Detection of active moulds on historical objects by means of the HS-SPME GC-MS method

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Biodeterioration of historical objects

Historical objects and art items are mostly made of organic materials which are susceptible to biodeterioration caused mostly by moulds. The objects with the deterioration symptoms should be disinfected. However, historical objects are sensitive to fumigation methods due to their organic composition and because they are already naturally aged. Thus, the decision for disinfection of objects should be taken after confirmation that the moulds are indeed active. This may be investigated with classical microbiological methods, but they are time-consuming, labour-intensive, require a contact with the surface of objects and give information more about the microbial contamination of the surface rather than the presence of active moulds. An alternative method of active moulds investigation may be the analysis of volatile metabolites that are emitted by moulds at every stage of their development. These metabolites are called microbial volatile organic compounds (MVOCs). They can be detected when moulds grow both on the surface and inside the material as they easily diffuse of the objects.

Materials & Methods

Microbial Broths

- **The modified Weary – Canby's broth (W&C)**
- **The modified Czapek – Dox broth (Cz&D)**

They did not contain sources of neither carbon nor nitrogen or sulphur, so that the main nutrient for moulds had to be organic material that was placed on the surface of the broth

The broths were prepared in the shape of a slope (using 5ml of broth) inside 20 ml sterile vials usually used for the headspace sampling

Materials

The model samples that were chosen to resemble historical objects were the following organic materials

- Whatman cellulose filter paper
- natural degummed silk
- natural wool fabric
- calf parchment

They were disinfected several times to degraded the materials and make them more similar to historical objects

They were cut into samples of 4 cm^2 that were placed on the surface of broths in vials

Historical sample

fragment of the leather binding of an 18th century old print

Disinfected, cut into two pieces, each of which was placed in sterile Petri dishes, without a broth, and then moistened, one piece was inoculated with mould

Fungi

- *Alternaria alternata* IHEM:18586
- *Aspergillus niger* IHEM:25949
- *Chaetomium globosum* IHEM:6552

The following set of samples were prepared:

Species	organic material	broth	Incubation and sampling
Alternaria alternata	silk	W&C	The incubation was carried out at 25°C. The MVOCs emitted by moulds were captured inside the vials to be sampled after that with the SPME - headspace method (fig. 1)
Aspergillus niger	calf parchment	Cz&D	
Chaetomium globosum	Whatman paper	Cz&D	
Chaetomium globosum	wool	W&C	
Aspergillus niger	leather		The incubation was carried out at 23°C. The SPME fiber was placed in a partly open Petri dish and then pushed out of the needle so that it was positioned just over the leather with or without mould (fig. 1)

Headspace - SPME

The so-called sandwich type (DVB/CAR/PDMS) SPME fibre was used for sampling of MVOCs. The volatiles collected in the vials were extracted on a fibre during 24 hours of headspace sampling. After that, they were analysed using GC-MS method. The same procedure of sampling was used for analysing the blanks (samples without moulds) as well as for a calibration procedure. The MVOCs emitted by *A. niger* growing on a historical leather sample were collected onto the fiber for 12 hours

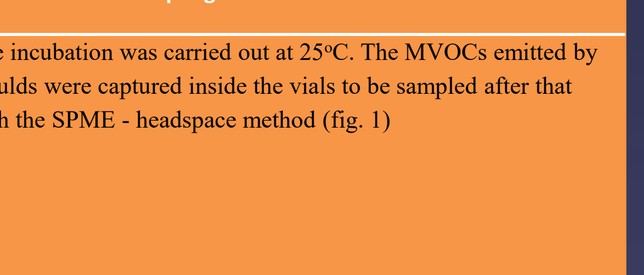
CG/MS analysis

The analysis of the MVOCs was carried out using a gas chromatograph (Trace 1310) coupled with a single quadrupole mass spectrometer (ISQ), both from Thermo Scientific Inc., (USA). RXi-5MS capillary column of 30 meters, 0.25mm ID, and 0.25µm of film thickness (Restek, USA) was used for separation.

The temperature program of the analysis:

The parameters of the MS detector:

MS transfer line 250°C, electron ionization (EI) with 70eV, and a 33 – 450 m/z mass range was used for detection in total ion current mode (TIC). Reference libraries (NIST/EPA/NIH Mass Spectral Library) available in the NIST MS Search program (version 2.0), were used to identify the MVOCs.



Results

A qualitative analysis of the chromatograms was carried out using the AMDIS program (Automated Mass Spectral Deconvolution and Identification System, version 2.70, may 13. 2011). The results indicated that each examined species of mould emitted more than 140 VOCs during their growth on the model and historical samples (figure 2). These volatiles belong to several groups of the organic compounds including: hydrocarbons, aromatic hydrocarbons, alcohols, aldehydes, ketones, organic acids, ethers, esters, terpenes, sesquiterpenes, organic compounds containing sulphur or nitrogen. Nevertheless the most important part of qualitative analysis was to identified long chain alcohols and ketones like: 1-octen-3-ol, 3-octanol, 1-octen-3-one, 3-octanone, 2-octanone (so-called C_8 complex), 2-hexanone or 2-heptanone as well as terpenes and sesquiterpenes like: limonene, bisabolene. These compounds are already reported in the literature as the indicators of active growth of moulds. Additionally it was crucial to find whether the profiles of MVOCs emitted by studied mould contained volatiles with the sulphur atom. The presence of sulfur containing compounds confirms that fungi actually did metabolize the specific protein material because fibroin, keratin and collagen contain amino acids having sulfur atoms. The results of the qualitative analysis have been presented in Table 1.

Figure 1. Sampling of MVOCs: a) from the vials with moulds inoculated on various samples a) just over the leather sample inoculated with *A. niger*

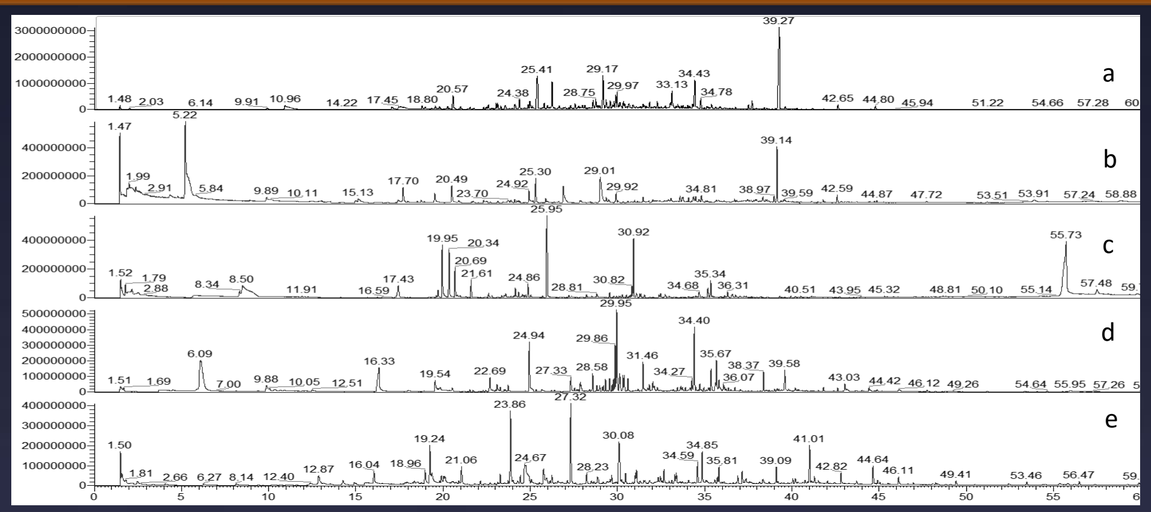


Figure 2. Chromatograms obtained for MVOCs emitted by molds growing on the various types of substrate: a) *Alternaria alternata*/silk/W&C; b) *Aspergillus niger*/parchment/Cz&D; c) *Chaetomium globosum*/paper/Cz&D; d) *Chaetomium globosum*/wool/W&C; e) *Aspergillus niger*/historic leather

Table 1. Qualitative and quantitative analysis of chromatograms obtained for MVOCs emitted by selected species of molds growing on the model material samples and the historic sample.

species/samples/broth	1-octen-3-ol, 3-octanol, 1-octen-3-one, 3-octanone, 2-octanone	2-hexanone 2-heptanone	terpenes sesquiterpenes	Compounds with sulfur	Total concentration of MVOCs [µg·m^{-3}]
***Alternaria alternata*/silk/W&C**	++	+	+++	+++++++++	14.17 ± 4.59
***Aspergillus niger*/parchment/ CzD**	+++	++	+++++++++	+++++	23.75 ± 9.66
***Chaetomium globosum*/paper/CzD**	+++++	++	++++	++++++++	10.78 ± 6.28
***Chaetomium globosum*/wool/W&C**	++	++	++++	++++++++	51.36 ± 2.33
***Aspergillus niger*/historic sample**	+++	++	++++++	+++	19.80 ± 6.84

+ indicates that the volatiles defined in the head-row of the table was identified in the chromatogram.

The amount of +'es indicates how many volatiles from the list were identified.

MDL = 0,496 µg· m^{-3}
MQL = 1,407 µg· m^{-3}

Conclusions

The obtained data indicate that all the species of mold emitted volatile compounds belonging to the C8 complex, regardless of the kind of material and microbiological broth on which they were cultivated

In all of the analyzed cases, fungi emitted ketones with six- and seven-carbons

In all of the analyzed cases, fungi emitted terpenes and sesquiterpenes

All of these volatiles are well known in the literature as the indicators of active growth of moulds. The obtained results support the hypothesis that the metabolic activity of molds growing on historical objects can be confirmed or denied based on the detection of the aforementioned three groups of MVOCs. It is a priceless tool for the conservators which have to decide about disinfection of objects.

The level of MVOC emission, measured both in closed and open system, was low; however, it was above the detection limit in all of discussed cases.

A. niger growing on the historic sample emitted three times smaller quantity of MVOCs compared with *Ch. globosum* cultivated on wool. The level of MVOC emission determined for *A. niger* growing on the historic leather sample was 0.83 times the emission that this fungus achieved when decomposing parchment constituents. It should be remembered, however, that in this measurement, sorption of MVOCs emitted by the mold was carried out in an open system and the sorption time was shortened. In spite of this open sampling, MVOC emission was above the method detection limit; additionally, it was higher than was measured for some other sets of mold – sample when samples were collected for 24 hours from closed systems, tightly screwed vials

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