

Controlled pore glass chromatography of humic substances from some organic soils of Austria and Bangladesh

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Chromatographie von Huminstoffen aus einigen Organischen Böden von Österreich und Bangladesh an Porenkontrolliertem Glas

1 Introduction

Organic matter, a complex and dynamic component of soil and a fundamental constituent of the biosphere, is one of the most important slowly renewable natural resources. It is considered as the single most important indicator of soil quality (SIKORA et al., 1996). Humic substances, the major component of organic matter, serve as a primary reservoir

of organic carbon in soils and the oceans for the global carbon cycle (WOODWELL et al., 1978). The pedogenic and agronomic importance of humic substances is well known since long time, its role in preserving global ecology is now well documented (MACCARTHY et al., 1990; old literature see e.g. SCHEFFER and ULRICH, 1960).

Organic soils are accumulators of organic and humic materials. In Austria, Terric and Eutric Histosols are cal-

Zusammenfassung

Die Huminstoffe von drei typischen Organischen Böden, einem Anmoor (Terric Histosol) und einem Niedermoor (Eutric Histosol) aus Österreich und einem Übergangsmoor (Dystric Histosol) aus Bangladesh, wurden mit einem komplexbildenden Ionentauscher und Wasser erschöpfend extrahiert und anschließend in Braunhuminsäuren und Fulvosäuren fraktioniert. Diese beiden Grobfraktionen und den unfraktionierten Extrakt untersuchten wir chromatographisch an einer Säule mit Porenkontrolliertem Glas mit einem Porendurchmesser von 16,7 nm; im abfließenden Säuleneluat wurden Kohlenstoff als DOC und gefärbte Substanzen als Extinktion bei 400 nm gegen den Verteilungskoeffizienten K_d registriert. Daraus errechneten wir die Spezifische Extinktion und benützten das Maximum derselben als Stoffkonstante der „reinen“ Huminstoffe. Die begleitenden Nichthuminstoffe errechneten wir als Differenz.

Die Ergebnisse zeigen in allen drei Huminstoffsystemen die Braunhuminsäuren als dominante Fraktion mit Anteilen von 56,05% für das Anmoor, 56,68% für das Niedermoor und 85,05% für das Übergangsmoor. Die Spezifischen Extinktionen der „reinen“ Huminstoffe ergaben für die Fulvosäuren 82,2 (Anmoor), 55,9 (Niedermoor) und 48,3 (Übergangsmoor); für die Braunhuminsäuren 210,1 (Anmoor), 173,9 (Niedermoor) und 251,1 (Übergangsmoor); für die Grauhuminsäuren 292,0 (Anmoor), 242,4 (Niedermoor) und 345,2 (Übergangsmoor). Die Fulvosäuren zeigten immer einen charakterischen Gipfel mit einem K_d -Wert von 0,70 bis 0,75; die Braunhuminsäuren hatten einen Doppelpeak mit K_d -Werten von 0,00 bis 0,10 und 0,60 bis 0,65; die Grauhuminsäuren schließlich ergaben ebenfalls Doppelpeaks mit K_d -Werten von -0,05 bis 0,00 und 0,35 bis 0,45. Die Nichthuminstoffe waren durch drei Fraktionen von niedrigem, mittlerem und hohem Molekulargewicht charakterisiert, wobei die mit mittlerem MG dominierten. Das Verhältnis von Huminstoffen zu Nichthuminstoffen war etwa 60:40. Die Farbquotienten (E400/E600) der drei Grobfraktionen der Huminstoffe waren: Fulvosäuren 8,18 bis 10,57, Braunhuminsäuren: 5,54 bis 5,67 und Grauhuminsäuren: 3,57 bis 3,95.

Schlagnworte: Huminstoffe, Organische Böden, Histosole, Chromatographie an Porenkontrolliertem Glas, „Rein-huminstoffe“, Spezifische Extinktion von „reinen“ Huminstoffen, Nichthuminstoffe.

Summary

Humic substances from three typical organic soils, two from Austrian Histosols and one from Bangladesh peat (Dystric Histosol), were exhaustively extracted with a chelating resin and water and subsequently fractionated into brown humic acids and fulvic acids, respectively. These gross humic fractions were chromatographically investigated using a column packed with controlled pore glass (CPG) of 16.7 nm pore diameter. Concentrations of both coloured material and carbon were simultaneously recorded in the chromatograms against partition coefficient (K_d). Specific extinctions at 400 nm were calculated and maximal values were taken as constants specific for 'pure' humic substances. Accompanying non-humic substances were obtained by difference.

Results revealed that the Terric and Eutric Histosol of Austria and Dystric Histosol of Bangladesh were typically dominated by brown humic acids (BHA). The percentages were 56.05, 56.68 and 85.05, respectively. The specific extinctions obtained for 'pure' gross humic fractions of these soils were: fulvic acids (FA) 82.2, 55.9 and 48.3; brown humic acids (BHA) 210.1, 173.9 and 251.1 and grey humic acids (GHA) 292.0, 242.4 and 345.2, respectively. The number of peaks and their corresponding K_d values in the different humic fractions as recorded in the elution curves were: FA with a characteristic single peak at 0.70–0.75, BHA with double peaks at 0.00–0.10 and 0.60–0.65 and GHA also with a couple of peaks at -0.05–0.00 and 0.35–0.45. The non-humic substances were characterized by three types of fraction, i.e. low, medium and high in molecular size, in which predominance of medium molecular sized non-humic materials was distinct. The humic to non-humic ratios were high, about 60:40. The ranges of colour quotients (E₄₀₀/E₆₀₀) for three studied soils were: FA 8.18–10.57, BHA 5.54–5.67 and GHA 3.57–3.95.

Key words: Humic substances, organic soils, Histosols, chromatography on Controlled Pore Glass, "pure" humic substances, specific extinction of "pure" humic substances, non-humic substances.

careous in nature. These soils, specially the latter one, occur in low-lying areas and are saturated with water throughout the year. Dystric Histosol (Peat) of Bangladesh can be characterized as organic soil of acidic nature. In general, it occurs in basin depressions under swamp grassland and is exposed to deep flooding very often. HUSSAIN (1992) reported that most peats in Bangladesh are derived from reeds and grasses, which are the major vegetation of marshy lands.

Fractionation and characterization by chromatography on CPG and spectrophotometry coupled with a C-monitor is a powerful tool in analysing the nature and characteristics of humic substances (DANNEBERG, 1976; 2002). In our previous paper, we analysed and reported on the humic systems of some typical Austrian Cambisols. The present paper reports the humic systems of some organic soils of Austria and Bangladesh, which may provide useful information for a better understanding of them.

2 Materials and methods

Soils: Two typical soils rich in organic matter from Lower Austria and a peat soil from Bangladesh were sampled for 0–20 cm in March–April, 1998. The collected soil samples

were air-dried and sieved (2 mm). Routine analysis was carried out with these samples following standard methods. The soil names, locations and some of their salient features are presented in Table 1.

Extraction and freeze dry preparation: The soils were exhaustively extracted for four consecutive days with a chelating resin (Chelex-100, Na-form) and water to get maximum possible yields (DANNEBERG, 1981; 2002). Extracted materials from each day were separated by high speed centrifugation, collected and combined in one container, deep-frozen, freeze-dried and stored in a refrigerator until further use.

Gross fractionation and optical analysis: The freeze dried extracts were dissolved in water, made to volume and aliquots were taken for gross fractionation into fulvic acids and brown humic acids according to DANNEBERG (2002). These, together with aliquots of the unfractionated extract, were freeze dried and kept under cooling for chromatography. Other aliquots, treated in the same way, were used for optical analysis; extinctions at wavelengths of 400 and 600 nm were recorded in buffered solution (pH = 10.0) with a spectrophotometer. Grey humic acids were not directly prepared since they can not be readily redissolved once they are precipitated; they are obtained by difference [GHA = Extract-(FA+BHA) in OD/g soil].

Table 1: Some characteristic physical and chemical features of the studied soils
 Tabelle 1: Einige chemische und physikalische Eigenschaften der untersuchten Böden

No.	Soil types/ names	Sand %	Silt %	Clay %	Soil pH CaCl ₂	Lime %	C-total %	Org. Matter %	N-total %	CEC cMol kg ⁻¹	Ash %
1	Terric Histosol Anmoor, Calc. Mitterndorf (KB 1) Austria	7	65	28	7.6	30	10.96	18.90	1.11	83.1	21.5
2	Eutric Histosol Niedermoor, Calc. Herrengas (KB 1) Austria	6	62	32	7.5	4.5	24.27	41.84	1.95	132.0	44.9
3	Dystric Histosol Peat, Non-calc. Faridpur (Satla series) Bangladesh	25	51	24	5.6	0	33.35	57.50	2.59	102.7	58.7

Chromatographic experiment:

Column set up: A glass column of 16 mm internal diameter was packed with controlled pore glass (CPG Inc., USA) with a pore diameter of 16.7 nm and a grain size of 6.0–125 µm (i.e. 120–200 mesh) to obtain a chromatographic column about 600 mm in length. The column was fitted with a flow adaptor. A compact and even packing of the CPG in the column was obtained by using a vibrator. The column was then equilibrated with the elution solution containing 0.02 Moles Na₂B₄O₇ and 0.05 Moles NaCl per litre in CO₂-free distilled water. The column outlet was connected to a continuous flow UV-monitor and to a fraction collector or, in turn, to a C-monitor. The UV-monitor and the C-monitor both were connected to a two-channel plotter.

Calibration parameters of the column were determined using a test solution of 1% benzyl alcohol (to determine V_t) and 0.5% blue dextran 2000 (obtained from Pharmacia, Sweden, to determine V_o) in elution solution.

C-monitor set up: An instrument designed for the determination of dissolved organic carbon (DOC) in natural water (TOCOR2, Maihak) was used as a C-monitor (DANNEBERG, 1979; 2002). The instrument was calibrated with standard solutions containing known amounts of potassium-hydrogen-phthalate, dissolved in CO₂-free water as a source of organic carbon.

Chromatographic analysis: The chromatographic experiment was carried out according to DANNEBERG (1981; 2002). Since soil humic substances contain a heterogeneous mixture of organic substances with different molecular sizes and shapes and for which each group of compounds may exhibit a different relationship between log MW and elution volume (K_d), calibration of column on the basis of

molecular weight has not been tried (SADAO and BARTH, 1999).

Freeze dried aliquots of unfractionated extract, FA and BHA were dissolved in a proper volume of elution solution. 2 ml of these sample solutions were introduced separately onto the column through a sample applicator loop and eluted with elution solution using a flow rate of approximately 1 ml/min. The column effluent was first passed through the UV-monitor to have a control of the proper run. It was then either introduced into the the C-monitor or it was collected in a fraction collector, the fractions being subsequently analysed with a spectrophotometer. Thus for every sample two runs were carried out, one for optical analysis and one for carbon determination.

Carbonate estimation: To estimate the amount of carbonates which were co-extracted from soils containing lime, separate runs were carried out with Na₂CO₃ solutions of different concentrations, keeping all other experimental set up the same. The closest of these chromatographically obtained and integrated values were then subtracted from the integrated value of the total extracts to correct for its carbonate content (DANNEBERG and ULLAH, 1982).

Calculation: The direct results of chromatographic experiments were recorder-plottings of either extinction or carbon concentration versus elution volume. Since elution volume depends on the dimensions of column and tubing connections, it is not suitable for comparison; therefore, it is converted to partition coefficient, K_d. This is calculated using the following formula:

$$K_d = \frac{V_e - V_o}{V_t - V_o} \quad \text{Where, } V_e = \text{Elution volume} \\ V_o = \text{Void volume and} \\ V_t = \text{Total volume}$$

To arrive at both extinction values and carbon concentrations, corresponding to equal Kd values, results were interpolated for given and round values of Kd. From this set of data, specific extinctions were calculated for unit of C ($E^{1\%C}_{1\text{ cm}}$) as a measure of purity of humic substances. The maximum value is considered the purest fraction and was used for the computation of 'pure' humic substances, i.e., to convert extinction values to corresponding concentrations of carbon using Lambert-Beer's law (SCHNITZER, 1971; DANNEBERG, 1981; 2002).

Non-humic substances were simply obtained by differences; i.e.,

$$C_{\text{non-humic substances}} = C_{\text{total}} - C_{\text{'pure' humic substances}} \text{ } [\mu\text{g C}].$$

All calculations as well as graphic presentations of results were carried out with EXCEL-97.

3 Results and discussion

The content of coloured substances in the total extracts and their gross humic fractions of the soils under study as obtained through optical analysis at E400 are presented in Table 2.

Results revealed that the extracts contained high amounts of coloured substances. Of them, Dystric Histosol has the maximum. In all these soils, brown humic acids are the predominant fraction and the grey humic acids are the minimum. The percent distribution of brown humic acids in Terric Histosol and Eutric Histosol of Austria and Dystric Histosol of Bangladesh are 56%, 57% and 85%, respectively. These results are well consistent with those of previous workers (ORLOV, 1995; STEVENSON and COLE, 1999).

When the humic acid:fulvic acid ratio (HA/FA) is computed, it is observed that the values in Austrian Histosols are comparatively low, but in Dystric Histosol (Peat) of Bangladesh, it is quite high. According to ANDREUX (1996),

the ratio of HA and FA increases with increasing degree of polymerization, which is higher for HA than for FA.

The elution curves of the different humic fractions (FA, BHA and GHA) of the soils under study are presented in the figures 1, 2 and 3.

A typical sharp and vertically elongated single peak is obtained in the chromatograms of 'pure' FAs in all the soils (Fig. 1a, 2a and 3a). The Kd value of the peaks of Terric and Eutric Histosols from Austria and of Dystric Histosol from Bangladesh are 0.7, 0.65 and 0.7 respectively. The Kd value positions signify that FA have eluted properly at the end of the chromatogram and have low molecular size.

The elution curve of 'pure' BHA consists of two peaks, one is tiny at the exclusion side and the other occupies the major portion of the diagram. The Kd values of flat-like tiny peaks are Terric Histosol (Austria): 0.05, Eutric Histosol (Austria): 0.10 and Dystric Histosol (Bangladesh): 0.00; these tiny peaks indicate the presence of a high molecular sized part in the chromatogram. On the other hand, the corresponding Kd values of the large peaks are 0.65, 0.60 and 0.60 (Fig. 1b, 2b and 3b). This result suggests that the most part of the BHA fractions of these soils is constituted of medium molecular humic materials.

The coloured humic fractions of GHA show distinct double peaks as well (Fig. 1c, 2c and 3c). The first peak is situated at the exclusion side with Kd values of 0.00, -0.05 and 0.00 for Terric Histosol, Eutric Histosol and Dystric Histosol, respectively. These peaks are comparatively sharper and larger than the first peaks of BHA. The corresponding Kd's of the second large peaks are 0.40, 0.35 and 0.45, occupying most of the area of the curve. These results suggest the presence of a larger part of GHA having high molecular size.

In all fractions, at least three peaks are obtained as accompanying non-humic substances (Fig. 1, 2 and 3). According to their Kd values, they can be characterized as high, medium and low molecular non-humic substances in which

Table 2: Contents of coloured material [OD/g] in humic extract in some organic soils of Austria and Bangladesh

Tabelle 2: Gehalt an gefärbten Substanzen [OD/g] in den Huminstoffextrakten der untersuchten Böden aus Österreich und aus Bangladesh

No.	Soils	Total extracts	FA	BHA	GHA	HA/FA ratio
1	Terric Histosol (Austria)	16.95 100%	5.65 33%	9.50 56%	1.80 11%	2.00
2	Eutric Histosol (Austria)	38.20 100%	11.35 30%	21.65 56%	5.20 14%	2.37
3	Dystric Histosol (Bangladesh)	94.30 100%	9.68 10%	80.20 85%	4.43 5%	8.74

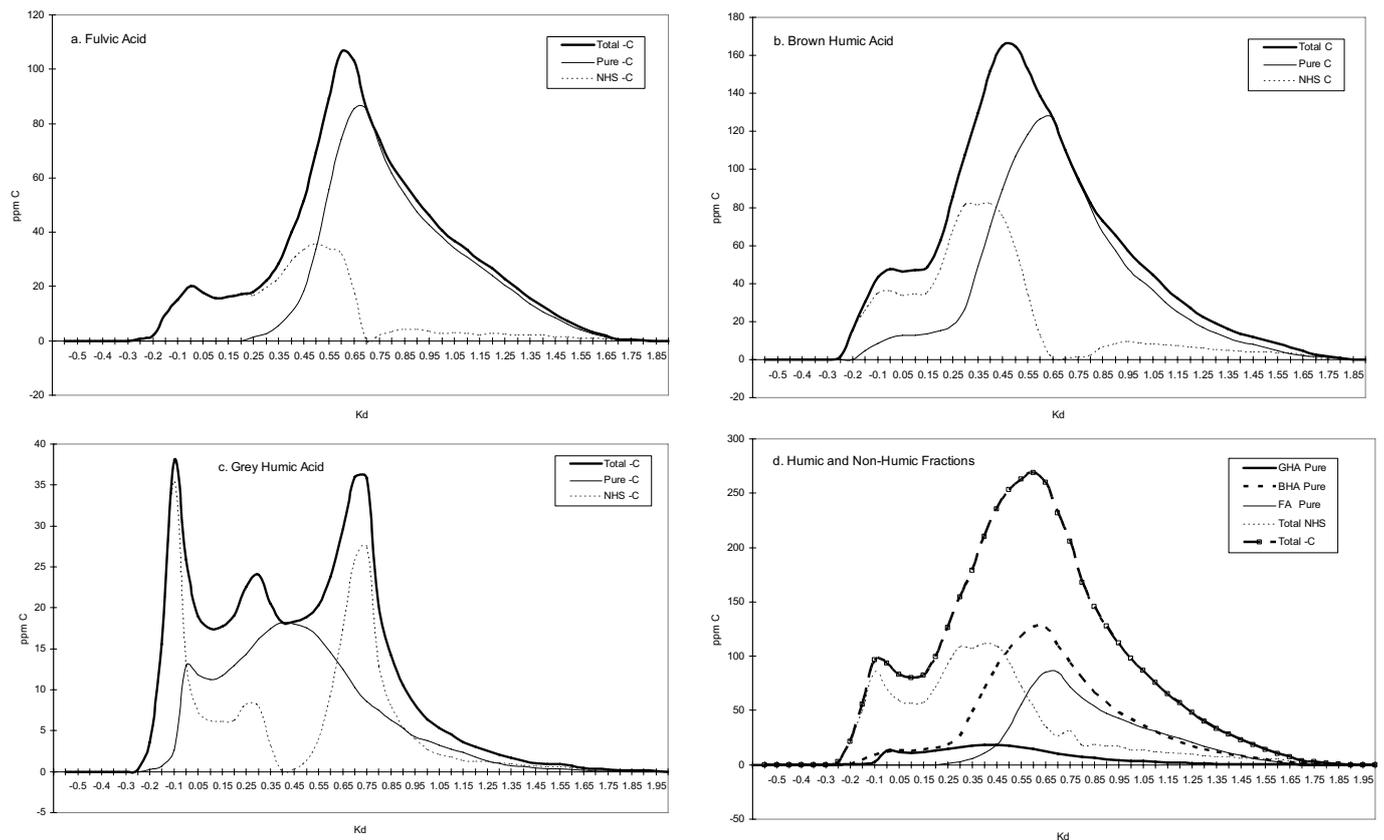


Figure 1: Size exclusion chromatograms of humic fractions of Terric Histosol
 Abbildung 1: Chromatogramme der Huminstofffraktionen aus Anmoor (Terric Histosol)

medium sized materials dominated in FA and BHA whereas high and low molecular sized non-humic fractions dominated in GHA.

When all the humic and non-humic fractions are combined into one diagram (Fig. 1d, 2d and 3d), it is evidently noticed that the second peak of 'pure' BHA constitutes most of the chromatogram's area in all these soils, followed by FA's in Terric and Eutric Histosols of Austria. The results are well comparable with the optical values. Non-humic substances in all three soils are clearly obtained in three fractions, in which the middle peaks in all cases are comparatively large.

It is to be mentioned that the materials of chromatographic column as found in the literature are in most cases somewhat different, so correct comparison is indeed difficult. However, the shape and pattern of elution diagrams of different humic fractions are in well conformity and K_d -values are more or less close to the figures stated in the literature (DANNEBERG, 1981; DANNEBERG and ULLAH, 1982; KUTSCH and DANNEBERG, 1985; GERZABEK et al., 1989; MOHAMAD et al., 1993; TAN, 1998; NOVOTNY et al., 1999).

Table 3 summarizes the chromatographic results by integration of the elution peaks and presentation of all three fractions obtained in terms of mg carbon per g of soil and, to arrive at a complete picture, as percent of extracted organic matter.

The results of the specific extinctions of 'pure' FA, BHA and GHAs of the studied soils are presented in Table 4.

It is evident that the values of specific extinction of humic fractions of the soils under study follow a definite trend, i.e., 'pure' GHA show the maximum in all cases, somewhat distantly followed by 'pure' BHA, whilst 'pure' FA are the minimum. The same sequence of specific extinctions is also reported by other authors (DANNEBERG and ULLAH, 1982; KUTSCH and DANNEBERG, 1985; MOHAMAD et al., 1993). It is to be noted that GHA are of higher molecular weight and so exhibit higher extinction values than BHA (LADD, 1969). Among the FA of different soils, Terric Histosol has the highest (82.20). The relatively high extinction values associated with the low molecular weight fractions are considered to be due to their higher contents of more extensively conjugated light absorbing components (LADD, 1969). SCHNITZER (1971) reported that the values of FA are 5 to 10 folds lower

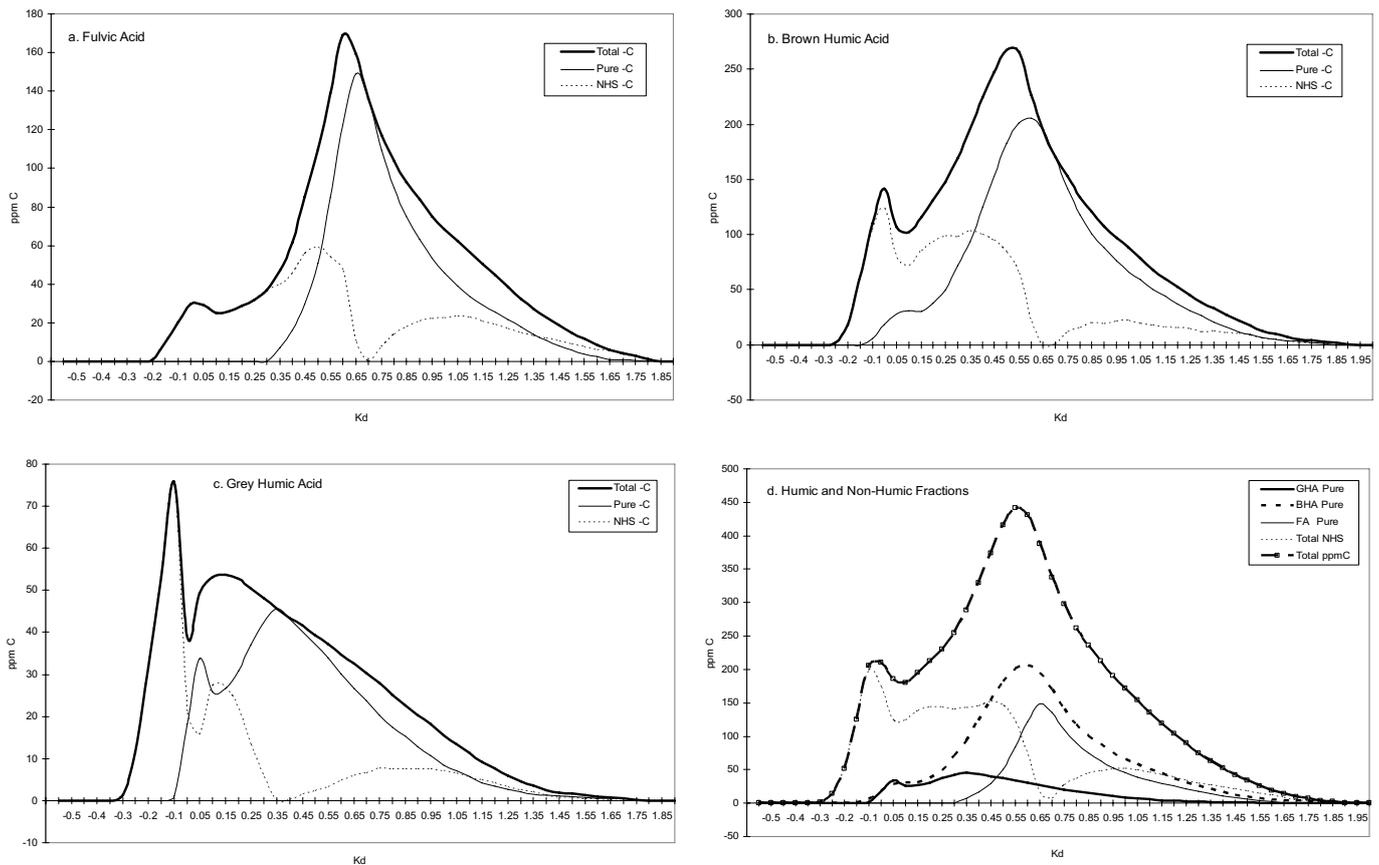


Figure 2: Size exclusion chromatograms of humic fractions of Eutric Histosol
 Abbildung 2: Chromatogramme der Huminstofffraktionen aus Niedermoor (Eutric Histosol)

Table 3: Chromatographic results of gross humic fractions of the soils under study
 Tabelle 3: Ergebnisse der chromatographischen Untersuchung der Grobfraktionen der Huminstoffe aus den untersuchten Böden

Gross fractions	Terric		Histosol		Eutric Histosol		Dystric Histosol	
	mg C/g	%	mg C/g	%	mg C/g	%	mg C/g	%
Sum HS 'pure'	12.31	64.01	20.23	60.82	49.55	60.36		
'Pure' FA	4.12	21.42	5.76	17.33	5.08	6.19		
'Pure' BHA	6.94	36.08	11.63	34.96	39.96	48.67		
'Pure' GHA	1.25	6.51	2.84	8.53	4.51	5.49		
Sum NHS	6.92	35.99	13.03	39.18	32.55	39.34		
FA I	0.38	1.95	0.53	1.59	0.13	0.16		
FA II	1.27	6.58	2.01	6.03	1.59	1.94		
FA III	0.20	1.03	1.35	4.06	1.73	2.11		
BHA I	0.68	3.53	2.13	6.42	2.52	3.07		
BHA II	2.81	14.59	3.90	11.72	15.09	18.38		
BHA III	0.48	2.48	1.24	3.72	8.99	10.95		
GHA I	0.40	2.07	0.97	2.90	1.01	1.23		
GHA II	0.12	0.61	0.44	1.32	0.25	0.31		
GHA III	0.61	3.16	0.47	1.42	1.23	1.50		
Total	19.24	100.00	33.26	100.00	82.10	100.00		
Lime	7.47	2.49*	2.57	5.72*	–	–		

* Per cent value of total lime contents.

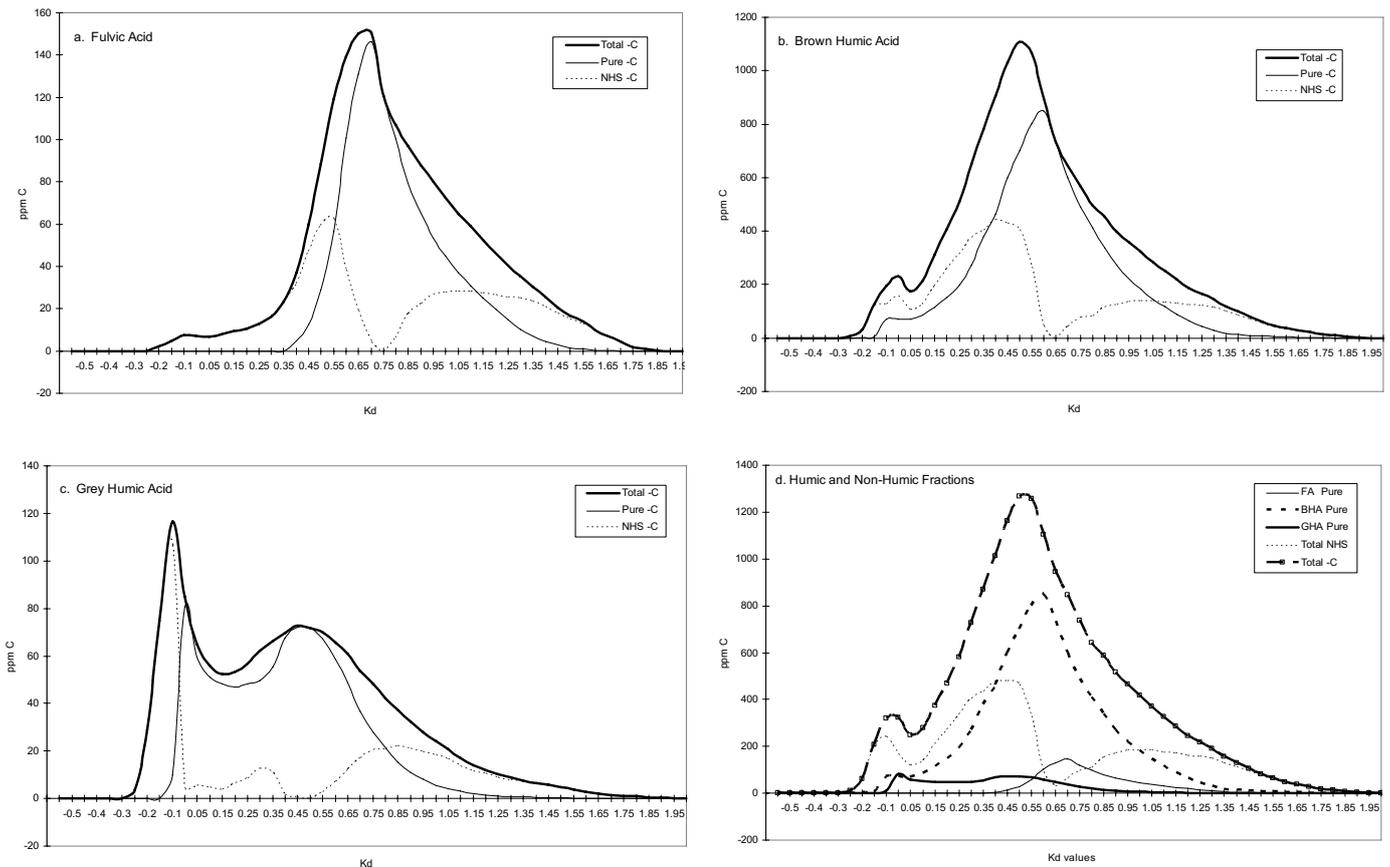


Figure 3: Size exclusion chromatograms of humic fractions of Dystric Histosol
 Abbildung 3: Chromatogramme der Huminstofffraktionen aus Übergangsmoor (Dystric Histosol)

Table 4: Specific Extinctions of the ‘pure’ humic fractions of the soils under study

Tabelle 4: Spezifische Extinktionen der „reinen“ Huminstofffraktionen der untersuchten Böden

No.	Soils	‘Pure’ FA	‘Pure’ BHA	‘Pure’ GHA
1	Terric Histosol (Austria)	82.20	210.10	292.00
2	Eutric Histosol (Austria)	55.90	173.90	242.40
3	Dystric Histosol (Bangladesh)	48.30	251.10	345.20

than for HA. MOHAMAD et al. (1993) obtained 50–90 for ‘pure’ FA in matured composts with comparatively lower values in young composts and a wide range of values for ‘pure’ BHA (80–280). All the specific extinction values in the present investigations are obtained at Kd of the peak maxima or close to them and within the peak areas.

The colour quotients (E400/E600) of the ‘pure’ humic fractions, i.e., recorded at the position with the maximum

specific extinction values in the chromatogram, are presented in Table 5.

From the results, it is revealed that the colour quotients of high molecular GHA ranged between 3.57 and 3.95, those of medium sized BHA between 5.54 and 5.67, while low molecular FA show values from 8.18 to 10.57. These results

Table 5: Colour quotients (E400/E600) of the ‘pure’ humic fractions of the soils under study as obtained from chromatographic experiments.

Tabelle 5: Farbquotienten (E400/E600) der „reinen“ Huminstofffraktionen der untersuchten Böden, erhalten aus den chromatographischen Experimenten.

No.	Soils	‘Pure’ FA (peak)	‘Pure’ BHA (2 nd peak)	‘Pure’ GHA (2 nd peak)
1	Terric Histosol (Austria)	8.18	5.56	3.57
2	Eutric Histosol (Austria)	8.74	5.54	3.95
3	Dystric Histosol (Bangladesh)	10.57	5.67	3.91

are in good agreement with previous findings (TAN, 1998; MARTIN et al., 1998; NOVOTNY et al., 1999; TRUBETSKOJ et al., 1999). DANNEBERG and SCHMIDT (1978) reported that the ratio increases with decreasing molecular sizes as humic substances are fractionated chromatographically through CPG column. TAN and GIDDENS (1972) reported that the humic fractions with higher partition coefficients as separated by Sephadex gel exhibit larger E4/E6 ratio's.

Among the colour quotients values of Histosols under study, the humic fraction of Terric Histosol of Austria represents the lower values than the others, which suggests that those fractions of this soil are more aromatic and highly humified than the other Histosols. The lower ratio of colour quotients as well as higher value of specific extinction of GHA in Dystric Histosol of Bangladesh suggest that this fraction appears to be highly polymerized and has undergone more humification.

It is interesting to mention some similarities in Kd position of different non-humic materials among the studied gross humic fractions in some cases. These similar Kd positions and their corresponding fractions, as shown in figures 1, 2 and 3, are:

FA I and BHA I at Kd = 0 in Terric Histosol of Austria,
FA I and BHA I at Kd = 0 in Eutric Histosol of Austria,
FA I and GHA I at Kd = -0.05 and FA III and BHA III at Kd=1.05 in Dystric Histosol of Bangladesh.

This situation might show similarities not only in molecular size but also in the chemical nature of these non-humic fractions; however, this necessitates further investigation.

As can be seen from table 3, a relatively lower amount of 'pure' humic substances corresponding with higher contents of non-humic impurities is observed in Eutric Histosol of Austria (61:39) and Dystric Histosol of Bangladesh (60:40) as compared to Terric Histosol of Austria (64:36). This situation might be associated with the prevailing intermittent water saturated condition for most parts of the year, for which the decomposition process is supposed to be hampered. In general, the ratio of humic to non-humic materials in soil is 67:33 (DANNEBERG and ULLAH, 1982; SCHNITZER and SCHULTEN, 1998). A lower ratio was reported by DANNEBERG et al. (1983) in the highly humified compost and by GERZABEK and ULLAH (1989) in a rice soil of Bangladesh.

4 Conclusion

The humic systems of the three organic soils under study are quite similar to each other. Obviously they constitute a special 'type' of humic system, characteristic for organic soils and well distinguishable from other 'types'.

DANNEBERG and SCHMIDT (1978) and later DANNEBERG and ULLAH (1982) investigated the humic system of chernozems. A high content of GHA was the main characteristic of this 'type' of humic systems and constitutes the main difference to the humic systems of organic soils. Further, the GHA of chernozems seem to be higher in particle size than that of organic soils, since they show a higher part of material excluded from the column.

Concerning the humic systems of composts of different origin and of different degree of maturity sufficient data have accumulated for a proper characterization (DANNEBERG, 1981; DANNEBERG et al., 1983; MOHAMAD et al., 1993). The main characteristic of this 'type' of humic systems is a high content of non humic substances in the extracts, which decreases with increasing maturity of composts but normally remains higher than in humic systems of soils. Humic systems of organic soils have, as shown in this study, a content of non humic substances near to that of soils.

Comparable data of the humic systems of cambisols have been published by MOHIUDDIN and DANNEBERG (in print). These show the humic systems of cambisols and of organic soils to be somewhat similar, both being dominated by BHA. The main difference is the total amount of extracted coloured material, being at least two times higher for organic soils than for cambisols.

Most of the mentioned features leading to a proper distinction of several 'types' of humic systems can be deduced from only optical analysis, not necessarily requiring chromatography. The results of chromatography in all cases confirm the optical data. Chromatographic analysis is required for the determination of specific extinctions of 'pure' humic substances as typical constants.

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