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MINI-REVIEW

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Relevance of airborne fungi and their secondary metabolites for environmental, occupational and indoor hygiene

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Abstract Airborne fungal contaminants are increasingly gaining importance in view of health hazards caused by the spores themselves or by microbial metabolites. In addition to the risk for infection, the allergenic and toxigenic properties, as well as the inflammatory effects are discussed in this review as possible health impacts of bioaerosols. A major problem is the lack of threshold values for pathogenic and non-pathogenic fungi, both in the workplace and in outdoor air. While the relevance of mycotoxins has been intensely studied in connection with contamination of food and feed, the possible respiratory uptake of mycotoxins from the air has so far not been sufficiently taken into account. Toxic secondary metabolites are expected to be present in airborne spores, and may thus occur in airborne dust and bioaerosols. Potential health risks cannot be estimated reliably unless exposure to mycotoxins is determined qualitatively and quantitatively. Microbial volatile organic compounds (MVOC) have been suggested to affect human health, causing lethargy, headache, and irritation of the eyes and mucous membranes of the nose and throat. The production of MVOC by fungi has been discussed in connection with domestic indoor microbial pollution, but the relevance of fungal metabolites in working environments remains insufficiently studied.

Keywords Airborne fungi · Mycotoxins · MVOC · Volatile compounds · Indoor air · Compost · Occupational hygiene

Introduction: health hazards associated with exposure to microorganisms

In recent years, research on environmental health has intensified, but up-to-date and in-depth literature on the relevance of airborne fungi and their microbial metabolites is sparse. Although a clear correlation between levels of fungi in the air and health impacts has not been shown in epidemiological studies (Bornehag et al. 2001; Burge 2001), fungi in indoor air must be regarded as potential health hazards. Airborne microfungi impact human health in four different ways: (1) they can infect humans, (2) they may act as allergens, (3) they can be toxigenic, or (4) they may cause inflammatory reactions. The microfungi of concern in environmental and occupational hygiene are mostly non-pathogenic or facultative pathogenic (opportunistic) species. Non-pathogenic species, such as the penicillia and most soil fungi, are ubiquitous. They do not cause infections but are relevant as allergens and mycotoxin producers. The opportunistic pathogens, e.g. some dermatophytes, the thermotolerant aspergilli (especially *Aspergillus fumigatus*), *Scopulariopsis* sp., and some *Mucorales*, are also ubiquitous, but can only affect human health when predisposing factors are involved. Pathogenic fungi showing high virulence, such as *Cryptococcus neoformans* and *Coccidioides immitis*, are non-ubiquitous species and will not be treated here.

If airborne fungal spores are inhaled down to the bronchia and alveoli, they will be lysed and the human body thereby exposed to primary and secondary metabolites. In some cases, mycotoxins are clearly involved in pathogenesis. Inhalation exposure has been suggested to cause acute kidney failure (ochratoxin), central nervous system damage (tremorgenic mycotoxins), and damage of the upper respiratory tract (*Stachybotrys chartarum*) (Miller 1994).

Natural species composition in outdoor air and the anthropogenic air spora

Microfungi such as *Cladosporium* spp., *Alternaria* spp., *Epicoccum nigrum*, and *Botrytis cinerea* are known to be an integral part of the fungal air spora outdoors (Table 1). They cover more than 90% of the total fungal spore load. The fungal spore load of penicilli and aspergilli is in the

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Table 1 Levels of airborne fungi (CFU m^{-3}) in different environments and their impact on health. *I* infectious, *A* allergenic, *T* toxigenic, + species regularly present in low numbers, *n.r.* not relevant for air hygiene, – no data available

Species	Type of impact:	Outdoors	Hospitals	Dwellings (wards)	Occupational environments
Alternaria alternata	A T	10^{1}-$10^{2(c,f)}$	–	$10^{1(f)}$	+
Aspergillus candidus	A	+	–	<5	$10^{4(d)}$
Aspergillus flavus	I A T	n.r.		<5	10^{4}-$10^{5(d)}$
Aspergillus fumigatus	I A T	<10–20	4–25[b]	+	$10^{7(d)}$
A. fumigatus, A. flavus	I A T	See above	1–12[a]	See above	See above
Aspergillus versicolor	A T	<5[e]	–	<5[e]	$10^{6(d)}$
Cladosporium spp.	A	$10^{3(c,e)}$	–	$10^{2(e)}$	10^{2} -$10^{3(d)}$
Epicoccum nigrum	A	$10^{1(f)}$	–	$10^{1(e)}$	+[d]
Eurotium herbariorum	A	<10[e]	–	<5[e]	+[d]
Penicillium brevicompactum	A	<10[e]	–	$10^{1(e)}$	$10^{4(d)}$
Penicillium chrysogenum	A	+	–	Frequent	$10^{2(d)}$
Penicillium glabrum	A	<5[e]	–	<10[e]	$10^{4(d)}$

[a]Hospenthal et al. (1998)
[b]Jaffal et al. (1997)
[c]Lacey (1996)
[d]Fischer (2000b)
[e]Verhoeff (1994)
[f]Senkpiel et al. (1996)

range of 2–10% and 1–3%, respectively (Lacey 1996). The species composition in outdoor air has been studied mostly by using slit-samplers and direct microscopy (assessing total numbers of spores). Exact ratios of species from genera such as *Aspergillus* and *Penicillium* in outdoor air are still unknown, but such data would provide a basis for establishing guide values for evaluating fungal spore counts in various environments. Species-differentiated data, especially for the penicilli and aspergilli, can only be assessed by air sampling in combination with subculturing of fungi (viable spores counts). Numerous techniques have been developed including impaction (Andersen sampler, 1-, 6-, 8-stage; Reuter centrifugal sampler, SAS-sampler, Loreco FH3, Klotz FH5), impingement (all-glass impinger, AGI 30) and filtration (Sartorius MD-8, MD-8 AirPort).

There is only limited knowledge on the emission of fungal propagules from composting plants and their dispersal into the environment. Concentrations of up to 5×10^{2} CFU m^{-3} for *A. fumigatus* and *Aspergillus niger* have been observed at distances of up to 2 km downwind from a facility (Ostrowski et al. 1999), whereas the number of CFU was below 10–20 CFU m^{-3} air in non-affected locations. In a residential area near an open-windrow composting plant in New York, a maximum of 1.4×10^{4} CFU/m^{-3} of *A. fumigatus* has been found at 540 m distance (New York State Department of Health 1994). While there are currently no epidemiological investigations on the incidence of *A. fumigatus* in different ecosystems in outdoor air, spore counts above 10 CFU m^{-3} do not seem to be likely unless there is an anthropogenic source (Haas et al. 1999).

Relevance of fungal infections

Nosocomial infections

In operating theatres, rooms, and corridors of wards in hospitals, intensive measures to maintain air hygiene are undertaken to reduce airborne fungal spores to almost zero. Activities such as in-hospital construction, renovation, excavation, and carpeting are regarded to be associated with outbreaks of nosocomial aspergillosis. Contaminated hospital ventilation systems, damp wood, or potting soil have been discussed as sources for pathogenic fungi. An association between concentrations of *A. fumigatus* and *Aspergillus flavus* exceeding 1 CFU m^{-3} in oncology wards and an increasing incidence of aspergillosis has been controversially discussed (Arnow et al. 1991; Hospenthal et al. 1998). For immunocompromised patients, numbers even below 10 CFU thermotolerant aspergilli m^{-3} have been discussed as health hazards, since aspergillosis is fatal despite appropriate treatment in more than 50% of cases (Denning 1996). In an investigation carried out in wards of an hospital in the United Arab Emirates, spore numbers of up to 25 CFU m^{-3} air for *A. fumigatus* are classified as low (Jaffal et al. 1997), an amount which is questionable even if thermotolerant species are likely to be more frequent in desert countries.

Infections in occupational environments

The level of opportunistic thermotolerant fungi in air has become a matter of discussion, as the number of workplaces in the waste-handling industry increases and emissions of microorganisms from these facilities are being critically evaluated. It is extremely difficult to estimate health risks, since severe infections have not been described for workers, and only people suffering from immune deficiencies are at risk for infection.

In addition to *A. fumigatus*, also *A. niger*, *Aspergillus terreus*, *Aspergillus flavus*, *Emericella nidulans*, *Aspergillus niveus*, *Aspergillus restrictus*, and *Eurotium amstelodami* are potentially pathogenic to humans. In waste-handling facilities, extremely high concentrations of up to 10^{7} CFU m^{-3} air for thermotolerant fungi have been found (Fischer 2000b). There are no reports in the literature indicating that high fungal spore counts of pathogens increase the risk for infection of workers with a normal immune status. However, long-term exposure to living or dead particles containing fungal toxins, e.g., aflatoxins, gliotoxin, ochratoxin, patulin, and trichothecenes is expected to eventu-

ally suppress or modulate the immune response in healthy people. Since most investigations on immunomodulation by mycotoxins have focused on oral application (Bondy and Pestka 2000), further research remains necessary to elucidate the role of exposure by pulmonary uptake.

Allergenic relevance of airborne spores

Fungi must generally be regarded as potential allergens and have probably been underestimated because of inadequate, non-standardized diagnostic antigen preparations. *Alternaria* and *Cladosporium* are considered to be the most important fungal airborne allergens in outdoor air, whereas *Aspergillus* and *Penicillium* have recently been recognized as significant indoor air allergens (Li and Hsu 1997). In contrast to pollen allergy, the correlation between fungal spore counts and positive skin-test frequencies has been generally less marked. Conidia of *Cladosporium*, which are present in large numbers in outdoor air, appear to be less sensitizing than species such as *Alternaria*, which is normally found in lower numbers (Cosentino et al. 1995). Dose-effect responses between airborne spore concentrations and health effects have not been described. However, exceptional rates of admission for asthma tended to occur on days with high total mould spore counts, although no specific taxon has been consistently implicated (Newson et al. 2000).

Indoor air

In buildings with mould problems, the incidence of penicillia and aspergilli is often higher than in outdoor air. The genera *Penicillium* and *Aspergillus* are more closely associated with respiratory allergic symptoms and allergen sensitization than the common outdoor moulds *Cladosporium* and *Alternaria*. Although *Cladosporium cladosporioides* has not been associated with indoor air complaints (Cooley et al. 1998), asthma has been associated with sensitization to allergens of other fungi (*Alternaria*) and house dust, with a prevalence of 25.4% for moulds among eight different allergens tested (Boulet et al. 1997; Norbäck et al. 1999). This low value is obviously due to *Alternaria* being the only fungal allergen tested, both as indoor and outdoor allergen. In households of patients with asthma bronchiale and positive reactions to intracutaneous application of fungal allergens, spore counts have been found to be four to 40 times higher than measured outdoors, with a prevalence for *Penicillium* spp. and *Aspergillus* spp. After renovation or change of residence, complaints of patients decreased clearly (Senkpiel et al. 1996). Statistically significant differences in frequency of distribution have been found for *Cladosporium* and *Penicillium* between atopic and control groups. In contrast to total spore counts, *Penicillium* spore counts are related to home dampness (Li and Hsu 1995), which itself is associated with allergenic symptoms in children with asthma and rhinitis (Li and Hsu 1997). Consequently, species of the genera *Penicillium* and *Aspergillus* should be the focus of future studies. It seems that *Aspergillus* spp. are somewhat more frequent in warmer climates than in cold and temperate regions, but species-differentiated data are still lacking.

Occupational environments

In occupational environments where waste, biowaste, or compost are handled, spore counts of *Penicillium* and *Aspergillus* are two to four orders of magnitude higher than in domestic environments. This may indicate a high risk for employees to acquire respiratory allergic diseases or sensitization to different moulds. Various cases of allergic alveolitis due to massive fungal exposure have been described for a series of professions in the fields of agriculture, forestry, and food production (Table 2). Repeated exposure to high concentrations of bioaerosols and exposure over long periods may be critical (Lacey and Dutkiewicz 1994). Frequent complaints of gastrointestinal symptoms by biowaste collectors have been associated with exposure to bioaerosols (Lundholm and Rylander 1980; Ivens et al. 1997). Long-term exposure to fungal spores exceeding 10^6 spores m^{-3} air has been found to be related to respiratory symptoms and symptoms of organic-dust toxic syndrome (ODTS) in sawmill workers (Eduard et al. 1993). Moreover, health problems have been found in workers employed in garbage-sorting facilities, and respiratory disorders and atopy occurred in Danish refuse workers (Malmros et al. 1992; Sigsgaard et al. 1994). Waste collectors are at increased risk for pulmonary diseases, infections, and skin and mucous membrane irritation, possibly caused by exposure to bioaerosols (Poulsen et al. 1995).

Table 2 Forms of allergic alveolitis and causal agents according to Hawksworth et al. (1995)

Form of allergic alveolitis	Causal agent
Farmer's lung	Thermophilic actinomycetes, sometimes *Aspergillus flavus, Aspergillus versicolor, Eurotium rubrum*
Cheese-washer's lung	*Penicillium casei*
Malt-worker's lung	*Aspergillus clavatus, Aspergillus fumigatus*
Suberosis (workers in cork industries)	*Penicillium glabrum*
Maple-bark stripper's lung	*Cryptostroma corticale*
Sawmill worker's lung	*Rhizopus rhizopodiformis, Penicillium* spp., *Aspergillus fumigatus, Trichoderma viride*
Sequoiosis	*Aureobasidium pullulans, Graphium* sp.
Mushroom picker's lung	*Pleurotus ostreatus, Pholiota nameko, Aspergillus fumigatus, Doratomyces stemonitis*
Allergic alveolitis from citric acid fermentations	*Aspergillus fumigatus, Aspergillus niger, Penicillium* spp.

Compared to waste collectors, the high exposure of compost workers to bioaerosols was significantly associated with a higher frequency of health complaints and diseases as well as higher concentrations of specific IgG antibodies for *A. fumigatus*, *A. niger*, and *P. crustosum* (Bünger et al. 2000).

Inflammation relevance

Besides the endotoxins of gram-negative bacteria, components of the fungal cell wall such as glucans and structurally related compounds seem to cause inflammations of the airways (Thorn and Rylander 1998). This is of major relevance in occupational environments due to high amounts of β-glucans and possible interactions with endotoxins in workplaces (Lacey and Dutkiewicz 1994). Fungal spores have been shown by in vitro testing to trigger the production of inflammatory mediators in macrophages (Ruotsalainen et al. 1998; Murtoniemi et al. 2001). Publications on cytotoxicity testing of spore-associated mycotoxins (using human embryonic fibroblast lung cells, ciliated respiratory tract cells of chicken, and feline fetus-lung cells) are too numerous to be referred to here in detail. However, interactions between glucans and spore-associated mycotoxins (Fischer et al. 2000a) need to be studied further with respect to inflammation of the airways.

Relevance of (M)VOC

The low concentration range – indoor air

The role of fungi in affecting the quality of indoor air has become increasingly controversial. There is, however, general agreement that volatile organic compounds (VOC) and microbial volatile organic compounds (MVOC) are involved in the sick-building syndrome (SBS). In experiments with humans exposed to VOC mixtures, irritation of the eyes and upper respiratory tract, as well as inflammatory reactions have been described among several other symptoms (Wiesmüller 1998). In buildings with visible fungal growth, compounds such as dimethyldisulfide, 2-methyl-1-butanol, 1-octen-3-ol, and isobutyl ether have been found in concentrations of 10–100 ng m^{-3} air (Keller et al. 1998). Total concentrations of 18 MVOC correlated to some extent with odor perception in dwellings when cases have been classified in three groups: (1) fungus-like odor not recognizable (<35 ng m^{-3}), (2) slight fungus-like odor (50–1,720 ng m^{-3}), (3) strong fungus-like odor (160–12,300 ng m^{-3}). The authors concluded that concentrations ≥50 ng m^{-3} air may indicate an indoor contamination with moulds, while outdoor concentrations are usually ≤10 ng m^{-3} air (Keller et al. 1998). In Germany, a maximum tolerable value for the total VOC concentration (TVOC) of 0.3 mg m^{-3} air has been defined for indoor air. During the last few years, a general trend has been observed concerning different types of VOC. Both the numbers and the concentrations of aldehydes have tended to increase, whereas chloro-carbohydrates have decreased (Seifert 1999). In older studies on dose-effect concentrations associated with TVOC values, irritations in humans have not been observed at concentrations of less than 0.2 mg m^{-3} air, but above 25 mg m^{-3} air headache and neurotoxic effects occurred (Mølhave 1991). Recent investigations have only partly confirmed these earlier results. Positive dose-response relationships have been found for mixtures of VOC using an experimental "eye-exposure system" that may serve to either determine threshold concentrations inducing sensory irritation or to validate predictive animal models (Hempel-Jorgensen et al. 1999). Korpi et al. (1999) determined the potential of three microbial volatiles, 1-octen-3-ol, 3-octanol, and 3-octanone, to decrease the respiratory frequency of mice by 50% (RD_{50} value). The data supported the conclusion that a mixture of MVOC may have synergistic effects, which constrains the interpretation and application of recommended indoor air levels of individual MVOC. Nonetheless, tolerable indoor air levels calculated from the individual RD_{50} values are considerably higher than the reported indoor air levels. This indicates that the contribution of known MVOC to the above-mentioned symptoms is less important than previously supposed. A promising technique for assessing MVOC profiles to predict fungal contamination in indoor air has been described (Ström et al. 1994). Experiments with house dust or common indoor moulds grown on plasterboard and cardboard showed that the profiles may be used to identify indoor contaminants at the species level (Sunesson et al. 1996; Wilkins et al. 2000). Even on poor substrates, such as house dust, production of MVOC has been observed (Pasanen et al. 1997; Wilkins et al. 1997). Since the number of prevalent mould species developing in contaminated buildings is limited to about 10–20, these could be identified by pattern recognition of their MVOC profiles (Larsen 1995; Fischer et al. 1999a). A compilation of relevant MVOC is given in Table 3.

Table 3 Microbial volatile organic compounds found to be characteristic for fungi in indoor and occupational environments

Furanes: 2-/3-Methylfuran[a–d], 2-pentylfuran[a,c,d]
Alcohols: 2-Methyl-1-propanol (isobutanol)[a,c–e], 2-pentanol[a,c,e], 3-methyl-1-butanol[a,c–e], 2-methyl-1-butanol[a,c–e], 1-octen-3-ol[a–d], 3-octanol[a,c,d]
Aldehydes: 2-Hexanone[a,b], 3-hexanone[d], 2-heptanone[a–c], 3-octanone[a–d]
Ester: Ethylisobutyrate[a,d], ethyl-2-methylbutyrate[a,d]
Ether: Methylisobutylether[c], 2-methylisopentylether[c]
Sulfur compounds: Dimethyldisulfide[a,c–e], dimethyltrisulfide[c]
Terpenes, terpene alcohols: Borneol[c,d], β-farnesene[c,d], Geosmin[a,c,d]

[a]Ström et al. (1994)
[b]Pasanen et al. (1997)
[c]Keller et al. (1998)
[d]Fischer et al. (1999a, 2000b)
[e]Wilkins et al. (2000)

The high concentration range – occupational environments

If humans in indoor environments are affected by relatively low levels of VOC, it is likely that employees in waste-handling facilities respond to levels of (M)VOC in these environments that are one to two orders of magnitude higher. However, concentrations of the most abundant aliphatic hydrocarbons and alkylbenzenes in the air of composting facilities have been observed to be below the maximum concentrations recommended by national and international guidelines. In The Netherlands, the threshold limit value for limonene, which occurred in concentrations of up to 150 mg/m^3 air in composting facilities, is 560 mg/m^3 air (Heida et al. 1995). Current regulations do not cover relevant (M)VOC, and further research will be necessary to evaluate the relevance of the great diversity of compounds in workplaces. A promising technique for assessing MVOC profiles in occupational environments has been developed (Fischer et al. 1999a). A great diversity of volatiles, both microbial- and plant-derived, has been identified in different workplaces in composting facilities, and the spectrum of compounds changed with the composition of fungal species in the air (Fischer 2000b). Future studies on the exposure to bioaerosols must include those volatiles that are possibly derived from microorganisms. Until now, there has been no evidence that MVOC are toxicologically relevant (Kreja and Seidel 2002), but further epidemiological research is necessary to elucidate their role as irritants and health hazards to humans.

Occurrence and activity of mycotoxins in bioaerosols

Reports on health effects due to ingestion of mycotoxins are of concern only in livestock breeding and food microbiology. In air hygiene, the inhalation of such compounds must be considered. The production of mycotoxins basically depends on the type of substrate available. Mycotoxins are excreted into the substrate or can be present in fungal cells. Consequently, two routes for mycotoxins becoming airborne are possible: (1) the dust may be contaminated by mycotoxins excreted by the fungi; (2) the conidia (and spores fragments) contain toxic metabolites which become air-borne by propagation. It seems that the amount of mycotoxins basically depends on the number of conidia present in airborne dust (Fischer et al. 1999b, 2000a). Thus, only in highly contaminated environments such as workplaces mycotoxins are relevant as health hazards. The pulmonary uptake of mycotoxins and its effects have not been investigated sufficiently in animal and in vitro experiments. In addition, epidemiological studies are needed to increase our knowledge of possible symptoms and health impacts in connection with exposure levels.

Exposure to airborne mycotoxins in workplaces in agriculture was intensely studied during the 1980s (Burg and Shotwell 1984; Sorenson et al. 1984, 1987), and its relevance for both environmental health and occupational medicine was clearly defined in the early 1990s (Hendry and Cole 1993; Miller 1994). Until now, the only toxins detected in airborne dusts and bioaerosols have been trichothecenes of *Stachybotrys chartarum*, aflatoxins of *Aspergillus flavus*, and metabolites of *A. fumigatus* (Table 4). However, investigations on the potential of distinct species to produce mycotoxins on semi-natural substrates indicated that compounds in addition to those already found in native bioaerosols may occur (Fischer et al. 2000a).

During harvesting and handling of airborne dust from maize, concentrations of up to 107 ng aflatoxin m^{-3} air were reached, whereas dust from peanuts contained 250–400 ng aflatoxin per g dust (Burg and Shotwell 1984). During on-farm grain handling activities, aflatoxin B_1 was found in concentrations of up to 4,849 ng m^{-3} (Selim et al. 1998). At these concentrations, workers could inhale 40–2,500 ng aflatoxin in a 45-h workweek. Sorenson et al. (1984) found amounts of 0.4–7.6 ng m^{-3} air in work places. These authors calculated that, assuming a breathing rate of 1 m^3 h^{-1} and an airborne aflatoxin concentration of

Table 4 Occurrence of airborne mycotoxins

Mycotoxins	Species	Substrate / type of sample	Concentration	Reference
Aflatoxins	*Aspergillus flavus, Aspergillus parasiticus*	Grain dust (maize)	206 ppb⇒107 ng m^{-3}	Burg and Shotwell (1984)
Aflatoxin B_1	*Aspergillus flavus, Aspergillus parasiticus*	Respirable peanut dust	22.7 – 612.4 ppb⇒0.4–7.6 ng m^{-3}	Sorenson et al. (1984)
Satratoxins G, H	*Stachybotrys atra*[a]	Artificially generated aerosols/dusts from laboratory experiment	9.5 ng mg^{-1} dust	Sorenson et al. (1987)
Aflatoxin B_1	*Aspergillus flavus*	Airborne grain dust	0.04 – 4,849 ng m^{-3}	Selim et al. (1998)
Trypacidin, tryptoquivaline	*Aspergillus fumigatus*	Bioaerosols, total dust from composting facility	Semi-quantitative analysis: 5–50 µg per 10^9 conidia, no standards available	Fischer et al. (1999b)
Ochratoxin	*Aspergillus ochraceus, Penicillium* spp.	Dust from heating ducts	Up to 58–1,500 ppb	Richard et al. (1999)

[a]*Stachybotrys atra* Corda 1837 = *Stachybotrys chartarum* (Ehrenb. Ex Link) Hughes 1958. *Stachybotrys atra* is a facultative synonym of *Stachybotrys chartarum*, thus based on different type material

0.2 ng m^{-3}, a worker could inhale 1.6 ng in an 8-h workshift and 8.0 ng in a 40-h workweek. Similar or even higher levels of exposure to microfungi can be found in waste-handling facilities; however, aflatoxins could not be extracted from samples of biowaste and compost, although strains of *A. flavus* isolated in situ proved to be partly toxigenic (Déportes et al. 1997). These results can be attributed to the fact that extraction of aflatoxins from compost is difficult, as was proven by spiking the samples. Aflatoxin does not seem to be relevant in indoor air, since its formation during fungal growth on building materials has not been observed (Rao et al. 1997; Ren et al. 1999).

Stachybotrys chartarum produces trichothecenes, e.g. satratoxines H and G, verrucarins B and J, trichoverrins, and atranones A and H, some of which have proven to be of toxicological relevance following exposure to 10^3–10^4 CFU m^{-3} air (Sorenson et al. 1987). Few clinical reports on human cases of toxicosis due to *Stachybotrys chartarum* have been described in the literature. Symptoms observed in connection with contamination by *Stachybotrys* spp., include coughing, rhinitis, sore throat, nose bleeding, moderate fever, diarrhea, headaches, dermatitis, fatigue and general malaise (Schiefer 1994), and a not yet validated report of acute pulmonary hemorrhage in infants (Etzel et al. 1998). A clear cause-effect relation between occurrence of the fungus and symptoms observed in patients is difficult to establish, which can partly be attributed to the fact that isolation from substrates with extremely high amounts of airborne fungal spores is difficult. Detection of conidia by direct microscopy is necessary. Moreover, it has recently been shown that three chemotypes can be distinguished in *Stachybotrys chartarum*, all differing with respect to their toxic and inflammatory potential as characterized by cytotoxicity assays (Andersen et al. 2002). However, there is no doubt that mycotoxins produced by *Stachybotrys chartarum* are the most toxic ones known.

The occurrence of mycotoxins from *A. fumigatus* in native dusts and bioaerosols sampled in a composting facility has been demonstrated (Fischer et al. 1999b). The compounds, i.e. tryptoquivaline (tremorgen) and trypacidin, were estimated to be present in an amount of 5–50 µg per 10^9 conidia. The detection of these compounds coincided with an extraordinary high density of conidia in air (10^7 CFU m^{-3}). Compounds such as gliotoxin, verruculogen, and the fumitremorgens A and B have so far not been detected in the bioaerosols.

A report of ochratoxin in house dust was published by Richard et al. (1999). Samples of dust collected from heating ducts yielded up to 1,500 ppb of ochratoxin A. In air samples taken previous to this sampling, *Aspergillus ochraceus* and *Penicillium* spp. had been found. The production of the mycotoxin could not solely be ascribed to *A. ochraceus* as the penicillia present in the dust were not identified to the species level. It has often been falsely assumed that ochratoxin is produced by several *Aspergillus* and *Penicillium* species (Richard et al. 1999; Robbins et al. 2000), which is not true for the penicillia (Frisvad and Thrane 1993).

The number of species that have been studied in experiments on exposure to mycotoxins in air is still limited. If data from the last two decades are critically evaluated, taxonomic, toxicological, and chemical expertise must be considered to reliably estimate possible health effects. The recent literature on health effects of mycotoxins in indoor air does not provide compelling evidence that exposure at levels expected in most mould-contaminated indoor environments is likely to result in measurable health effects (Robbins et al. 2000). Therefore, if effects of substrates are taken into consideration, occupational environments can be taken as model system to investigate the relation between microorganisms and microbial metabolites in air (Fischer 2000b). The most important aims for further research on mycotoxins will be to analyze extracts of aerosol samples for microbial metabolites, to study the effect of pulmonary exposure to relevant mycotoxins, and to study their effects on mammalian systems in vivo and in vitro.

Conclusions and perspectives

Since it is not possible to quantify the risk of infection by thermotolerant fungi, guide values or threshold limit values should be established that are based on the natural fungal air spora in outdoor air. Therefore, the species composition must be thoroughly identified in urban and rural regions. New insight into pathogenic and allergenic effects can only be achieved by epidemiological investigations, both in environmental and occupational medicine, and by assessing relevant compounds and species. To achieve a profound assessment of all environmental factors involved, investigations must include microbiological, taxonomic, and toxicological expertise.

The role of fungi as allergens has probably been underestimated because of inadequate, nonstandardized diagnostic antigen preparations. There is a strong need to characterize the allergenic potential of different species even within more complex genera such as *Penicillium*. Allergists and environmental microbiologists must use the same names for microorganisms so that medical data can directly be compared to data from exposure assessments. A striking example is *Penicillium chrysogenum* Thom 1910, one of the most common representatives of its genus on interior finishes. In allergy diagnosis, the old name, *P. notatum* Westling 1911, is still in use for this species.

The production of mycotoxins is to a wide extent species-specific. Therefore, proper identification of species according to current taxonomic concepts is a prerequisite for a reliable estimation of expected exposure levels. In numerous publications, the production of certain mycotoxins has been ascribed to wrongly identified species or vice versa (Samson 1992), which has to be considered when older publications are referred to.

Since the number of composting facilities has risen during the last years, emissions from these sources may possibly influence the natural air spora in outdoor air. Human activities in urban areas such as waste collection may contribute to additional emissions that can eventually alter

the ratios of individual species. The importance of such emissions becomes apparent by calculating the proportion of land where the air spora may be altered due to such emissions. In Germany, approximately 650 composting facilities exist. The total area exposed to such emissions is 2,600 km^2, that is nearly 1%. This calculation does not include microbial emissions from other types of activities or facilities, e.g. landfill areas, livestock facilities, sewage treatment plants, food-processing and recycling plants, and farmland. Taking the longevity of fungal propagules into account, the proportion of land potentially influenced by *anthropogenic* emissions of microorganisms must not be underestimated.

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