

HUMIC ACIDS FOR MEDICAL USE: 1. UNDERSTANDING THE INFLUENCE OF PEAT FORMATION ON HUMIC ACID QUALITY

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SUMMARY

Humic acids are potent agents against certain types of human pathogenic viruses such as herpesviruses. However, their ill-defined chemical structure seriously hampers structure-effect studies and has so far prevented their wide spread use. Here we present on-going work to isolate high quality humic acids suitable for medical purposes from peat, i.e. with a high content of active compounds. We study the influence of peat formation variables on the chemical and antiviral characteristics of humic acids. In this abstract, data obtained from peat samples from different depths of the *Dierhäger Moor*, a raised bog on a terrestrialisation mire (fig. 1), are presented.

KEYWORDS: Humic acids, peat formation, palaeoecological analysis, organohalogen compounds.

INTRODUCTION

Humic acids, a class of naturally occurring organic polyelectrolytes, have not gained widespread use in the treatment of virus diseases, despite their well-known antiviral potential. This obvious contradiction is based in their structure, which is generally assumed to consist of a collection of molecules containing aromatic moieties connected by oxygen, nitrogen, or carbon bridges. (Stevenson, 1994) This ill-defined structure renders detailed structure-effect relationship studies virtually impossible. The situation is complicated further by the fact that basic properties of humic acids (elemental composition, molecular weight, functional group content, etc.) differ between humic acids obtained from different sampling sites and hence depend on the conditions of peat formation. Therefore it is urgent to establish how the major factors of peat formation (parent material, pH, trophic status, contaminant input, temperature, soil horizon, hydrogenetics) influence the antiviral and chemical characteristics of the formed humic acids (yield, degree of humification, cation exchange capacity, functional group content, molecular weight distribution, toxic impurities).

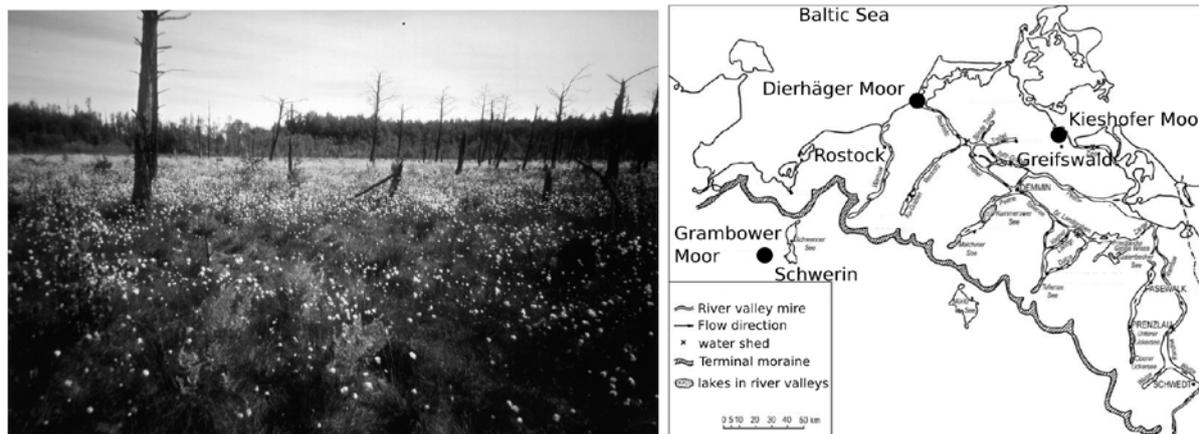


Figure 1: Dierhäger Moor, NE Germany (map after: Succow & Joosten (2001)).

MATERIALS AND METHODS

Chemicals

Solvents used for the extractions were of synthesis grade. Water ($\sigma < 5.7 \mu\text{S/m}$), used for HPLC/SEC, or humic acid isolations, was purified using a *SG Reinstwassersystem Typ Clear UV*. Organic solvents used for HPLC/SEC were of HPLC grade or better. Other chemicals were of analysis grade. All chemicals were used as received. *Sampling* - Peat samples from the *Kieshofer Moor* (0-2 m), and the *Dierhäger Moor* (0-3 m) have been obtained. In the case of the *Kieshofer Moor*, the yields of humic acids were very low. A core of the upper 2 m of the *Dierhäger Moor* was divided into 17 samples that were used for chemical and for palaeoecological analysis, alternatingly (table 1). The sampling depths and basic chemical and compositional parameters of the crude peat are given in table 1.

Table 1. Basic chemical parameters of the analysed peat samples. Ash content and organo-extractable impurities are calculated relative to dry mass.

depth/cm	pH	water	ash	org. extract	depth/cm	pH	water	ash	org. extract
30-42	4.1	86.8 %	7.0	4.8	128-140	4.0	89.1 %	1.7	5.3
48-60	3.8	84.1 %	2.5	6.9	148-160	4.0	88.9 %	1.3	6.9
68-80	4.2	89.3 %	2.0	5.0	168-180	4.1	88.4 %	1.8	7.9
86-98	4.0	91.8 %	4.1	9.5	187-199	4.2	89.7 %	1.3	6.2
108-120	4.1	85.7 %	2.9	7.5					

Macrofossil analysis - Macrofossil samples were boiled in 5 % NaOH (10 min) and sieved into three fractions (>1 mm, 0.5 mm-1 mm, 0.2 mm-0.5 mm). Macrofossil types are expressed as absolute figures or as volume percentages (as % of the sieve residue, estimated in the following steps 0.1 %, 1 %, 2 %, 5 %, 10 %, and then every ten percent). *Organic extraction* - The freeze-dried peat sample was extracted in a Soxhlet extractor with a mixture of methyl acetate and *c*-hexane (3:2) until the extract was colourless. Residue and extract were dried in vacuum. *Aqueous extraction* - A suspension of the dried peat in water (10 ml per g wet peat) was warmed to 30°C under stirring. pH was adjusted to 9.0 with NaOH and stirred under pH control for 2 h. The sludge was separated by centrifugation (2,800 x g, 10 min), and floating particles were removed by filtration. *Humic acid precipitations with oxalic acid* - 1.0 l of the aqueous extract was warmed to 30°C, after which 0.33 l of an 0.50 M aqueous solution of oxalic acid was added. After stirring for 30 min, the precipitate was collected by centrifugation and washed with water until it was free of oxalic acid (typically 3-4x, controlled by HPLC). The residue was suspended in water and dissolved by adjusting to pH 7.0. The resulting solution was centrifuged (16,500 x g, 3 h) to remove undissolved material and then freeze-dried. *Humic acid precipitations with hydrochloric acid* - 1.0 l of the aqueous extract was warmed to 30°C and the pH adjusted to 1.5-2.0 by stepwise addition of 10% hydrochloric acid. After stirring for 30 min, the precipitate was collected by centrifugation and washed with water once. The residue was suspended in water and dissolved by adjusting to pH 7.0. The resulting solution was centrifuged (16,500 x g, 3 h) to remove undissolved material and then freeze-dried. *HPSEC/HPLC* - A Varian Prostar HPLC system equipped with autosampler, gradient pump, column oven, and diode array detector was used. HPSEC was performed on a PSS BIO MCX column (1,000 Å, 5 µm) that was calibrated using polystyrene sulfonate standards (77 k, 32 k, 17 k, 8 k, 4 k, 2 k, 1 k) (PSS GmbH, Mainz, Germany). Additionally, Alizarin S Red (320), *p*-amino salicylic acid (153) and *p*-hydroxy benzoic acid (138) were used as low molecular weight standards. A solution of 283.9 mg Na₂HPO₄, 87.7 mg ethylenediaminetetraacetic acid and 467.5 mg NaCl in 700 ml water was filtered, mixed with 300 ml of *n*-propanol, degassed, and used as eluent. NaNO₃ and [Fe(EDTA)]Cl were used as flow markers, respectively. The detection was performed at 254 nm. HPLC was performed on a LiChroCART® 250-4; RP-18 (5 µm) column (Merck). A solution of 7.80 g NaH₂PO₄ in 1.00 l water was adjusted to pH 2.8, filtered, degassed and used as eluent. The detection was performed at 202 nm.

RESULTS AND DISCUSSION

The *Dierhäger Moor* is a raised bog founded on a terrestrialisation mire (fig. 1). The palaeoecological analysis (fig. 2) showed that the vegetation development started with an Equisetum reed in a shallow lake (DMC-A) followed by a terrestrialisation with brown mosses and Menyanthes (DMC-B) and sedges (DMC-C). Later, an outspreading of Eriophorum vaginatum (cotton grass) and Ericaceae took place indicating a transition to a more or less rain water-fed system (DMC-D). The following Sphagnum peat (DMC-E) is interpreted as a peat formation of a raised bog.

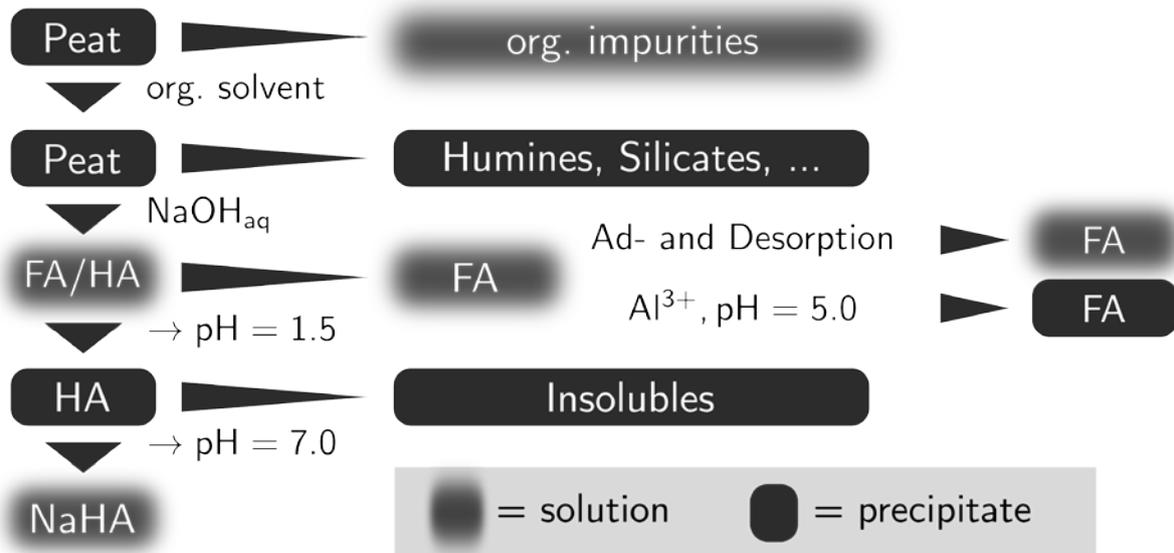


Figure 2: Macrofossil composition of the Dierhäger Moor (Analysis: D. Michaelis 2011).

The chemical composition is within the limits of humic substances reported in the literature (table 2) (Klavins, 2010). The especially low N-content of humic acids from the lower DMC-F, DMC-E, DMC-D2 and upper DMC-D1 horizons indicates a low degree of pollution originating from anthropogenic sources. The yields spread over a wide range from 2.0-16.8 % for carbon and 4.1-28.5 % for nitrogen, indicating that the conditions have differed vastly between different periods of peat formation. This is particularly apparent in DMC-D-horizon where a layer with low humic acid content is enclosed by layers exceptionally rich in humic acids. A comparison between the C/N- and C/O-ratios of DMC-C- and DMC-D2-horizon humic acids indicates, that the former are richer in nitrogen and poorer in oxygen.

CONCLUSIONS

Both palaeoecological and chemical analysis support the view that the *Dierhäger Moor* is a raised bog founded on a terrestrialisation mire. The chemical characteristics and macrofossil data obtained reveal clear differences in humic acid yield and compositions between different types of peats within the profil. A more comprehensive discussion will be presented in the poster when the residual analytical data have been obtained.

Table 2: Chemical parameters of humic acids, isolated from peat from the Dierhäger Moor. C-, N-yield and C/N, C/O, and C/H-ratios were determined for HCl-precipitated acids. Molecular weights, E₂/E₃, and E₄/E₆-ratios were determined for sodium humates obtained from humic acids precipitated with oxalic acid. C-/N-yield refer to the ratio between the carbon isolated as humic acids and the carbon contained in the peat. (n.d. = not determined yet).

depth/cm	horizon	C-yield	N-yield	M _w /kDa	M _n /kDa	C-%	N-%	C/N	C/O	C/H	E ₄ /E ₆	E ₂ /E ₃
30-42	DMC-F	2.4 %	4.1 %	3.1	0.8	54.1	3.0	21	1.93	0.96	8.3	2.7
48-60	DMC-F	7.4 %	13.6 %	6.6	0.9	55.3	1.2	52	1.87	1.22	6.8	2.5
68-80	DMC-E	8.7 %	15.1 %	6.6	0.9	54.9	1.2	53	1.84	1.18	6.3	2.5
86-98	DMC-E	7.7 %	13.4 %	9.2	0.9	55.0	1.5	44	1.88	1.12	6.6	2.6
108-120	DMC-D1	ca. 11 %	n.d.	7.4	0.9	n.d.	n.d.	n.d.	n.d.	n.d.	6.3	2.6
transition from terrestrialisation mire to raised bog												
128-140	DMC-D2	2.0 %	4.3 %	4.1	0.7	56.1	1.4	48	1.96	1.16	6.4	2.6
148-160	DMC-D2	16.6 %	28.5 %	11.5	1.0	57.2	1.0	64	2.03	1.19	5.6	2.6
168-180	DMC-D2	4.1 %	5.7 %	3.2	0.8	56.7	2.1	32	2.08	1.03	6.7	2.8
187-199	DMC-C	3.6 %	5.0 %	3.1	0.7	57.3	2.6	26	2.18	1.02	7.0	2.9

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