression (PLSR) method in connection with the mid infrared Fourier transform (FTIR) spectra of a series of soil humic substances for the prediction of different HS properties. The results with humic acid (HA) and fulvic acid (FA) fractions of soil HS from different environments show the possibility to predict several properties of an unknown soil HA with satisfying reliability above all the elemental composition.

*Keywords:* Soil humic substances; mid-infrared; PLS regression

## INTRODUCTION

The vital role of humic substances (HS) in aquatic and terrestrial ecosystems has stimulated manifold efforts to uncover the mechanisms by which these refractory organic substances realize their environmental functionalities. Especially in studies concerning ecosystem dynamics, a number of analyses are needed to describe the usually complex and mostly heterogeneous environmental compartment under investigation. The measurements of the required parameters should be rapid but precise enough to allow reliable conclusions. Therefore, spectroscopic methods have been often used for these purposes. However, in the case of HS additional difficulties arise from the extreme molecular heterogeneity and complexity of these substances<sup>[1,2]</sup>, that are frequently available only in very small

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quantities, e. g. from waters. So it will be not ever possible to perform all the measurements needed for the characterization of the HS. An expedient from this situation could be to use an analytical method which needs only a small quantity of substance for the measurement, but contains so much information, that other properties than the measured one can be predicted by calculation. Such an approach is already known from early work in applied infrared spectroscopy. Some of the topics in this line are the characterization of coal<sup>[3,4]</sup>, peat<sup>[5]</sup> and soil<sup>[6]</sup> and the prediction of environmental fate parameters for chemicals<sup>[7]</sup> using methods of multivariate data analysis.

The aim of our study was to evaluate the possibility to predict various properties of soil HS from infrared spectra. We started our investigations with soil HS, because in this case we could extract amounts of HS enough to carry out analyses with as many methods as possible. Additionally we included results from measurements of aqueous soil extracts for comparison with an ecologically meaningful parameter.

## EXPERIMENTAL

### Material

Soil samples of the A horizon were taken from 19 different environments in urban and industrial regions surrounding Leipzig, Halle, and Bitterfeld (Germany). Nine samples originated from floodplain areas (series A and series K), six samples from a municipal landfill (series T) and four samples from fallow land (series V). Sample sites differed in pollution, inundation, topology, and tillage. Air-dried samples were sieved through a 2 mm mesh.

### Preparation of fulvic (FA) and humic (HA) acids

HS were extracted by use of 0.1M NaOH and separated into FA and HA according to the recommendations of the IHSS (International Humic Substances Society), following the procedure described by Kuwatsuka *et al.*<sup>[8]</sup>

### Analyses

The FA and HA were characterized by elemental analyses (LECO CHN932), UVNIS spectra (Varian Cary 3, 0.05 M NaHCO<sub>3</sub> solution) and <sup>1</sup>H-NMR spectra (Varian Unity 400, 0.5 M NaOD/D<sub>2</sub>O solution).

In addition the contents of dissolved organic carbon (DOC) and of HS were estimated for the aqueous soil extracts to determine the HS/DOC ratio, which is an ecologically important quantity (compare with the "humus coefficient", mentioned by Prakash et al.<sup>[9]</sup>).

The samples were prepared as KBr pellets (about 1 mg of the HA or FA was mixed with about 300 mg of coarse disperse KBr by milling for 2 min and resulting powder pressed under vacuum for 4 min to a thin pellet with a diameter of 13 mm). Absorbance spectra were obtained with a FTIR spectrometer (System 2000 FTIR, Perkin Elmer) in the frequency region from 3800 to 450  $\text{cm}^{-1}$  using resolution of 2  $\text{cm}^{-1}$  and data interval of 0.2  $\text{cm}^{-1}$ . 128 scans were accumulated. The spectra were scaled to 1 mg sample/cm and normalized to the carbon content of the compound.

### Data analysis

In the last years the partial least squares regression (PLSR) has been established as a statistical standard approach, that we used for the prediction of chemical properties of the prepared HS from the IR spectra.

If multivariate statistical modelling tools, based on the maximum likelihood principle, were applied to real data with many collinear variables and a modest number of observations, grave identification and convergence problems were often encountered. In the basic papers of this field<sup>[10-15]</sup> it has been shown that PLSR solves the multivariate prediction problem for collinear data with satisfactory predictive ability.

PLSR is a non least squares biased linear regression method that relates a set of predictor variables, **X**, to a set of response variables, **Y**. A least squares regression is performed on a set of uncorrelated variables, **T**, that are standardized linear combinations of the original predictor variables, **X**. The variables **T** are called the **X** latent variables. PLS models the responses as

$$\mathbf{Y} = \mathbf{TBQ} + \mathbf{E}$$

where **B** is a diagonal matrix containing the least squares coefficients from the regression on the latent variables, **Q** contains the weights of the responses, and **E** is the residual matrix. The latent variables are calculated on at a time, maximizing their covariance.

We worked with the chemometrics programs PLSplus for GRAMS/386 (PLS-1 approach), Galactic Industries Corporations and SCAN 1.0 from the Minitab Inc. The SCAN program works with X-weights, X-latent variables (X-scores), Y-weights and Y-latent variables (Y-scores). The X-weights resemble to the usual PLS-loadings (spectral loadings) and the Y-weights refer to chemical loadings.

The optimal number of latent variables was determined by means of validation statistics. A measure of the goodness of the prediction is calculated from the cross-validated residuals (labelled as standard error of prediction):

$$\text{SECV} = \sqrt{\frac{1}{n-1} \sum_{i=1}^n (Y_{a_i} - Y_{p_i})^2}$$

where  $Y_{a_i}$  are the actual and  $Y_{p_i}$  the predicted values.

Moreover, the SCAN program facilitates the calculation of regression coefficients for the original predictors and in this way the analysis of the importances of these regression coefficients. For each response, the importance of a predictor is measured by the standardized regression coefficient (equals to the corresponding regression coefficient multiplied by the standard deviation of the response). We used the predictor importances for the detection of the spectral ranges contributed mostly to the model. These importances are suitable for our purpose because the predictor importances reflect the whole regression model and not only a contribution to single factor loadings.

In order to handle the SCAN program, the IR spectra of our 19 samples, each of them consisting of 16751 points, were transformed into variable pattern with 838 points by means of an average moving procedure of PLSplus.

## RESULTS

In the Tables I and II the data concerning elemental analysis, the N/C, O/C, and H/C ratios and the ash content at 750° C (4 hours) of the HA and FA, respectively, are reported. Some data, commonly used for the characterization of HS, were calculated from the UV/VIS and <sup>1</sup>H-NMR spectra of the HA solutions and shown in Table III together with the HS/DOC ratios of aqueous soil extracts.

The infrared spectra of HS are generally poorly resolved and show a considerable degree of similarity. Differences between the IR spectra of the samples (Figure 1a and b) happened mainly in the frequency region between 2950 and 2850 cm<sup>-1</sup> (aliphatic CH<sub>3</sub> and CH<sub>2</sub>) and between 1750 and 1500 cm<sup>-1</sup> (C=O of CO<sub>2</sub>H, C=O of ketonic carbonyl, hydrogen-bonded C=O, COO<sup>-</sup>, NH-deformation of amide II type), which therefore are commonly used for the interpretation of IR-spectra of HS.

However, we used the full spectra to test the predictive ability of the IR spectra for various properties of HS, because we assume that the structural informations of these polyfunctional macromolecules are more or less spread out over the whole IR spectral region.

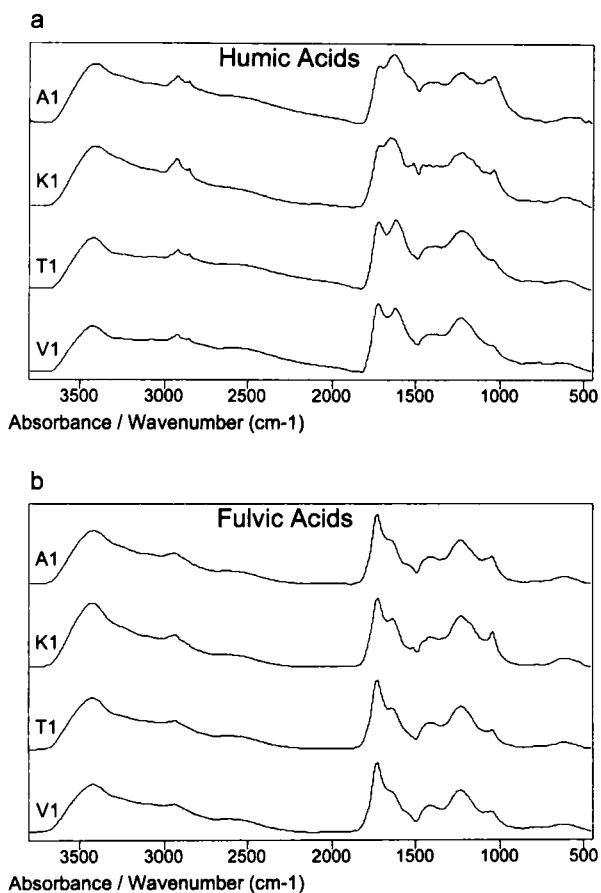


FIGURE 1 FTIR-spectra of humic (a) and fulvic acids (b) from soils (one was chosen from each group)

TABLE I Elemental composition and ash content of the humic acid fractions from soils

Sample	Elemental composition (%) <sup>a</sup>					Atomic ratio			Ash(%)
	C	H	N	O	S	N/C	O/C	H/C	
A1	51.38	4.40	3.59	39.18	1.45	0.0599	0.572	1.028	6.64
A2	51.05	4.12	4.05	39.75	1.03	0.0680	0.584	0.968	0.97
A3	51.35	3.97	3.43	39.97	1.28	0.0572	0.584	0.928	3.82
A4	50.12	5.22	4.51	38.78	1.37	0.0771	0.580	1.250	2.63
A5	51.77	4.97	4.16	38.25	0.85	0.0689	0.554	1.152	1.32
K1	52.43	5.41	3.90	35.47	2.79	0.0637	0.507	1.238	1.04

Sample	Elemental composition (%) <sup>a</sup>					Atomic ratio			Ash(%)
	C	H	N	O	S	N/C	O/C	H/C	
K2	51.46	5.62	4.82	35.77	2.33	0.0803	0.521	1.311	2.98
K3	50.77	5.24	4.56	38.03	1.40	0.0770	0.562	1.239	2.62
K4	49.96	5.40	4.41	38.99	1.24	0.0756	0.585	1.297	6.36
T1	54.58	4.06	2.11	37.46	1.79	0.0331	0.515	0.893	0.30
T2	52.61	4.27	2.12	39.28	1.72	0.0345	0.560	0.974	1.29
T3	54.41	4.20	1.86	37.45	2.08	0.0293	0.516	0.926	0.66
T4	55.15	4.68	3.11	35.08	1.98	0.0483	0.477	1.018	2.85
T5	57.25	3.87	1.42	35.74	1.72	0.0213	0.468	0.811	3.73
T6	53.65	4.68	2.92	36.13	2.62	0.0466	0.505	1.047	0.77
V1	54.78	3.70	2.84	37.97	0.71	0.0444	0.520	0.811	0.76
V2	53.58	3.84	2.69	39.08	0.81	0.0430	0.547	0.860	0.83
V3	55.91	3.70	2.92	36.74	0.73	0.0448	0.493	0.794	3.31
V4	56.50	3.66	3.07	35.96	0.81	0.0466	0.477	0.777	1.70

<sup>a</sup>ash free basis, O content was calculated from the weight difference to 100%.

TABLE II Elemental composition and ash content of the fulvic acid fractions from soils

Sample	Elemental composition (%) <sup>a</sup>					Atomic ratio			Ash(%)
	C	H	N	O	S	N/C	O/C	H/C	
A1	45.42	3.15	3.46	46.44	1.53	0.0653	0.767	0.833	2.45
A2	44.84	3.87	3.35	46.76	1.18	0.0640	0.782	1.052	3.10
A3	42.88	3.76	2.51	49.55	1.30	0.0502	0.867	1.052	2.45
A4	45.16	4.67	3.19	45.92	1.06	0.0657	0.763	1.241	2.88
A5	45.39	4.43	3.14	45.79	1.25	0.0593	0.757	1.171	2.78
K1	45.92	4.47	2.27	44.70	2.64	0.0424	0.730	1.168	4.61
K2	44.49	4.27	2.87	45.75	2.62	0.0553	0.771	1.152	0.75
K3	44.37	5.11	3.77	45.19	1.56	0.0728	0.764	1.382	1.74
K4	44.50	4.85	3.62	45.91	1.12	0.0697	0.774	1.308	2.04
T1	44.93	2.69	2.41	48.59	1.38	0.0460	0.811	0.718	4.10
T2	44.83	3.70	2.35	47.70	1.42	0.0449	0.798	0.990	4.60
T3	45.42	3.58	2.64	46.70	1.66	0.0498	0.771	0.946	3.27
T4	44.92	4.01	2.68	47.01	1.38	0.0511	0.785	1.071	1.36
T5	44.25	3.04	1.46	50.17	1.11	0.0283	0.850	0.824	1.34
T6	44.47	3.43	3.08	47.19	1.83	0.0594	0.796	0.926	2.88
V1	45.14	3.72	1.49	48.47	1.18	0.0283	0.805	0.989	4.15
V2	44.42	3.11	1.40	48.81	1.26	0.0270	0.824	0.840	3.10
V3	43.46	3.50	0.41	51.40	1.23	0.0296	0.887	0.966	2.64
V4	43.09	4.34	1.88	49.13	1.56	0.0374	0.855	1.209	2.41

<sup>a</sup>ash free basis, O content was calculated from the weight difference to 100%.

TABLE III Data from UV/VIS and from  $^1\text{H-NMR}$  spectra of the HA fractions and "Humic Coefficient" (HS/DOC) of aqueous soil extracts

Sample	UV/VIS Molar Absorbance <sup>a</sup>		$^1\text{H-NMR}$ Relative intensity (% of total area)				HS/DOC
	E (280) <sup>b</sup>	E <sub>4</sub> /E <sub>6</sub> <sup>c</sup>	Aliphatic <sup>d</sup> protons 0.4–1.7 ppm	Aromatic protons 6.5–8.1 ppm	HCO <sup>e</sup> 3.3–4.6 ppm	Arom./Aliph. protons	
A1	51.38	3.59	32.0	13.3	28.2	0.416	0.572
A2	51.05	4.05	31.7	10.9	31.8	0.344	0.584
A3	51.35	3.43	31.2	12.6	28.2	0.404	0.584
A4	50.12	4.51	30.8	12.8	31.3	0.416	0.580
A5	51.77	4.16	33.0	13.0	27.4	0.394	0.554
K1	52.43	3.90	34.9	15.4	23.4	0.442	0.507
K2	51.46	4.82	35.3	13.0	25.5	0.369	0.521
K3	50.77	4.56	33.3	12.7	29.7	0.382	0.562
K4	49.96	4.41	30.4	12.0	30.9	0.395	0.585
T1	54.58	2.11	29.9	16.3	20.0	0.545	0.515
T2	52.61	2.12	29.1	16.8	19.4	0.577	0.560
T3	54.41	1.86	27.7	17.2	18.6	0.621	0.516
T4	55.15	3.11	29.3	16.4	22.1	0.560	0.477
T5	57.25	1.42	31.0	24.6	12.8	0.794	0.468
T6	53.65	2.92	30.3	16.6	20.7	0.548	0.505
V1	54.78	2.84	31.3	21.7	22.5	0.693	0.520
V2	53.58	2.69	26.6	22.1	23.3	0.831	0.547
V3	55.91	2.92	29.7	23.4	22.7	0.788	0.493
V4	56.50	3.07	28.8	21.9	23.5	0.760	0.477

<sup>a</sup>normalized to the carbon content, <sup>b</sup>related to the aromatic carbon content.

<sup>c</sup>E (465)/E(665), inversely proportional to molecular size, <sup>d</sup>upfield aliphatic protons only.

<sup>e</sup>protons on a carbon adjacent to a heteroatom.

The quality of the prediction can be viewed from the plots of the predicted component values versus the actual ones (see Figure 2a – d with the properties H/C, E<sub>4</sub>/E<sub>6</sub>, HS/DOC and the ratio of aromatic to upfield aliphatic protons calculated from the  $^1\text{H-NMR}$  spectra).

The importances and the first and second factor loadings for the nitrogen content of HA are compared in Figure 3. With respect to the pattern of the curves of importances and to the positions and absolute values of maxima in this curves the results can be divided into two groups. One group shows characteristic patterns whose maxima can be assigned to absorption maxima of structural groups which are related to the corresponding property (Figure 4 a and b). The importances of the other group show small absolute values of maxima and a great similarity between the curves (see property HS/DOC Figure 4). Numerical results of the PLS calculations has been arranged in the Table IV.



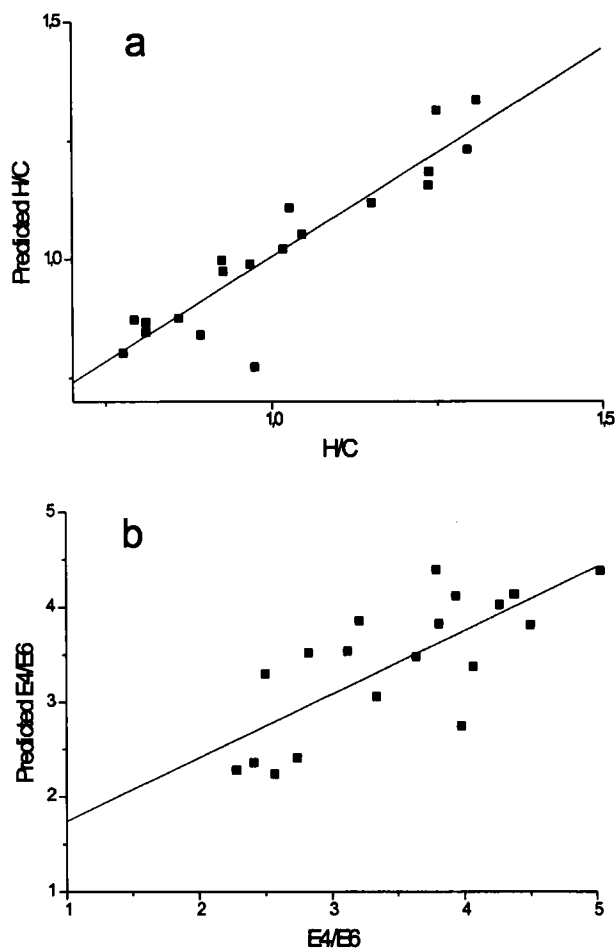


FIGURE 2 Regression plot for PLS; predicted versus actual values for the HA properties: a – H/C, b – E4/E6, c – aromatic/upfield aliphatic protons, d – HS/DOC

The numerical values of properties vary little within the sample series but differ between the properties often in orders. For this reason the predictive ability of the IR spectra for each property was proved on the basis of a validation set of samples using a relative  $SECV$ -value<sup>[16]</sup> ( $SECV_{rel} = SECV \times 100/c$ , where  $c$  is the mean of the actual values of each property). The smallest prediction errors were obtained for the properties H/C, N/C, and O/C, the nitrogen content and the proportion of upfield aliphatic protons and of the HCO groups determined from the  $^1H$ -NMR spectra of HA and for O/C of FA.

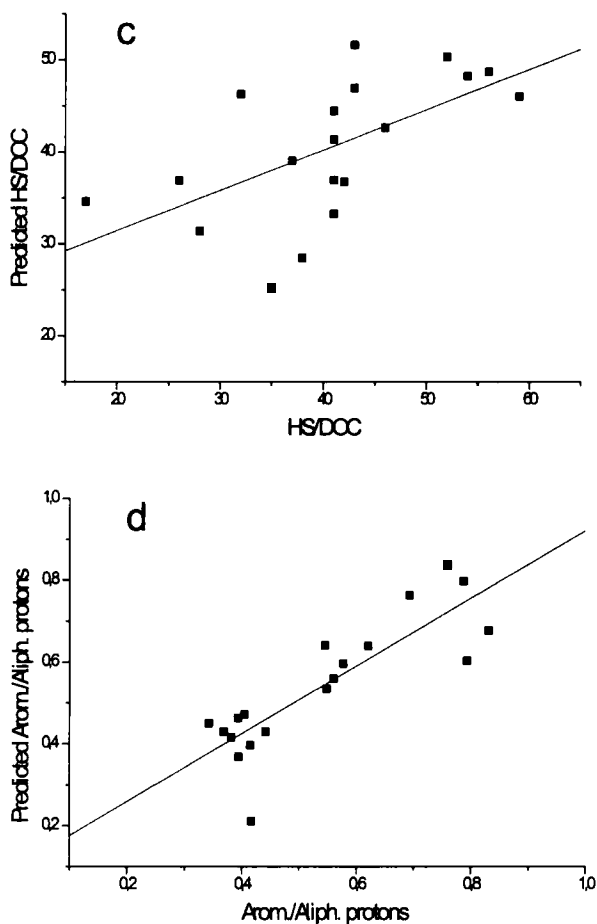


FIGURE 2 Continued

Evidently a relation exists between the pattern of the importances and the prediction ability, which is distinct in the two groups mentioned above. Within the first group only slight errors emerge for the predicted values showing a small variance of the errors (Figure 5a for O/C), because of a strong correlation between the properties and the IR spectra. In the second group higher prediction errors appeared showing a large variance of the errors (Figure 5b for HS/DOC). Since characteristics such as HS/DOC or aromatic proton content from  $^1\text{H-NMR}$  have only a little response to the IR spectra, we excluded these parameters from further considerations.

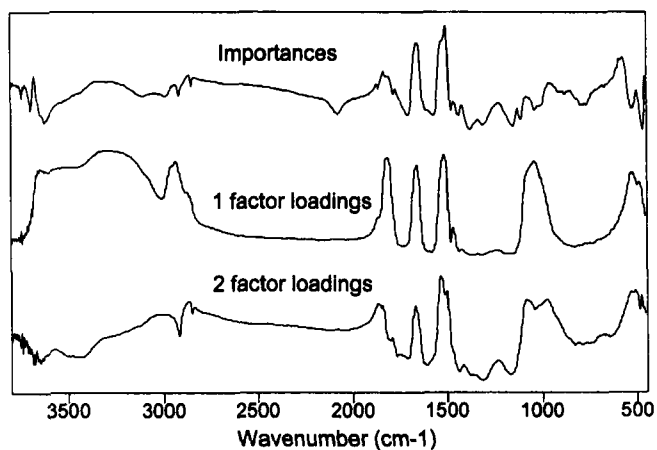


FIGURE 3 Importances, first and second factor loadings from PLSR for the nitrogen content of HA

TABLE IV Results of the PLS correlation using full spectra

<i>Property<sup>a</sup> (HA)</i>	<i>Factors</i>	<i>R<sup>2</sup></i>	<i>SECV</i>	<i>Mean value</i>	<i>rel. SECV (%)</i>
H/C	2	0.851	0.070	1.017	6.9
N/C	9	0.932	0.0048	0.0537	8.9
O/C	8	0.700	0.024	0.533	4.5
N	9	0.920	0.286	3.294	8.7
S	10	0.836	0.262	1.506	17.4
E (280)	1	0.486	156.8	998.1	15.7
E <sub>4</sub> /E <sub>6</sub>	5	0.560	0.549	3.495	15.7
Protons:					
Aliphatic	15	0.728	1.212	30.85	3.9
Aromatic	3	0.606	2.845	16.46	17.3
HCO	2	0.711	2.704	24.31	8.4
Arom./Aliph.	3	0.711	0.0912	0.541	16.9
HS/DOC	1	0.360	8.51	40.6	20.9
<i>Property<sup>a</sup> (FA)</i>	<i>Factors</i>	<i>R<sup>2</sup></i>	<i>SECV</i>	<i>Mean value</i>	<i>rel. SECV (%)</i>
H/C	1	0.307	0.150	1.044	14.4
N/C	5	0.737	0.0078	0.0498	15.7
O/C	13	0.604	0.029	0.758	3.8
N	6	0.754	0.401	2.57	15.6
S	8	0.824	0.195	1.488	13.1

<sup>a</sup>abbreviations see Tables I, II, and III.

From early studies<sup>[17]</sup> it is known that one can use information from the importances to improve the prediction results by means of a suitable selection of the spectral windows. Exemplarily we included only such spectral regions in further calculations for which large values of importances were observed. So we achieved improvements of the forecast of the H/C value from 6.9 % with full spectra calculation to 5.3 % using spectral selection, and for the HCO proportion determined from the <sup>1</sup>H-NMR spectra from 8.4 % to 6.0 %, respectively.

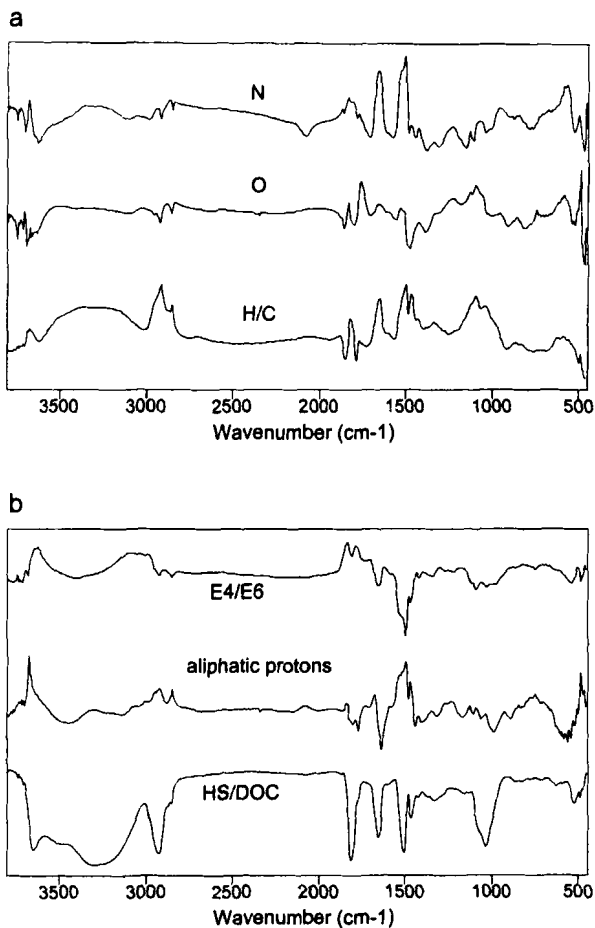


FIGURE 4 Importances for N, O and H/C (a), E4/E6, upfield aliphatic protons and HS/DOC (b) of HA

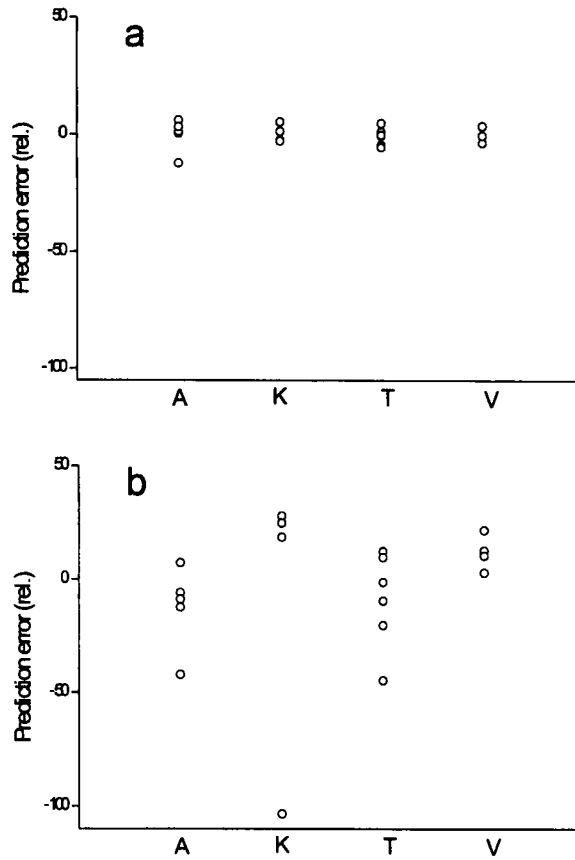


FIGURE 5 Relative prediction error of the single soils referring to the soil series (A,K,T,V) for O/C (a) and HS/DOC (b) of HA

## CONCLUSIONS

In this study we showed that several properties of soil HS, above all the elemental composition as well as elemental ratios, are readily predictable from the FTIR spectra using the PLSR method. From the elemental ratios ecologically meaningful conclusions can be drawn concerning the soil humus quality, e. g. the degree of humification.

The aliphatic and aromatic proton contents of HA, which are important for the interactions with hydrophobic xenobiotics in the environment, cannot be predicted with satisfying reliability. This is also true for the absorbance at 280 nm (related

to the aromatic carbon), the  $E_4/E_6$  ratio (inversely proportional to the molecular size), and the HS/DOC ratio, which is a measure of the stability of a soil ecosystem. The reason for these unsatisfying results could be that the corresponding properties of the HS are generally underestimated in the IR spectra.

The PLS regression worked better with HA than with FA fractions, probably because of the distinct preparation methods for these fractions, by which the natural differences of HS sometimes may be equalized. In the HA preparations more structurally different proportions of HS remain preserved.

It is possible that the predictive power of the applied procedure can be improved, for instance by inclusion of more samples with a greater variability of properties of HS and therefore greater differences in the IR spectra.

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