Murderous multicolored molds?

Studies on detection and occurrence of toxigenic micromycetes and mycotoxins in indoor environments

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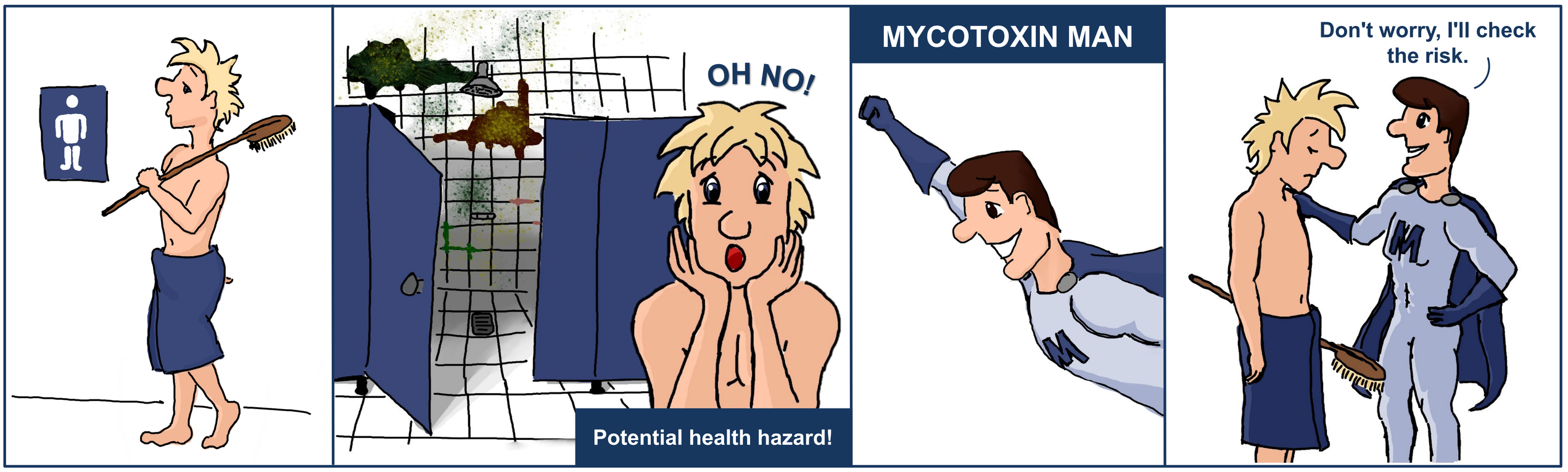
introduction

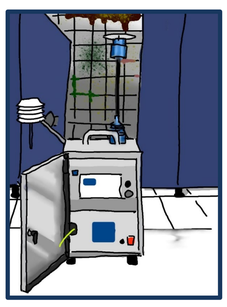
Indoor mold is generally recognized by the public as not beneficial to human health. As the non-expert encounters a “moldy” problem assessment of the situation is mostly based on the extent and color of the mold – often with the misconception that particularly colorful or black molds are particularly harmful. However, what exactly is the non-expert afraid of and is the initial assessment correct? In most cases, one does not really know. From a scientific point of view [1-3], there is the risk of developing a mycosis when inhaling large amounts of spores or an allergic respiratory response associated with fungal allergens and there is the risk of being exposed to mycotoxins. These secondary metabolites exhibit strong chronic toxicity with carcinogenic, mutagenic, teratogenic or immunosuppressive effects, their formation depending on favorable environmental conditions like temperature, humidity or nutrient availability [4,5]. As many molds producing colored pigments also form other secondary metabolites, estimating their potential hazard by color may not be entirely incorrect. However, it’s not always mycotoxins that are produced, even if the fungi might be capable of doing so.

A better picture of the on-site situation is provided by airborne microbial sampling and identification of potentially toxin-producing species. To evaluate whether relevant mycotoxins have actually been formed by the collected fungi a simultaneous airborne particle sampling with subsequent mycotoxin determination should be carried out. The project presented here aims to establish an appropriate service for the German armed forces to assess the exposure to toxigenic fungi and mycotoxins by mold-infested infrastructure.

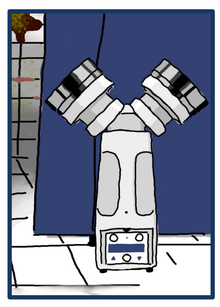


airborne particle sampling

For airborne particle sampling a MVS 6.1 (Comde-Derenda) was used at a flow rate of 2.36 m³/h. Sampling was carried out for 8 to 10 h depending on the expected exposure time in the respective room giving a total of 18.9 to 23.6 m³. As sampling should cover typical conditions of use office buildings are usually sampled during daytimes, whereas living spaces are more likely to be considered in the evening or at night. Particulate matter was retained by Whatman GF/A filters (1.6 µm) without pre-selecting any particle size. Subsequently filters were extracted using water/acetonitrile (80/20, *v*/*v*) and subjected to LC-MS/MS analysis.



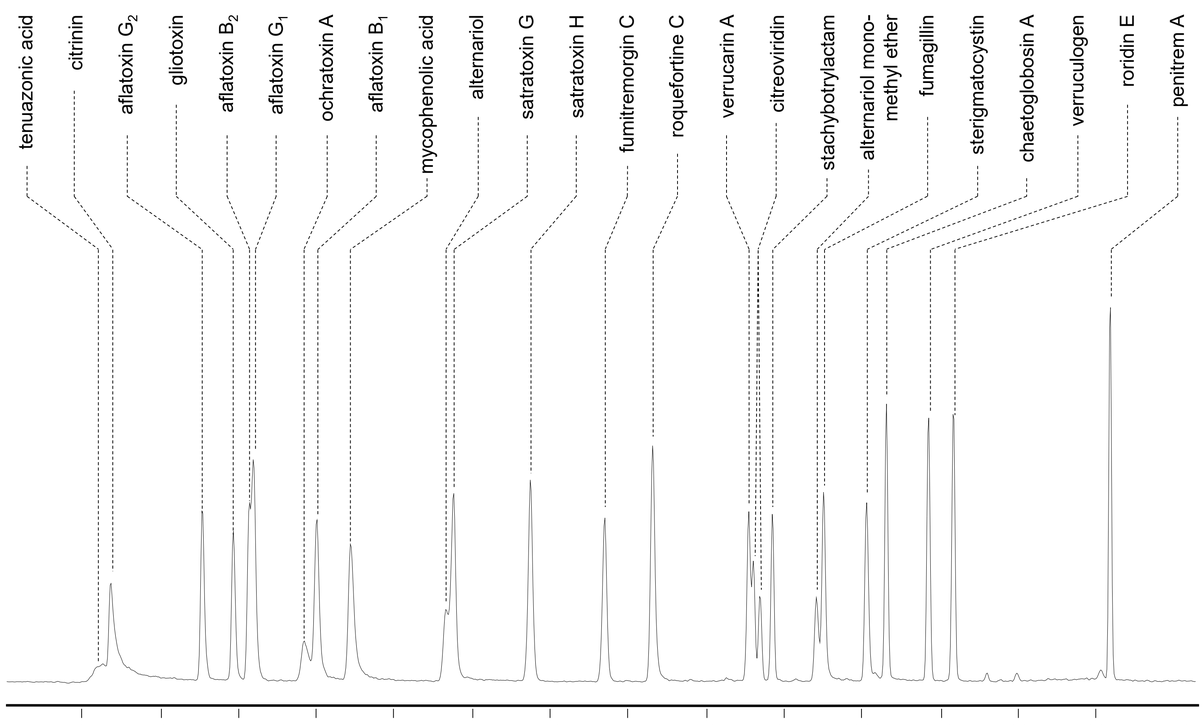
airborne microbial sampling



For sampling of airborne fungi a DUO SAS SUPER 360 (VWR) was loaded with malt extract agar containing 0.25% novobiocin (MEA⁺) and dichloran-glycerol agar (DG-18). While both culture media are suitable for fungi identification, water activity of DG-18 is lower leading to a different spectrum of fungal growth. Sample volume was adjusted to the given environment (the more infested the lower the sample volume to avoid media overloading) but was usually set between 0.25 and 0.5 m³/plate. MEA⁺ and DG-18 media were subsequently incubated at 25 °C for 7 days.

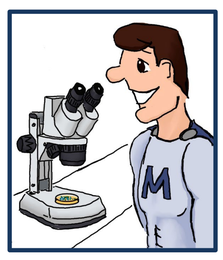
mycotoxin analysis

From the large number of possible secondary metabolites 24 mycotoxins were selected based on their relevance to indoor environments and toxicity and analyzed using an in house LC-MS/MS screening method. As composition changes from sample to sample dust analysis can be quite challenging. Mycotoxin quantification therefore requires isotope dilution, standard addition or in-sample calibration analysis.

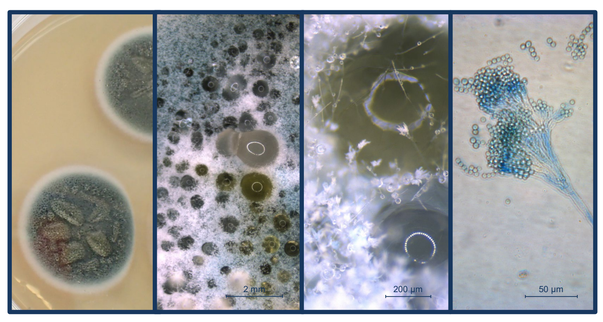


LC-MS/MS analysis was carried out using a 1260 Infinity LC (Agilent) connected to a 6470A (Agilent) triple-quadrupole mass spectrometer run in ESI positive and negative mode. Chromatographic separation was achieved using a 2.1 × 150 mm, 1.7 µm Kinetex Evo C_{18} column (Phenomenex) using gradient elution (A: 10 mM ammonium formiate aq., B: 7% 10 mM ammonium formiate aq. in acetontrile, min, %B: 0/30, 7/37, 22/65, 23/90, 24/90, 24.01/30, flow rate 0.2 mL/min).

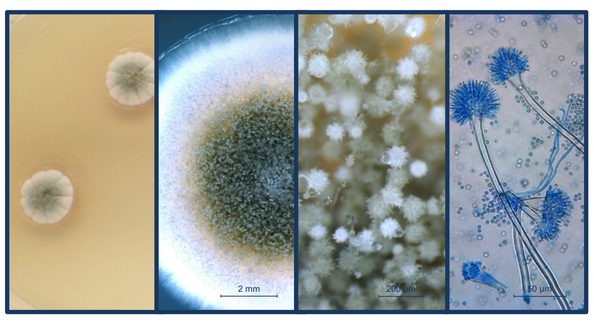
species identification



Microscopic identification focused on fungi relevant to interiors, which are mainly from the genera *Aspergillus*, *Penicillium*, *Alternaria* and *Stachybotrys* [2,3]. While some fungi can already be identified at species level based on morphological characteristics, other fungi require further subculture on selective media. Questionable results were verified using MALDI spectra obtained using an autoflex speed MALDI-TOF (Bruker) and matched against a filamentous fungi database (Bruker).



Penicillium chrysogenum. From left to right: colonies on MEA⁺, 10× magnification (stereo microscope), 80× magnification (stereo microscope), 400× magnification (light microscope, colored with lactophenol cotton blue, adhesive tape technique). The petrol-colored colony with a clearly outlined white border and large droplets of exudate, some of which are yellowish in color, are characteristic features. The brush-like conidia are typical for *Penicillium sp*.



Aspergillus versicolor. From left to right: colonies on MEA⁺, 10× magnification (stereo microscope), 80× magnification (stereo microscope), 400× magnification (light microscope, colored with lactophenol cotton blue, adhesive tape technique). The green-orange coloration of the colony fading into a white border are characteristic features. The spherical conidia are typical for *Aspergillus sp*.

literature

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