Hair ice

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Abstract Hair ice grows on the surface of wet, rotten hardwood at temperatures slightly below 0°C. The attraction of winter-active insects as well as the formation of very thin fibres at the beginning of hair-ice melting is presumably attributed to inherent organics. First analysis of melted, brownish hair-ice liquid confirms a high total organic carbon content in the mg/l range. By coupling of Ultra Performance Liquid Chromatography with mass spectrometry (UPLC-MS) a chromatogram with non-resolved peaks was received. Averaged spectra different retention time show peaks spreading over a mass range 100-650 Da with favored intense, odd-numbered peaks, similar to dissolved organic matter (DOM). In the next step, the desalted/ concentrated sample was measured in an Electrospray Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (ESI-FTICR-MS). The received mass list was converted to correspondent formulae, plotted in a van Krevelen diagram and classified as lignin as main ingredient by comparison with two references.

Introduction

Hair ice is a rather unknown, natural phenomenon. In contrast to generally known frost needles, originating from atmospheric water and expanding e.g. from plant surfaces in all directions, hair ice grows from the basis of wet, rotten hardwood. The hair-like, flexible, linear structures may reach up to 10 cm in length without any ramifications whereas its diameter is near 0.02 mm. Hair ice appears to be related to the biological activity of a fungus mycelium within the wood.

Hair ice can attract winter-active Collemboles (snow flea, *Isotoma nivalis).* At the beginning of hair-ice melting a very thin fibre becomes apparent, which carries brownish pearl-like water drops. Therefore, it is supposed that organic substances are inherent, which could possibly act as freezing catalyst as well as recrystallization inhibitor. The aim of this work was the chemical characterization of organic substances contained in hair ice.

Experimental

Start of the analysis was TOC and TN determination of melted hair ice (locality Wiliwald/ Moosseedorf, Switzerland, 500 m NN), followed by several screening methods for identification of different distinct substance classes: proteins (e.g. Anti-Freeze Proteins) and lipids by Electrospray Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (ESI-FTICR-MS), small carboxylic acids (e.g. known in root exudates) by Capillary Electrophoresis (CE) and small volatile substances by (head space) Gas Chromatography-Mass Spectrometry (HS GC-MS).

Next, more general screening was made by coupling of Ultra Performance Liquid Chromatography with mass spectrometry (UPLC-MS).

After desalting/ concentration of the sample by solid-phase extraction (SPE, C18 hydra, Machery & Nagel) the sample was again analyzed by flow injection into an ESI-FTICR-MS (LTQ-FT Ultra, ThermoFisher Scientific), now under modified conditions concerning characterization of complex samples in comparison to the first measurements. Main recording parameters were magnetic field strength: 7 T, resolution: 400.000 at m/z 400 Da, AGC target: 5E5, data averaging: 7 scans with 50 µscans each, syringe pump flow: 8 µl/min, transfer capillary: 275°C, mass range: 200 – 1000 Da.

For comparison a snow sample from same region was partly included in the investigations.

Guttation droplets from a tree fungus (*Fomitopsis pinicola*, locality Brachbach/ Sieg, Germany) will complement the investigations.

Results and Discussion

First analyses of the melted hair ice show a high total organic carbon (TOC) value of 235 mg/l in contrast to 11 mg/l total nitrogen. Most of the inherent nitrogen (70 %) exists thereby as ammonium. Screened by different (mass spectrometric) methods, no evidence was for the initially expected organic substances like proteins, lipids, small volatile substances or carboxylic acids.

By UPLC-MS, a non-resolved chromatogram from a melted hair-ice sample was received. Averaged spectra from different regions are looking similar with a broad peak spreading over the mass range 100-650

Da with favored intense, odd-numbered peaks. Such spectra are similar to dissolved organic matter (DOM), known e.g. from terrestrial and marine waters, soil extracts or aerosols.

The second FTICR-MS measurement with conditions commonly applied for complex samples shows a bimodal distribution (maxima at 500 and 610 Da, respectively), typical for aqueous-methanolic solutions with additional four outstanding peaks [Figure 1]. The detailed evaluation of this spectrum revealed the typical presence of molecular families, however with strong underrepresented (one) nitrogen containing molecules (their signals are still weaker than the isotopic peaks of prominent CHO peaks).

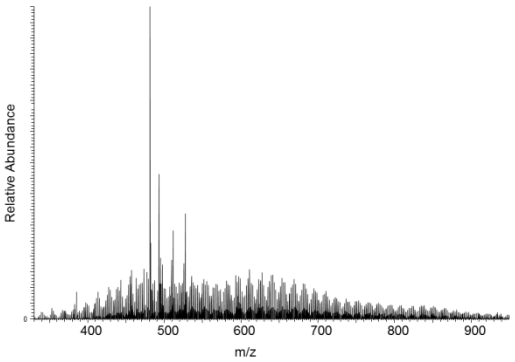

Figure 1. High-resolved hair-ice spectrum in the negative mode

After automatic post processing based on Scilab for assignment of the data, the received sum formulae were graphically presented in Kendrick and van Krevelen plots. By comparison with two references (1,2), which presented various biopolymer substance classes in such plots, lignin beside tannin could be detected as the main hair-ice components [Figure 2]. In contrast, snow samples don't show any signals.

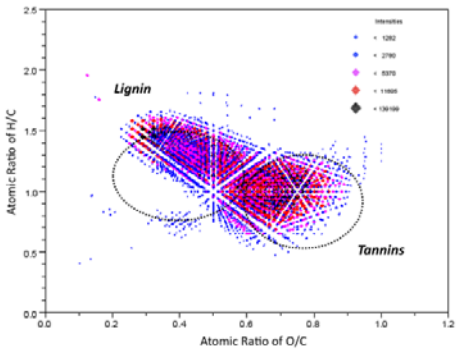

Figure 2. Van Krevelen Plot of the CHO compounds from hair ice for peak intensities > 600 counts

The four prominent peaks, each CHO compounds with 24 carbon atoms are supposed/ explained as more stable lignin fragments.

In summary, we conclude that hair ice is caused by incomplete wood destruction by fungi. Especially tannin beside lignin as more difficult degradable material is for several organism also toxic. Therefore it is concluded that fungi eject these substances from wood. Under the right meteorological conditions (temperature, wetness) they possibly act as numerous crystallization nuclei and simultaneously as recrystallization inhibitors under formation of such flexible, linear structures.

Guttation droplets of a tree fungus show a different spectrum – predominant single peaks at each nominal mass in the mass range 439 – 505 Da (multiple oxygenated hydrocarbons with C-number 27, each) [Figures 3, 4]. In contrast to hair ice, in the positive mode a lot of predominant (single) nitrogen containing peaks were additionally detected.

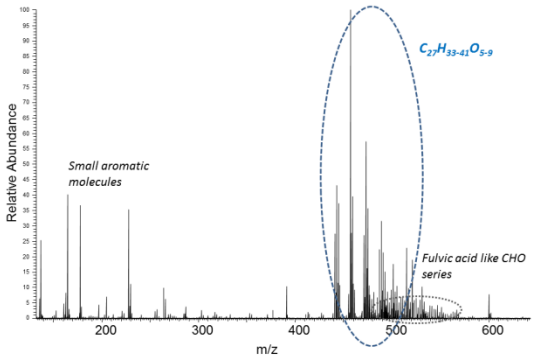

Figure 3. High-resolved spectrum (negative mode) of guttation droplets

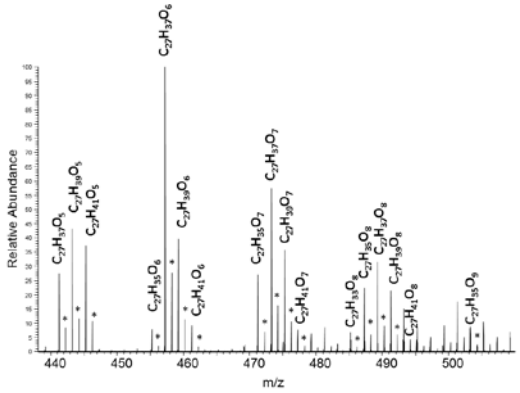

Figure 4. Cutout of the high resolved spectrum (negative mode) of guttation droplets

Identification of the hair-ice relevant fungus/ fungi out of numerous to date found and assigned species is under progress.

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