

## CHEMICAL PROPERTIES CONTRIBUTING TOWARDS THE ANTIMICROBIAL ACTIVITY OF THE MAIN PEAT-FORMING MOSSES

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### SUMMARY

Peat properties influence peat-forming plants, grasses, shrubs and first of all moss (commonly different *Sphagnum* species). Other studies have suggested that biologically active ingredients in mosses determine their stability in respect to degradation and influence moss peat properties in northern bogs. We have studied the presence and concentration of polyphenolics in the composition of peat-forming mosses, their elemental composition (C, H, N, O) and major spectral properties (FT-IR, <sup>13</sup>C NMR) in respect to their antimicrobial activity. Significant differences among different *Sphagnum* and other moss species were found. Substances (possibly of a phenolic origin) that influence the scavenging activity of free radicals can be considered as a factor providing significant contribution towards the antimicrobial activity of the studied mosses.

**KEYWORDS:** peat, mosses and bryophytes, humification, antimicrobial activity

### INTRODUCTION

Peat properties are largely influenced by peat-forming plant properties, grasses, shrubs and mosses, among which different *Sphagnum* species play a special role. It has been hypothesized that biologically active ingredients in mosses determine their stability in respect to degradation and influence moss peat properties greatly in northern bogs. Despite the abundance of peat-forming mosses, the number of studies on their composition is low, especially in respect to basic structural elements. Trace element composition of mosses – considering the impact of anthropogenic pollution and possibilities to use bryophytes as bio-indicators of human pollution – as well as peat profiles for reconstruction of pollution history has been studied relatively widely. At the same time, the number of studies using methods common in structural characterization of biomaterials, such as pyrolysis-gas chromatography (Py-GC/MS) or <sup>13</sup>C NMR, is low and the information is scattered (Kracht and Gleixner, 2000).

Carbohydrates are considered to be among the major ingredients of peat-forming mosses, whereas the percentage of lignins has been found to be small, despite controversies also in this respect. It has been suggested that carbonyl groups which contain carbohydrates could be a major factor determining biological stability and ability to bind amino acids in the structure of proteins, thus inactivating them (Bland *et al.*, 1968). However, other studies pointed at evident mistakes of these statements and suggested that the major structural unit included

uronic acid residues (Hajek et al. 2011). In several studies of peat-forming mosses, a number of biologically active substances have been found, for example, phenolics sphagnum acid as well as lipids, and even antibiotics (Rauha *et al.*, 2000). Bryophyte extracts have demonstrated antimicrobial, antifungal, cytotoxic and many other kinds of biological activities (Asakawa, 2007).

The aim of this paper is to analyze the elemental composition and antimicrobial activity of peat-forming mosses – the major living material forming northern bogs

## MATERIALS AND METHODS

### Samples

Fresh moss material was collected in the vegetation season in 2011. Until analysis the moss samples were stored at -20°C; before analysis the material was cleaned and dried to constant weight at room temperature.

### Characterization of mosses

Elemental analysis (C, H, N, S and O) was carried out in triplicate using an Elemental Analyzer Model EA-1108, and the found values were normalized with respect to ash content. Ash content was measured after heating 50 mg of each sample at 750°C for 8 h. Fourier transform infrared spectra were collected using Nicolet AVATAR 330 spectrophotometer in KBr pellets. UV/Vis spectra were recorded on a Thermospectronic Helios  $\gamma$  UV spectrophotometer in a 1-cm quartz cuvette. Fluorescence spectra were recorded using a Perkin Elmer LS 55 fluorescence spectrometer, on aqueous solutions of each sample. <sup>13</sup>C NMR spectra were recorded at 75.47 MHz with a Bruker DRX300 NMR. The Py-GC/MS analysis was performed using a micro-furnace Frontier Lab Micro Double-shot Pyrolyser (Py-2020iD). The final pyrolysis temperature was 500°C, heating rate 600°C/s. The pyrolyser was directly coupled with a Shimadzu GC/MS-QP 2010 apparatus fitted with a capillary column RTX-1701 (60 m x 0.25 mm) with a 0.25  $\mu$ m stationary phase film. The injector temperature was set at 250 °C, ion source 250 °C with EI of 70 eV, the MS scan range m/z 15-350, using He as carrier gas at a flow rate of 1 ml/min and the split ratio 1:30. The weight of the sample was 1.00-2.00 mg. The oven program was 1 min isothermal at 60 °C, then 6 °C/min to 270 °C, and finally 10 min at 270 °C. The identification of individual compounds was performed on the basis of a GC/MS chromatogram using Library MS NIST 147.LI13.

## RESULTS

In comparison with peat, depending on the studied species, there was significant variation in the elemental composition of peat-forming bryophytes. The elemental compositions of the studied bryophytes were: C – 40-42%; H – 5.5-5.7%; N – 0.5-1.1%; S – ~0%. The O content within a range of 49-53% was determined by mass balance. For comparison, the elemental compositions of peat were: C – 49-57%; H – 4.6-5.7%; N – 1.6-2.8%; S – 0.5-1.5%; ash – 0.1-1.2%. The O content: 32-42%. In peat, C and H concentrations increased with depth. N concentrations decreased with depth, whereas S concentration was very variable down the profile.

The UV-Vis spectra of the examined mosses were not very informative and rather reflect the presence of photosynthetic pigments. Fluorescence spectra in emission scanning mode were used to obtain some information on the condensed aromatic structures in mosses that contain fluorophoric structures. All the fluorescence spectra produced have similar characters, showing maximum peaks at around 450 nm and weak shoulder curves at about 480 nm. The Fourier transform infrared spectra (Fig. 1) of the studied mosses were similar to one another in the main position of adsorption, while differences of various entities were apparent in the relative intensity of some bands, depending on origin of the sample. The FT-IR spectra of the analyzed HAs can be divided into regions depending on informativity and the presence of important functional groups. Absorption bands in the 3600-2800  $\text{cm}^{-1}$  spectral region were very broad; absorbance in this region is determined by the presence of  $-\text{OH}$  groups. Sorption at the wavelengths 2950 and 2850  $\text{cm}^{-1}$  identified the presence of  $\text{CH}_3-$  and  $\text{CH}_2-$  groups respectively. Typical intensive sorption lines were common for the region around 1700  $\text{cm}^{-1}$  (1725-1700  $\text{cm}^{-1}$ ), which is characteristic for carbonyl groups in aldehydes, ketones and carbonic acids. The actual sorption maximum greatly depends on the conjugation degree, presence of substituents and hydrogen bonding. In the spectral region 1690-1500  $\text{cm}^{-1}$  it was possible to identify the sorption maximum of amide bonds (1640-1620  $\text{cm}^{-1}$  and 1550-1540  $\text{cm}^{-1}$ ). In the region 1625-1610  $\text{cm}^{-1}$ , the sorption indicated the presence of aromatic  $\text{C}=\text{C}$  and carbonyl groups, and quinones. At the wavelengths 1470-1370  $\text{cm}^{-1}$ , there were bands typical for  $\text{C}-\text{H}$  and  $\text{O}-\text{H}$  bonding and sorption maximums typical for  $\text{C}-\text{O}$ . For the wavelengths  $< 1000 \text{ cm}^{-1}$  fingerprint patterns were evident. Sorption at 1080  $\text{cm}^{-1}$  shows OH deformation or  $\text{C}-\text{O}$  stretch of phenol and alcohol OH groups, and 1040  $\text{cm}^{-1}$  indicates  $\text{C}-\text{O}$  stretch of polysaccharide components. Changes in the relative intensities of sorption maximums in the FT-IR spectra (Fig. 1) indicate the character of changes during the peat development and humification process, especially if compared with the IR spectra of peat-forming plants. The character of changes of sorption between peat and bog plant HAs in the wide line around 3400  $\text{cm}^{-1}$  was remarkable. The absorption band centered at 1720  $\text{cm}^{-1}$  (indicating acidic carboxylic groups) was clearly stronger for humic acids from peat with higher degree of decomposition, especially compared with the intensity of the sorption line in bog plants. The absorption band centered at 1040  $\text{cm}^{-1}$  ( $\text{C}-\text{O}$  stretching, e.g. in polysaccharides) was equally weak for humic acids, even if their decomposition degree was low.

Table 1 lists the components in the bryophyte pyrolysates. The pyrolysates of the studied *Sphagnum* species are very similar, being dominated by simple phenolic compounds. The dominant compounds are: phenol, 4-ethenylphenol. Their origin is thought to be a tannin-like polyphenolic biopolymer with 4-isoprenylphenol, being at least partially bound to it. The importance of cellulose in *Sphagnum* structures is indicated by the high abundance of polysaccharide products; at the same time, no lignin markers were detected. Abundant polysaccharide products confirm the importance of cellulose in the structural make-up of mosses.

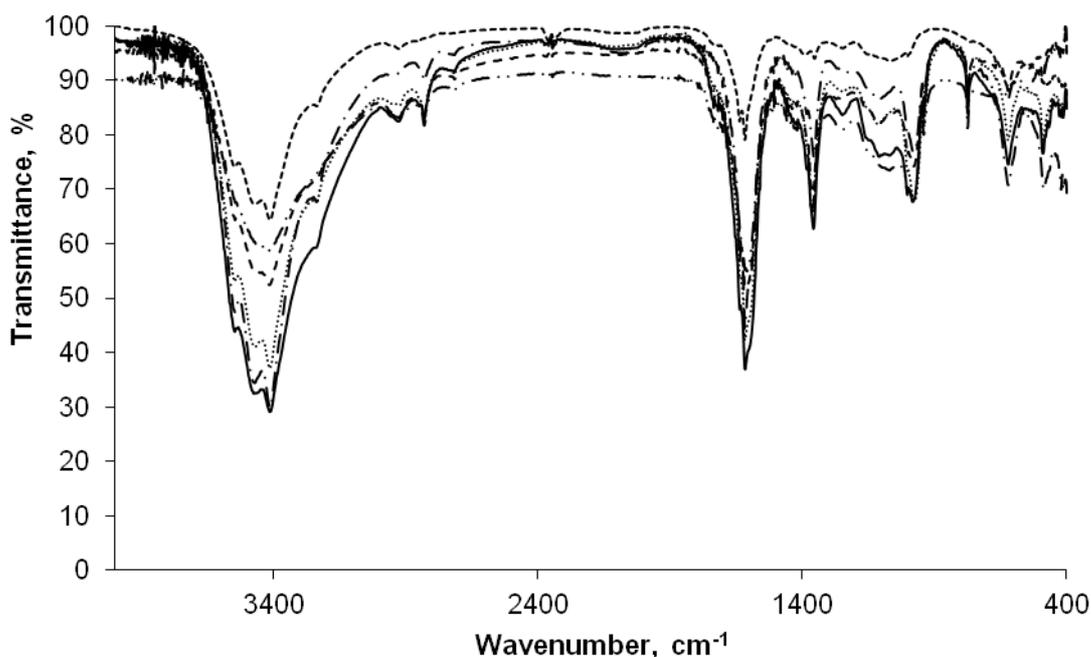


Fig. 1. FT-IR spectra of peat-forming plants

— *Pleurozium schreberi*      - - - Heather      - · - Sedge  
 - - - *Sphagnum magellanicum*      - *Sphagnum angustifolium*  
 ······ *Hylocomium splendens*

Table 1. Relative abundance (%) of the main groups of pyrolysis products of mosses

Origin*	SS	SZ	SG	SM	PA
MA	15.84	17.72	16.87	14.26	14.53
C	7.72	6.38	5.58	9.12	3.36
Ar	3.06	3.78	4.26	3.48	1.16
L	0	0	0	0	1.02
Lp	1.35	1.10	1.47	1.24	3.98
N	0	0	0.02	0	0.11

SS- *Sphagnum capillifolium*, SZ- *Sphagnum angustifolium*, SG- *Sphagnum girgensohnii*, SM- *Sphagnum magellanicum*, PA- *Plagioschila asplenoides*

\* - MA – multi-origin aliphatic compounds with C<6; C – furanes originated from carbohydrates, pyran and cyclopentene derivatives; Ar – aromatic compounds (except methoxylated phenols); L – methoxylated phenols; Lp – compounds originated from lipids C>6; N – N-bearing compounds

We have studied the presence and concentration of polyphenolics in the composition of peat-forming mosses in respect to their antimicrobial activity. The antimicrobial activity was tested on different microorganism species: *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and others. Total concentrations of phenolics were analyzed in moss species common for Northern Europe, and significant amounts of phenolics, evidently also contributing towards radical scavenging activities, were found as a result.

## CONCLUSIONS/DISCUSSION

Today, not only peat but also mosses have increasing importance as sources of valuable substances. Therefore, studies of their composition are of importance, for they provide better understanding of peat development processes. Within this study, using different analytical methods, common peat-forming mosses were studied in respect to peat formed during their decay. It can be concluded from the study that the phenolic substances of low molecular weight (especially considering the low concentration of lignins in mosses) are amongst the major groups of substances that influence biological activity of mosses. Significant differences among different Sphagnum and other moss species were found. Substances (possibly of a phenolic origin) that influence the scavenging activity of free radicals can be considered as a factor providing significant contribution towards the antimicrobial activity of the studied mosses.

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