FUNGI AS ALLERGENS IN INDOOR AIR: A DIFFERENCE IN SPECIES RANGE BETWEEN THE RETURN AND SUPPLY AIR SIDE OF HVAC AIR FILTERS

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ABSTRACT

Fungi on filters of HVAC systems are being increasingly suggested as a causal factor of sick building syndrome (SBS) symptoms. Differenciation of fungi is critical to understanding any relationship as different fungi species have different allergic potential and produce different by-products. The HVAC system of a large Berlin library was used to analysed airborne fungi before and after the filters using a six stage Andersen sampler and three different types of agar media. After the filters, both the airborne fungi counts were reduced (by 60% to 90%) and the range of species were significantly reduced with several species absent including *Trichoderma harzianum, Chrysonilia crassa, Aureobasidium pullulans*. The type of agar media also altered which fungal species were detected. The malt extract and xerophilic (malt +40% saccharose) agars appear to be most useful for detecting airborne fungi. DG 18 was shown to be less effective when used in the Andersen sampler. The allergenic potential of the fungal genera differenciated illustrates the possible role for fungi in SBS.

INTRODUCTION

The importance of heating ventilation and air conditioning (HVAC) systems are now discussed as both a disseminator and source of fungi. System components capable of have variously been reported in relation to sick building syndrome (SBS) symptoms including the air filtration material, cooling coils, humidification equipment, and duct work (1).

The air filtration material of HVAC systems in particular are gaining increasing focus in indoor air quality research as their purpose is to accumulate the animate (micro-organisms) and inanimate (dust) airborne particles in the air stream. When the filters eventually become loaded with inorganic and organic components in the normal course of service life, they have most of the ingredients required for fungal colonisation. The potential health effects from exposure to airborne fungi include a wide range from allergic, to toxic, and odour reactions similar to those observed in SBS. Thus, fungi on filters of HVAC systems are being increasingly suggested to play a role in the SBS (2).

It is reasonable to speculated that micro-organisms may survive but also decay on filters. The fungi, their debris and by-products then have the potential to leave the filters and enter the supply air stream. This situation is not well understood but is important as it could contribute significantly to loading of metabolic or lysis products and increase the amount of allergens and mycotoxins in the indoor air. These are important potential risk factors for indoor air quality. However, few detailed studies on microbial colonisation on air filters been performed to date (3,4). This is partly due to the fact that differenciation of fungi is difficult to perform but

critical to understanding any relationship to SBS as various species have different allergic potential and generate different by-products.

A study was designed to detect the presence of viable fungi in the air before and after the filtration material in the HVAC system in a large library building. The methods chosen would need to perform several functions. First, it is necessary to show differences before and after the filters by the number of colony forming units present, and in the range of species present. And second, it remains necessary to determine which agar media is most useful for detecting a wide range in airborne fungi as there are no accepted guidelines or standards available for reference. This last point is critical to compare results between other studies as all agar types are currently used in research and industry are selective to some degree in which fungi they detect. The aim of this study was to determine which fungi can pass through the filters of the HVAC system and thereby have the potential to affect human health.

METHODS

Measurements location

The prefiltering device before the main filters a large HVAC system was chosen for the field experiments. The HVAC system serviced a large public library building in the centre of Berlin. The surrounding area was undergoing major reconstruction with substantial earthworks in progress during the experiment period. The air intake for the HVAC system was on the roof-top (adjacent to the prefilter equipment) and approximately 50 meters above ground level. The normal outdoor air intake was 120,000 cubic meters per hour (m^3/hr). Only outdoor air was passed through the prefiltering device in normal operation and no other HVAC equipment was before the filters including no pre heating device. A second „main" filtering device (which does not concern this experiment) was directly after the prefilter which filtered the return air and the prefiltered outdoor air. The normal prefiltering material used in the HVAC system was a fibreglass type EU 7. The samples were taken directly before and after the filters at a level from 60 cm high.

Analysis Of Airborne Fungi

A six stage Andersen sampler was chosen for the experiment because of the relationship between its construction to the human airway tract from the upper mucous lined airways down to the alveoli in the lungs. Meteorological measurements were also performed during the fungi sampling period. Air samples were taken once a month for half a year using the Andersen sampler impacting air onto agar filled petri-dishes. Three different types of agar media were tested in the sampler. Each measurement period was 10 minutes long at 28.3 litres per minute air flow rate and was also performed in triplicate (18 plates per medium per sample location) using three samplers in parallel. The plates were then incubated and the colony forming units (cfu) were counted and their genera and species differentiated.

Agar Media

The choice of medium was important for this experiment as no single media is able to show the entire spectrum of fungi. For this purpose, the three media were chosen for their different characteristics which were Malt extract, Malt extract with 40% saccharose, and DG 18. The two malt agars had the antibiotic spreptomycin/penicillin added, and the DG 18 agar had chloramphenicol added to limit bacterial growth. A brief description and recipe of each media is given below:

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Malt extract: most often used, rich in nutrients, a wide range of fungi can grow on it, but also fast growing are able to overgrow the hole plate in a very short time, so that differenciation is rendered more difficult xerophilic fungi like *Wallemia sebi* can't grow on malt. Recipe: 30 g Malt extract (Merck 5391), 18 g Agar, add 1000 ml distillated water, autoclave at 121°C for 15 min at 1 bar, pH at 5.0-5.5. Antibiotic-addition: Penicillin/Streptomycin mixture = 10.000 U Penicillin G (Penicillin G potassium, Merck 6993) / ml and 10.000 µg Streptomycin (Streptomycin-sulphate, Merck 10117) / ml, sterile filtered and 16 ml / 1000 ml added to Agar after sterilisation.

DG 18: DG 18 = Dichloran- 18% Glucose-Agar (Oxoid CM 729), DG 18 is suggested to be the most useful for xerophilic fungi. Developed by Hocking and Pitt as a medium with low water activity for the selection of xerophilic fungi. Dichloran restricts the growth of the colonies, so that counting and isolation is more easily. Recipe:: 5 g/l Pepton, 10 g/l Glucose, 1 g/l Potassium hydrogenphosphate, 0,002 g/l Magnesium sulphate, 15 g/l Agar, pH-value 5.6 ± 0.2, 15.75 g in 500 ml distillated water, dissolved with heat, 110 g Glycerol at, one Ampoule Chloramphenicol-Supplement SR 78 added, autoclaved at 121° C at 1 bar für 15 min, cooled to 50° C and, well mixed and poured into sterile Petri dish.

Malt extract with 40% saccharose: Another special medium for xerophilic fungi is malt with 40% saccharose. The high percentage of saccharose has the effect that water is bounded on the molecules and cannot used by the fungus. Many mesophilien fungi are not able to grow on this agar. Recipe: Malt extract Agar plus 40 % Saccharose: see recipe above für Malt extract, after this. 40 % Saccharose in 1000 ml distillated water, add to the autoclaved Agar.

Incubation

All cultures were incubated for 5 days at 20 ± 2 °C and 60 % RH in a darkened climate controlled room (no light incident). For the quantitative evaluation, colony forming units (cfu) were recorded. Cfu results are given in colony forming units per cubic meter (cfu/m^3) for air samples. Reference literature used for differentation (5, 6, 7, 8). The cfu were calculated separately for filametous and yeast like fungi by there morphologic view on the plates.

Differentiation of Fungi

Parallel to the quantitative evaluation of CFUs sample were used for differentiating the fungi. Colonies that not could be identified were reinoculated on to different agar media and again incubated for 6 days at 20 ± 2° C. Fungi were differentiated using light microscopy. The yeasts were not identified to genera and all yeast like growing fungi were included in this group. Some fungi are able to grow as well like a mould as a yeast (*Aureobasidium pullulans, Geotrichum candidum*). These fungi were counted to the moulds.

RESULTS

Genera Differenciation

Table 1 shows the fungal species on the three different agar media before and after the filters. A total of 28 genera of moulds and the artificial group of yeasts were detected on all three agar media. 24 fungal species were differenciated.

Differences In Agar Media

Varying numbers of genus and species were detected on the three different media. In the air before the filters there were 26 species detected on Malt agar, 12 species on DG 18, and 13

Table 1 Fungi species on three different agar media before and after the filters.

Genus And Species	Before The Filter			After The Filter		
	Malt	DG18	Malt 40	Malt	DG18	Malt 40
Acrodontium crateriforme	❑			❑		❑
Alternaria alternata	❑	❑	❑	❑	❑	❑
Aspergillus spp.	❑	❑	❑	❑	❑	❑
Aspergillus candidus				❑		
Aspergillus fumigatus	❑			❑		❑
Aspergillus niger	❑	❑	❑			
Aspergillus ochraceus	❑					
Aureobasidium pullulans	❑		❑			❑
Botrytis cinerea	❑	❑	❑	❑	❑	
Cladosporium spp.	■	■	■	❑	■	❑
Cladosporium cladosporioides	■	■	■	❑	❑	❑
Cladosporium herbarum	❑	❑	❑	■	❑	❑
Cladosporium macrocarpum	❑	❑		❑		
Cladosporium sphaerospermum	❑	❑				❑
Chrysonilia crassa	❑	❑				
Eurotium spec.			❑			
Fusarium spp.	❑			❑		
Fusarium cerealis	❑					
Geotrichum candidum	❑			❑		
Paecilomyces variotii						❑
Penicillium spp.	■	■	■	❑	❑	❑
Penicillium claviforme	❑					
Penicillium isariiforme	❑			❑		
Phoma glomerata	❑			❑		
Rhinocladiella spec.	❑					
Rhizopus stolonifer	❑		❑			
Trichoderma harzianum	❑					
Wallemia sebi			❑		❑	❑
Yeast spp.	■	■	❑	❑	❑	❑

Legend: ■ = more than 50 cfu/m^3, ❑ = less than 50 cfu/m^3.

species on Malt 40%. However, behind the filters only 16 species were detected on malt, 9 species on DG 18, but 13 species were still detected on the malt +40%.

Colonies of special genus and species only occurred on certain media. On the malt agar, the species *Aspergillus ochraceus, Aspergillus candidus, Fusarium spp., Fusarium cerealis, Geotrichum candidum, Penicillium claviforme, Penicillium isariiforme, Phoma glomerata, Rhinocladiella spec.*, and *Trichoderma harzianum* occurred where they were not detected on DG18 or Malt +40%. Similarly on DG18 and malt +40%, the species *Eurotium spec. and Wallemia sebi* occurred where they were not detected on the malt agar.

Before The Filter Results

Certain genus and species were only detected in the air before the filter. These include the species *Aspergillus niger, Aspergillus ochraceus, Chrysonilia crassa, Eurotium spec.,*

Fusarium cerealis,
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These include the
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Fusarium cerealis, Penicillium claviforme, Rhinocladiella spec., Trichoderma harzianum, Rhizopus stolonifer, and *Aspergillus ochraceus.*

After The Filter Results
all species behind the filters were present in the air before the filters. That is, no new species were detected. However, the fungi counts and range in genera were significantly altered in the air after the filters.

Fungi Genera Present
Of all the fungi differenciated, only a few genera and one species are strongly represent which were the group of yeasts, *Cladosporium spp., Cladosporium cladosporioides*, and *Penicillium spp.*.

In the air before the filters, a high amount of *Cladosporium spp,. Cladosporium cladosporioides*, *Penicillium spp.*, and yeasts spp. were detected on all three media. All other species were only detected in low amounts on all three media. However, in the air after the filters only *Cladosporium herbarum* on malt, and *Cladosporium spp.* on DG 18 were detected in high amounts. All other species were only detected in low amounts.

The efficiency of the filters in reducing fungi appeared to be related to the outdoor concentrations of fungi. At times when high cfu counts were detected in the outdoor air, the filters were observed to reduce fungi by approximately 90%. However, when low amounts of fungi were detected in the outdoor air, the filters appeared to be much less efficient with fungi counts reduced by only 60%.

DISCUSSION

Probably the most important finding in the experiment was the extension of the range of species detected when using the combination of the three agar media. However, it should be kept in mind that this extended range still only reveals a small window on the wide range of airborne fungi that exist.

Most of the species detected occurred at relatively low counts. However, the allergic potential of fungi detected behind the filters can have a significant impact on human health. This point also illustrates the need for correct differenciation of fungi as their allergic potential depends on which species of a genera are present (for example *Aspergillus fumigatus*) and on the mass of dispersed spores (for example *Cladosporium spp.*).

While fungi with allergenic potential were detected behind the filters, the filtration media appears to relatively efficient at arresting fungi particles. This is illustrated in the results where some species only appear in the air before the filters. The species absent behind the filters are mostly those with large spores. An example are the large spores of *Alternaria spp.* and *Fusarium spp.* where *Fusarium* genus more commonly produces macro conidia and rarely produces micro conidia.

Several improvements to the incubation methods would make it possible to identify more species. One improvement would be to use Ultra Violet light during incubation to stimulate certain fungi to sporulate. Another improvement would be to use several other temperatures during incubation including 27 °C and 37 °C to make species such as *Aspergillus fumigatus* sporulate.

Overall, the DG 18 agar appears less usable for airborne fungi measurements in an Andersen sampler as only 12 species were detected on this media leaving 16 species absent. No explanation for this difference is obvious from the methods or the results. To obtain the best results from Andersen sampler, it appears necessary to use a combination of two agar media such as the malt extract agar and a xerophilic agar (for example the malt +40%) used in this experiment. This combination would allow the greatest range in fungal species to be detected.

CONCLUSION

This study showed that air filtration media in HVAC systems can reduce airborne fungal counts by 60% to 90%. The efficiency of fungi reduction appears related to the concentration of fungi. That is, greater efficiency of filtration occurs at higher concentrations. The filtration material was also effective in reducing the range of fungi species. Several species were absent in the air behind the filters including *Trichoderma harzianum, Chrysonilia crassa, Aureobasidium pullulans*.

The type of agar media used in the Andersen sampler significantly altered the range in fungal species detected. The malt extract and xerophilic (malt +40% saccharose) agars appear to be most useful for detecting airborne fungi. DG 18 was shown to be less effective to detect airborne fungi when used in the Andersen sampler. It appears necessary to use a combination of a malt extract agar and a xerophilic agar to detected the greatest range in airborne fungi.

The allergenic potential of the fungal genera differenciated illustrates the possible role for fungi in SBS.

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