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Investigation of soil humic substances from different environments using TG–FTIR and multivariate data analysis

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Abstract

The pollution of terrestrial ecosystems is reflected by the structural characteristics of the humic substances they contain. We studied the thermal behaviour of humic substances (fulvic acids and humic acids) from differently polluted soils in an attempt to identify possible links to structural changes in the substances. Using a coupled analysis technique comprising thermogravimetry/Fourier transform infrared spectroscopy and multivariate data analysis, correlations with elemental composition were calculated and classifications with model classes ascertained. The findings showed that stress situations in soils are reflected in the thermal characteristics of the humic acid fractions. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Soil pollution; Humic substances; TG–FTIR; Classification

1. Introduction

The vital role of humic substances (HS) in aquatic and terrestrial ecosystems has prompted numerous efforts to uncover the mechanisms by which these refractory organic substances realise their environmental functionalities [1,2].

For decades the chemical structure of humic substances has been the subject of investigations, with virtually all the analytical techniques available being used. The only way to analyse such macromolecular material without its disintegrating is to employ spectroscopic methods [3,4]. Pyrolysis has proved suitable for the chemical characterisation of these substances as pyrolytic studies do not require chemical splitting, fractionation, clean-up or time-consuming derivation. Although analytical pyrolysis is not the ideal technique for exploring the overall structures of such complex macromolecules, it is nevertheless a suitable method for studying these substances by means of their decomposition behaviour. Information about the chemical structure of humic substances can be derived from the pyrolysis gases evolved. These pyrolysis gases can be qualitatively analysed using mass spectrometry following prior gas-chromatographic separation [5] or infrared spectroscopy [6]. However, infrared spectroscopy is especially suitable for identifying the low-molecular gases released such as water, carbon dioxide, carbon monoxide and ammonia even without prior gas-chromatographic separation, as they exhibit simple and well classifiable vibrational spectra.

It is known that stress factors such as impurities caused by inorganic and organic substances or even flooding affect the elemental composition and the structure of humic substances in the soil (fulvic acids

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Table 1
Atomic ratios calculated by the elemental composition of humic and fulvic acid fractions from soils

Sample	Humic acid			Fulvic acid		
	N/C	O/C	H/C	N/C	O/C	H/C
A1	0.0599	0.572	1.028	0.0653	0.767	0.833
A2	0.0680	0.584	0.968	0.0640	0.782	1.052
A3	0.0572	0.584	0.928	0.0502	0.867	1.052
A4	0.0771	0.580	1.250	0.0657	0.763	1.241
A5	0.0689	0.554	1.152	0.0593	0.757	1.171
K1	0.0637	0.507	1.238	0.0424	0.730	1.168
K2	0.0803	0.521	1.311	0.0553	0.771	1.152
K3	0.0770	0.562	1.239	0.0728	0.764	1.382
K4	0.0756	0.585	1.297	0.0697	0.774	1.308
T1	0.0331	0.515	0.893	0.0460	0.811	0.718
T2	0.0345	0.560	0.974	0.0449	0.798	0.990
T3	0.0293	0.516	0.926	0.0498	0.771	0.946
T4	0.0483	0.477	1.018	0.0511	0.785	1.071
T5	0.0213	0.468	0.811	0.0283	0.850	0.824
T6	0.0466	0.505	1.047	0.0594	0.796	0.926
V1	0.0444	0.520	0.811	0.0283	0.805	0.989
V3	0.0448	0.493	0.794	0.0296	0.887	0.966
V4	0.0466	0.477	0.777	0.0374	0.855	1.209

and humic acids) [7]. The goal of our investigations was therefore to find out whether these structural changes are reflected in the profiles of the pyrolysis gases evolved or in the profiles of the thermogravimetric mass loss curves in the form of their first derivatives by analysing humic substances from soils of differently polluted locations. To do so, we used the elemental composition of humic substances, which can be determined with great accuracy, as a reference point for structural changes (Table 1).

2. Experimental

2.1. Sample collection

Soil samples from the A-horizon of urban and industrial areas in the central German region around Leipzig were used for the investigations:

Series A: from river plains near the city of Leipzig with different levels of heavy metals (A1, A2, A3, A4, A5);
Series K: from a river plain near Bitterfeld, variously polluted by xenobiotics (K1, K2, K3, K4);
Series T: from the surface layer of a landfill in Leipzig, different hillside locations, low pollution (T1, T2, T3, T4, T5, T6);
Series V: from fallow land of a continuous field experiment in Bad Lauchstädt with little or no pollution (V1, V3, V4).

2.2. Preparation of humic substances

The humic substances were extracted from air-dried soils using the grain size fraction smaller than two millimetres with 0.1 M NaOH, and separated into fulvic FA and humic acids (HA) according to the recommendations of the IHSS (International Humic Substances Society), modified by Kuwatsuka et al. [8].

2.3. TG–FTIR analysis

During thermogravimetric analysis, about 20 mg of the samples were first isothermally heated for 15 min in the thermo-balance in a flow of nitrogen to 100°C, and then heated at a rate of 10 K/min up to 900°C. The evolved gases were simultaneously transferred into the gas cell of the FTIR spectrometer using a flushing gas flow of about 80 ml min^{-1}. The transfer line (volume about 0.5 ml) was heated to about 230°C and the IR gas cell (volume about 5 ml) to about 250°C. The infrared spectra were taken at a resolution of 4 cm^{-1} in the spectral range of 450–4000 cm^{-1}, and in each case four spectra were accumulated (measuring set-up: TGA7/System 2000, Perkin-Elmer). Elemental analysis of the fulvic acids and humic acids was already documented in a previous paper [4].

2.4. Data analysis

The data from the TG–FTIR experiment take the form of series of infra-red spectra in a temporal sequence. In accordance with the temperature programme of the TG experiment, temperature access is assigned to the time axis. The three-dimensional result files thus obtained (wave number, absorbance, temperature) can be used to identify the gases evolved by means of their specific absorption bands (Fig. 1). Moreover, the appearance of these gases can be monitored depending on the temperature in the

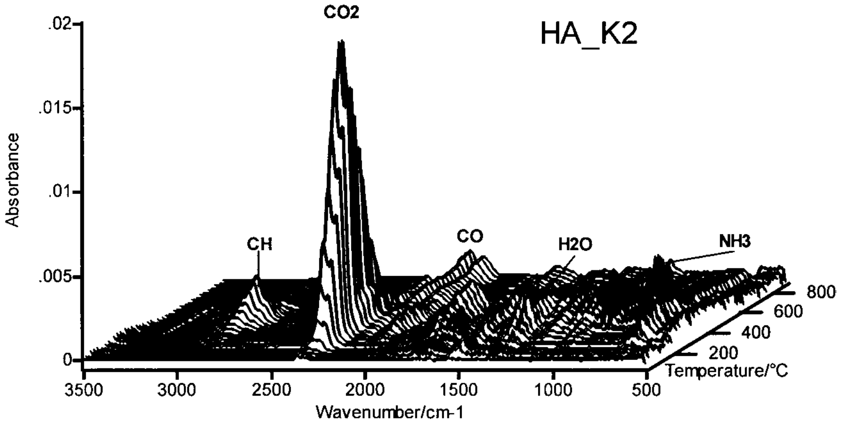

Fig. 1. TG–FTIR experiment with the humic acid fraction of soil K2.

spectroscopic 'windows' along the temperature axis as specific gas profiles.

In addition to the specific gas profiles for carbon dioxide, carbon monoxide, water, ammonia and saturated hydrocarbons, the first derivatives of the thermogravimetric mass loss curves (DTG curves) also served as source data for data analysis.

Correlations between these thermoanalytical curves and the elemental composition, and ultimately between the thermoanalytical curves and known stress situations of the soils, can be identified using the methods of multivariate data analysis [9]. These methods are typically full curves techniques where the data are fitted to many data points thereby improving sensitivity. They are mathematical robust and can provide practicable solutions to qualitative and quantitative applications. We used the add-on application PLSplus/IQ^{TM} for GRAMS/32 (GALACTIC INDUSTRIES COOPERATIONS) [10] for our calculations.

- In a preliminary examination, correlations were ascertained between the specific gas profiles as well as the DTG curves (all as full curves) and the values of the atomic ratios for all samples by means of partial least square regression (PLSR).
- Afterwards, the TG–FTIR data which showed good correlations were used to classify the samples from the individual locations. For this purpose, we applied principal component analysis (PCA) on the corresponding thermoanalytical curves (DTG curves, CO_2 profile and NH_3 profile, all of humic acids) of all samples. Taking into account additional knowledge from previous studies [4,11], the set of calibration data was compiled from typical representatives of the four series of variously polluted soils (see Section 2.1) with the samples A1, A2, A3, K3, K4, T1, T2, T3, V3 and V4. The remaining samples were classified using the Mahalanobis distance [12] (a statistical grounded distance measure).
- A second variant of classification comprised the introduction of an artificial variable (dummy variable) [9,13], and the calibration set selected as before was divided by this variable into two groups, to solve the classification as a two class problem. The samples A1, A2, A3, K3, K4 are assigned to a polluted soil group G1 and the samples T1, T2, T3, V3, V4 are assigned to an unpolluted group G2. The basis for this division was provided again by knowledge from previous studies [4,11]. The value of the stated dummy variable Y for the two groups (1,2) with n samples in each case (n_1, n_2) results after Eq. (1).

$$Y = \begin{matrix} -n_1/(n_1+n_2) & \text{for samples belonging to G1} \\ n_2/(n_1+n_2) & \text{for samples belonging to G2} \end{matrix} \tag{1}$$

These initial values for Y lead to a good estimation for the expected value of Y. On this base a partial least square model is obtained for regression between the selected thermoanalytical curves and the above defined dummy variable Y. By the means of this determined PLSR model the value of the dummy variable is recalculated for all the samples (including the samples of the calibration data set).

3. Results and discussion

3.1. Preliminary examination

The correlations calculated between the elemental levels of fulvic acids and humic acids of all samples expressed in the atomic ratios N/C, O/C and H/C (Table 1) and the TG–FTIR data show that certain qualitative differences between soils and their changes are evidently better reflected in the thermal behaviour of the humic acid fractions than in that of the fulvic acid fractions (Table 2). Judging by the standard error of cross validation (SECV) and the square correlation coefficient $R^2$of the PLS regression, as well as by the regression plot of actual values versus predicted values (Figs. 2 and 3), the best correlations were found in the humic acid fractions between the DTG curves as well as the specific gas profiles of carbon dioxide and of ammonia and the atomic ratios N/C and H/C. Comparable correlations were found in the fulvic acid fractions between the DTG curves and the specific gas profiles of ammonia and the atomic ratios N/C only. The results suggest that modifications in the structures of humic substances due to different environmental stress in soils can also be reflected in some thermoanalytical characteristics of these substances.

Table 3
Mahalanobis distances of the ‘unknown’ samples relative to the mean of the calibration group for classification using PCA

Sample	DTG (HA)	CO_2 (HA)	NH_3 (HA)
A4	1.354	3.210	1.908
A5	4.461	0.739	0.989
K1	40.38	1.200	3.101
K2	64.20	2.628	5.132
T4	7.714	2.130	0.332
T5	7.583	6.476	1.037
T6	11.37	2.377	0.378
V1	0.945	0.844	1.160

3.2. Classification by PCA

The allocation of the ‘unknown’ samples to the calibration data set from humic acids of variously polluted soils using the Mahalanobis distance of the PCA is shown in Table 3 for selected thermoanalytical characteristics. Concerning preceding investigations [4,11] the result for the specific gas profile of NH_3 of the humic acids can be interpreted especially consistent with other experimental findings. Because the limit for allocation to the calibration group is usually >3, the samples K1 and K2 can not be included. This finding answers the extremely high quantities of xenobiotics detected in the corresponding soils. The special position of these two samples can also be recognized in the spectroscopic behaviour of the humic acids [4,11]. Such an interpretation within

Table 2
Results of the correlations between TG–FTIR[a] data and atomic ratios using PLSR

	Humic acid						Fulvic acid					
	N/C		O/C		H/C		N/C		O/C		H/C	
	R^2[b]	SECV[c]	R^2	SECV	R^2	SECV	R^2	SECV	R^2	SECV	R^2	SECV
DTG	0.909	10.1	0.386	6.5	0.778	8.5	0.757	13.4	0.480	3.9	0.120	18.5
CO_2	0.737	17.7	0.347	6.2	0.545	13.0	0.359	23.7	0.535	7.1	0.085	18.0
CO	0.156	139.2	0.029	25.7	0.421	14.3	0.059	33.0	0.142	9.7	0.556	11.9
aliph. Hc.	0.161	64.3	0.252	8.2	0.339	36.2	0.247	26.4	0.048	9.9	0.175	43.5
H_2O	0.261	33.7	0.200	9.2	0.470	15.5	0.178	29.9	0.249	6.8	0.490	26.2
NH_3	0.614	20.8	0.094	7.5	0.869	6.5	0.515	20.3	0.380	4.4	0.104	13.5

[a] DTG: first derivative of the TG-curve. Specific gas profiles: CO_2, carbon dioxide; CO, carbon monoxide; aliph. Hc., aliphatic hydrocarbons; H_2O, water; NH_3, ammonia.

[b] R^2: square correlation coefficient.

[c] SECV: standard error of cross validation.

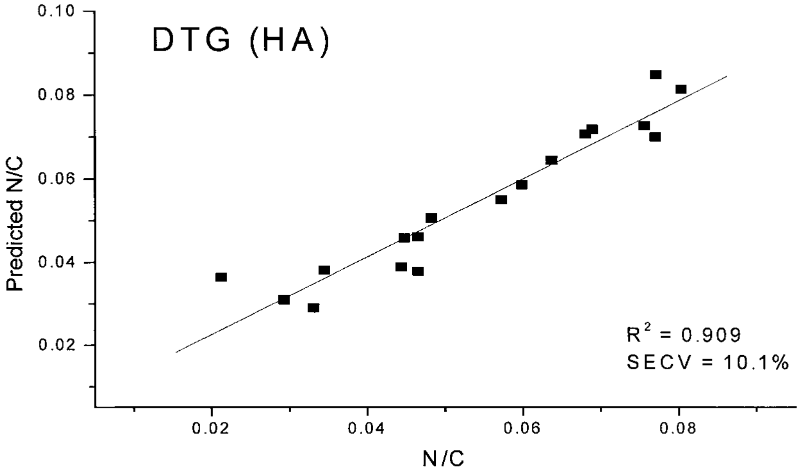

Fig. 2. PLS regression plots: predicted from DTG curves versus actual values for the N/C ratios of HA.

the above limits is not possible with the DTG curves and the specific gas profile of CO_2. Only the very high values of the Mahalanobis distance for K1 and K2 in the case of the DTG curves show the particular characteristics of these substances.

3.3. Classification by dummy variables

The results of the classification using the artificial variable Y and the PLSR are represented in the Figs. 4–6 and Table 4. The model predictive ability of the models is showing itself from the standard error of cross validation SECV and the square correlation coefficient R^2.

For the predicted allocation of the 'unknown' samples into the two groups is to emphasize:

The definition of the dummy variable suggests that for the polluted samples a negative value is predicted and for the unpolluted a positive value. To the majority of the samples this applies.

The allocation of the sample K2 using the specific gas profile of ammonia ought in turn to indicate high 'stress'.

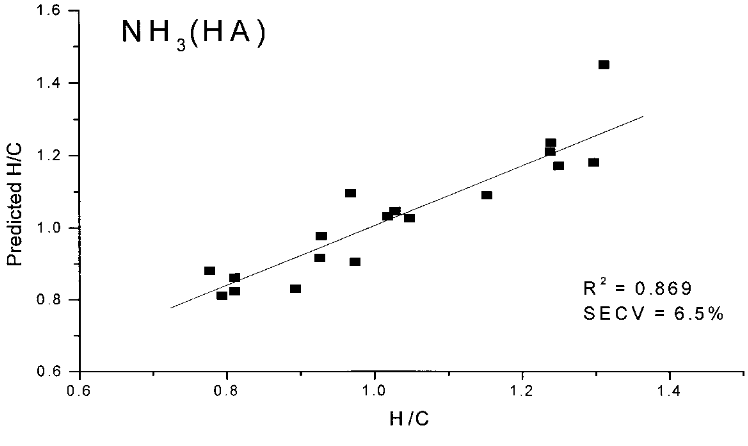

Fig. 3. PLS regression plots: predicted from specific gas profile of ammonia versus actual values for the H/C rations of HA.

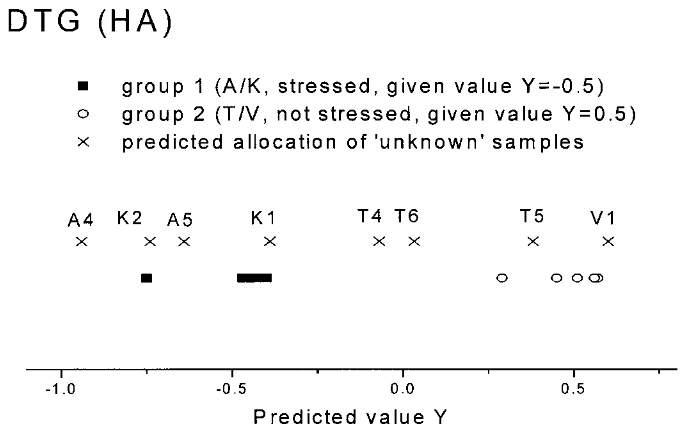

Fig. 4. Classification of soil humic acids as a two group problem using DTG curves.

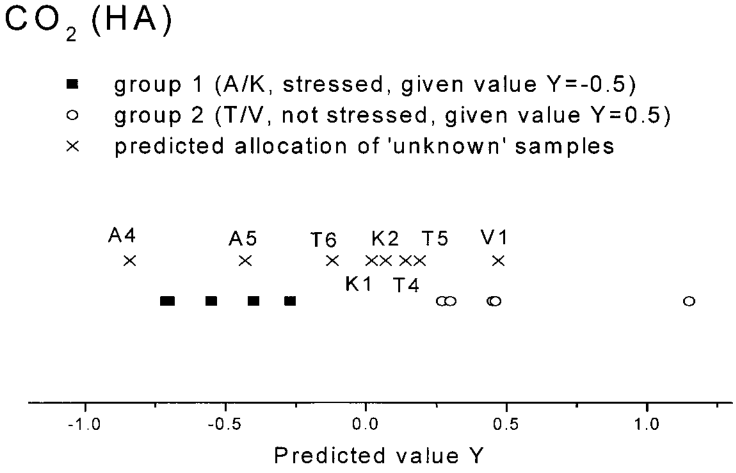

Fig. 5. Classification of soil humic acids as a two group problem using specific gas profile of carbon dioxide.

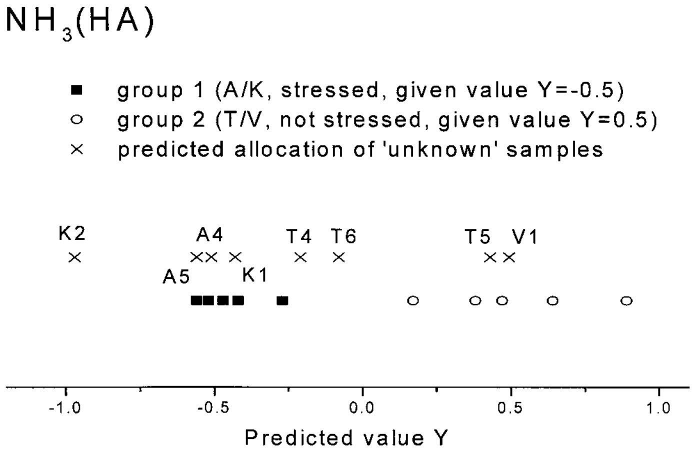

Fig. 6. Classification of soil humic acids as a two group problem using specific gas profile of ammonia.

Table 4
Results of the classification using thermoanalytical curves of soil humic acids, PLSR and a dummy variable

Sample	DTG[a]		CO_2[b]		NH_3[c]	
	Y stated	*Y* predicted	*Y* stated	*Y* predicted	*Y* stated	*Y* predicted
A1	−0.5	−0.40	−0.5	−0.55	−0.5	−0.42
A2	−0.5	−0.75	−0.5	−0.71	−0.5	−0.47
A3	−0.5	−0.42	−0.5	−0.40	−0.5	−0.27
K3	−0.5	−0.44	−0.5	−0.70	−0.5	−0.56
K4	−0.5	−0.47	−0.5	−0.27	−0.5	−0.52
T1	0.5	0.29	0.5	1.15	0.5	0.17
T2	0.5	0.57	0.5	0.45	0.5	0.64
T3	0.5	0.56	0.5	0.27	0.5	0.38
V3	0.5	0.51	0.5	0.30	0.5	0.89
V4	0.5	0.45	0.5	0.46	0.5	0.47
A4		−0.94		−0.84		−0.51
A5		−0.64		−0.43		−0.56
K1		−0.39		0.02		−0.43
K2		−0.74		0.07		−0.97
T4		−0.07		0.14		−0.21
T5		0.38		0.19		0.43
T6		0.05		−0.12		−0.08
V1		0.60		0.47		0.49
	SECV[d]=0.119 R^2[e]=0.944		SECV=0.258 R^2=0.807		SECV=0.190 R^2=0.868	

[a] DTG: first derivative of the TG-curve.

[b] CO_2: specific gas profiles of carbon dioxide.

[c] NH_3: specific gas profiles of ammonia.

[d] SECV: standard error of cross validation.

[e] R^2: square correlation coefficient.

Samples T4 and T6 adopt for all curves an intermediate position.

The allocation of sample A4 using the DTG curve and the specific gas profile of the carbon dioxide may reflect a particular location rather than a stress situation.

4. Conclusion

The characteristics of soil humic substances, which can be determined by TG–FTIR spectroscopy, reflect the degree of pollution of soils to a differing extent. Correlations with possible structural alterations in particular be seen in the thermal behaviour of the humic acid fractions. As also shown by previous investigations using infrared and fluorescence spectroscopy [4,11], above all highly polluted soils (in the present case samples K1 and K2) can be distinguished from less polluted soils. Using artificial variables for the two calibration groups which, during data analysis using previous information, allow the classification of ‘unknown’ soils with reference to a polluted and an unpolluted group, also prove to be helpful.

By using a higher number of samples and by optimising the TG–FTIR experiment by raising the quantity of samples, reducing the gas volume in the measuring set-up, and reducing the dilution rate in the transfer of the gases to the infrared spectrometer, the method described here ought to be even more suitable for identifying stress situations in soils.

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