MICROBIAL VOLATILE ORGANIC COMPOUNDS (MVOC) AS INDICATORS FOR FUNGAL DAMAGE

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ABSTRACT
Laboratory experiments demonstrated that the production and emission of volatile secondary metabolites by molds is strongly dependent on various influencing factors. A major influencing factor is the substrate. In the case of indoor environment construction material serves as substrate, so the MVOC spectrum encountered mainly depends on the infested site. Differences concerning the MVOC emission exist even between the various strains of the same type of fungus, which was proved by the present study with different strains of *Aspergillus versicolor (wild strains versus certified laboratory strains)*. Even the emitted MVOC spectrum was sometimes not constant when experiments were repeated. Field studies in 28 flats showed that typical MVOC were found in housing with considerable mold infestation in the µg/m^3 range. We found a correlation between humidity and a total of 8 selected MVOC. However, smoking also correlated to the concentration of the "MVOC" 3-methylfurane, so that interpreting the detection of "typical" MVOC should basically still be done with great care.

INDEX TERMS
Microbial volatile organic compounds (MVOC), indicator of fungal damage, emission profiles, substrate dependency, quality of indicators

INTRODUCTION
The growth of molds, bacteria and other microorganisms is frequently found in different parts of buildings. Almost all usual materials used for construction or furnishings can serve as a substrate. These include wallpaper, flooring, textiles and wood. For hygienic reasons, the growth of microorganisms in indoor spaces must always be suppressed or eliminated, since microorganisms emit a number of products whose negative impact on the health of the resident is known or has not yet been definitively clarified. This includes the spores of molds, which may cause allergies, asthma or contain mycotoxins. Microorganisms also produce many volatile organic compounds (MVOC) that sometimes provoke an unpleasant smell. Although even with strong microbiological infestation only very low concentrations of MVOC were measured, often only in the range of fractions of 1 µg/m^3, they can still contribute to poor air quality and/or sick building syndrome due to their partly very low odor threshold. Their toxicological relevance, however, has not yet been definitely clarified.

Various studies have shown that there is frequently no significant difference in the spore concentrations between microbiologically infested and non-infested buildings. This occurs especially when the fungal damage is concealed, since fungal spores are usually too large to

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penetrate e.g. through carpeting or insulating material. Therefore often no alarming concentrations of airborne spores or remarkable genera are observed even when mold damage covers a surface of several square meters. All the more suitable are the volatile compounds produced by microorganisms as an indication of concealed infestation, since these compounds easily diffuse through almost all construction materials. Therefore, MVOC are more frequently accepted as valuable indicators.

Many questions remain unanswered with regard to evaluating a measured MVOC spectrum, although some promising approaches have been made (Keller, 2001; Lorenz, 2001). Knowledge about emitted MVOC spectra is usually gained from laboratory experiments. At first such trials have to prove that the systems are free from blank values (Keller, 2001). It has also been recognized that the formation of so-called secondary metabolites is very strongly dependent on the substrate. Therefore trials, in which molds are cultured on pure nutrient agar or in which nutrient broth is added to construction materials as a "jump start", must be interpreted regarding the results with extreme care or have to be ignored at all.

METHODS

Experimental set

We used two-liter glass incubation chambers. All glass parts were at first cleaned with 2-propanol and acetone and sterilized by autoclave at a temperature of 121°C, thereby eliminating all volatile organic residues (VOC). 5 typical indoor building materials (see table 2) were inoculated with spores of fungi and placed in the incubation chambers under sterile conditions. After one week, thermal desorption tubes were connected directly at the outlet of the chambers. Air samples were taken in intervals during the second week. The supply air was purified by activated charcoal (250 mg) to avoid blank values. In order to prevent laboratory contaminants from being sucked into the chambers, the purified air was pumped into the incubation chambers and through the sampling tubes. All connections were made of glass, metal connectors had Teflon fittings.

MVOC monitored

The air samples of the second week were analyzed for the following compounds by GC/MS with selected ion monitoring (SIM):

dimethylsulfide, 2-methylfurane, 3-methylfurane, 3-methyl-2-butanone, 3-methyl-2-butanol, 2-pentanone, 2-pentanol, 3-methyl-1-butanol, pyrazine, 2-methyl-1-butanol, 2-methyl-1-butanol, dimethyldisulfide, 1-pentanol, 2-butanonoxime, 2-hexanone, 3-methoxy-1-butanol, furfurale, dimethylsulfoxide, 1-hexanol, 2-heptanon, 1-heptanol, 1-octen-3-ol, 3-octanone, 3-octen-2-ol, 3-octanol, 2-n-pentylfurane, 2-octanol, 2-ethyl-1-hexanol, cis-3-octen-1-ol, trans-2-octen-1-ol, 1-octanol, 1-nonanol, 4-hydroxyanisole, 1-decanol, 2,4,6-trimethylbenzaldehyde, diphenylsulfide.

MVOC spectrum in dependence of the building material

The following materials were inoculated with *Aspergillus versicolor* # 1943, a strain delivered by the German collection of microorganisms and cell cultures (DSMZ):

- green gypsum board (impregnated with a fungicide!)
- spruce wood
- pine wood
- wood chip wall paper

- wood chip wallpaper with a special heavy-duty glue, whose main constituents were starch ether and polyvinyl acetate.

MVOC spectrum according to different strains of one mold species

We also examined which variations occurred in the MVOC spectrum, when different strains of a certain type of mold were inoculated onto an identical substrate. The background situation here was that mainly certified strains from collections were used in most studies for standardization purposes, although it has not been proven that wild strains show the same behavior in the emission of secondary metabolites. In this study we used 2 strains of *Aspergillus versicolor* from the German collection of microorganisms and cell cultures (DSMZ, see also table 3) and 3 wild strains isolated from infestation cases.

Correlation between moisture and MVOC in moldy indoor environments

To emphasize the importance of the moisture control in indoor environments we examined the connection of air humidity and MVOC in 28 indoor spaces. Since both fungus spores, for example from outdoor air, and sufficient material serving as substrate is available in indoor spaces, moisture is normally the only limiting factor for the growth of fungi. Thus, we measured the air humidity, given in g water per m^3, and the occurrence of MVOC. A total value for MVOC was formed from the 8 compounds given in table 1; the selection is according to Lorenz (Lorenz, 2001). Only indoor spaces with a clear mold infestation were under investigation.

Table 1: A selection of MVOC according to Lorenz (Lorenz 2001) used for correlation with humidity

• 3-methylfurane	• 2-pentanol	• 3-methyl-1-butanol	• dimethyl-disulfide
• 2-hexanone	• 2-heptanone	• 1-octen-3-ol	• 3-octanone

RESULTS

MVOC spectra according to building material

Table 2 shows that only 2-ethyl-1-hexanol was produced by *Aspergillus versicolor* (DSMZ # 1943) on all five substrates. This compound, however, must also be considered as a ubiquitous VOC, which is emitted from many other (´non-viable`) materials. This strain of *Aspergillus versicolor* produced a variety of other MVOC, which are indicated in table 2. 3-Methylfurane, pyrazine and 2-methyl-1-butanol were emitted by *Aspergillus versicolor* on four substrates, 2-pentanol was produced on three substrates.

Table 2: Emission of MVOC by *Aspergillus versicolor* (DSMZ # 1943) on different substrates normally used as building materials

Material (substrate) / **MVOC**	Green* gypsum board	Spruce wood	Pine wood	Wood chip wallpaper w/o glue	Wood chip wallpaper with special glue**
3-methylfurane	+	++		+	+
2-methylfurane				+	
2-pentanol		++	++	+	
3-methyl-2-butanol	+			+	+
3-methyl-1-butanol	+			+	
Pyrazine	++	++	+	+	
2-methyl-1-butanol	+	++	+	+	
2-butanonoxim		+			
1-octen-3-ol	++			++	
3-octanone	+++			+	
3-octen-2-ol		++			
3-octanol	++				
2-octanol		++			
2-n-pentylfurane				++	
2-ethyl-1-hexanol	+++	++	++	++++	++
cis-3-octen-1-ol		++			

* The green gypsum board was impregnated with a fungicide.

** A special heavy-duty glue was applied, whose main constituents were starch ether and polyvinyl acetate.

+: 1 – 10 ng/week, ++: 10 – 100 ng/week, +++: 100 – 1000 ng/week

MVOC spectrum according to different strains of one mold species

In the examinations it was found that only 3 of 36 compounds were produced by 5 different isolates of *Aspergillus versicolor*. They were 3-methyl-2-butanol, 1-octen-3-ol and 2-ethyl-1-hexanol. Another 11 compounds were detected but not emitted by all 5 strains. 3-Methylfuran, pyrazine, 2-n-pentylfurane and 2-methyl-1-butanol were produced by 4 strains (see table 3).

The trials were based on at least 4 measurements, in which both parallel and independent repeat measurements were included. As a result we experienced that "reproducibility" was not always achieved at the repeat measurements, i.e., in some cases certain MVOC could not be detected in every trial round.

Table 3. Emission of MVOC by 5 different isolates of *Aspergillus versicolor*

Asperillus versicolor **MVOC**	DSMZ # 1943	DSMZ # 63292	Wild strain 1	Wild strain 3	Wild strain 4
2-methylfurane	+		+	+	
3-methylfurane	+	+	+		+
3-methyl-2-butanone			+		+
3-methyl-2-butanol	+	+	+	+	+
2-pentanol	+	+	+		
3-methyl-1-butanol	+				
Pyrazine	+		+	+	+
2-methyl-1-butanol	+	+	+	+	
1-octen-3-ol	++	++	+++	++	+++
3-octanone	+	+	++	+	++
2-n-pentylfurane	+		++	++	++
2-ethyl-1-hexanol	++++	++++	++++	+++	++++

inoculated on wood chip wallpaper
DSMZ: German collection of microorganisms and cell cultures
Wild strains were derived from indoor mold damages
+: 1 – 10 ng/week, ++: 10 – 100 ng/week,
+++: 100 – 1.000 ng/week, ++++: 1000 – 10.000 ng/week

Connection between moisture and MVOC in moldy indoor environments

The investigation in 28 moldy indoor environments showed that increased humidity correlated with a total of 8 MVOC (see fig. 1). Here each point represents a flat, which was infested with mold. The mold damage was thereby clearly visible. This figure supports the hypothesis that fungal damage is clearly connected with moisture indoors
and that MVOC are suited for predicting mold infestation in indoor spaces.

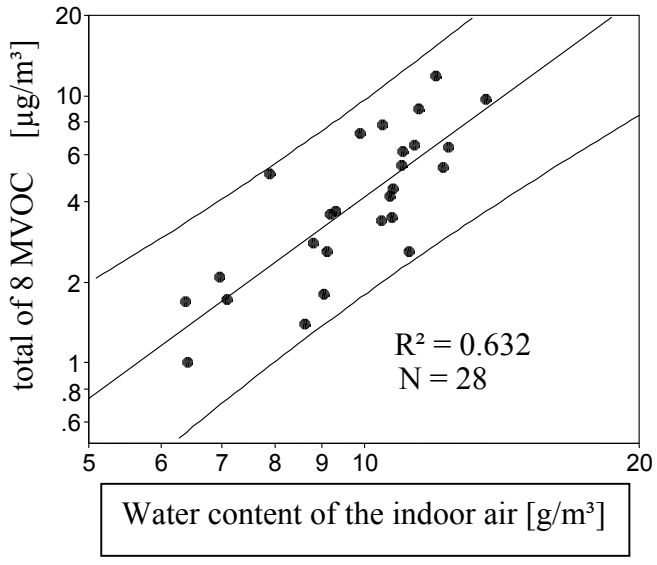
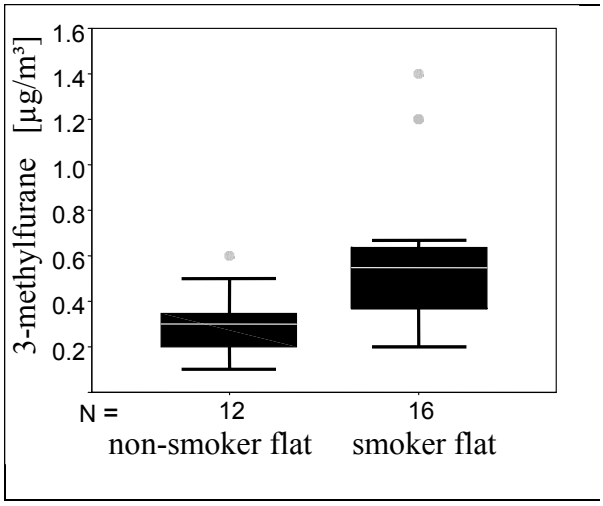


Figure 1.
Total of 8 MVOC in 28 flats with clearly visible mold infestation in relation to absolute air humidity.

Total of 8 MVOC =
Σ of 3-methylfurane, 2-pentanol, 3-methyl-1-butanol, dimethyldisulfide, 2-hexanone, 2-heptanone, 1-octen-3-ol and 3-octanone



One result from our field study is the finding that 3-methylfurane correlated with the smoking behaviour of the residents (see fig. 2)

Figure 2.
Concentrations of the "MVOC" 3-methylfurane in µg/m³, divided into non-smoker and smoker flats

DISCUSSION

The results of our study and those of other authors lead to the findings, that further laboratory and field trials are necessary to produce more knowledge about MVOC production. The following points shall be put into discussion:

1. Our results showed that MVOC production is strongly dependent from the substrate infested. Up to now there is only a punctual knowledge in literature concerning some indoor materials infested with some typical moulds. Therefore a broad range of typical substrates used indoors has to be tested with a broad range of different moulds typically occurring indoors in order to establish the spectrum of MVOC analyzed in doubtful indoor environments.
2. Our results show that the emission of MVOC clearly depends on the strain of the respective mould genera (laboratory strains versus strains from collections). In scientific work usually certified strains from official collections are used. This is done for better comparability and is therefore good laboratory practice. But normally these strains are several years old. Freshly isolated “wild strains” show a partly different metabolism and emit a different MVOC spectrum.
3. MVOC considered to be typical so far have to be re-assessed, as there are apparently other sources indoors. E.g. 3-methylfurane, which was found as a fungal product in the lab part of this study, as well as by many other authors [e.g. Börjesson, Stöllman and Schnürer, 1992], revealed a significant correlation with the smoking behavior of the inhabitants. Therefore 3-methylfurane should not be used as an indicator for mould infestations.
4. Our results showed, that the emission spectra of MVOC are not constant from trial to trial. For better understanding more knowledge should be provided about the question: “how reproducible and reliable is the formation of secondary metabolites?”

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