

FUNGAL COLONIZATION OF AIR-CONDITIONING SYSTEMS

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Abstract — Fungi have been implicated as quantitatively the most important bioaerosol component of indoor air associated with contaminated air-conditioning systems. Rarely, indoor fungi may cause human infections, but more commonly allergenic responses ranging from pneumonitis to asthma-like symptoms. From all air conditioner filters analyzed, 16 fungal taxa were isolated and identified. *Aspergillus fumigatus* causes more lethal infections worldwide than any other mold. Air-conditioning filters that adsorb moisture and volatile organics appear to provide suitable substrates for fungal colonization. It is important to stress that fungal colonization of air-conditioning systems should not be ignored, especially in hospital environments.

Key words: Indoor air, air-conditioning filters, micromycetes, human health

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INTRODUCTION

Fungi inhabit nearly all terrestrial environments. In this regard, the interiors of human dwellings and workspaces are no exception. The mycobiota of human-inhabited indoor environments consists of a distinctive group of organisms that collectively are not normally encountered elsewhere. Dust formation occurs as a result of the ongoing elutriation of airborne organic and inorganic particulate matter that originates from a multiplicity of indoor and outdoor sources (Scott, 2001). House dust is a fibrous material composed primarily of a matrix of textile fibers, hairs, and shed epithelial debris (Scott, 2001). Fungi commonly isolated from indoor air include *Alternaria*, *Aspergillus*, *Aureobasidium*, *Cladosporium*, and *Penicillium* species. Many of these species may contaminate indoor air through heating, ventilating, and air-conditioning systems (Simons et al., 1997). It is well known that ventilation systems, even those without water-containing components, may act as a potential microbial source in indoor air (Pasanen et al., 1997). Filters are porous soft insulation material very often used in air-conditioning systems. Direct microscopic examination of air filters reveals pollen particles, cellulose fibers, synthetic fibers, plant hairs, decayed leaves,

insect parts, dust mites, and many organic compounds (Moray and Williams, 1990). Cellulose and synthetic fibers probably come from indoor sources, while the other components most likely originate from outdoor sources. Insulation materials also absorb moisture and volatile organics and provide suitable substrates for fungal colonization (Simons et al., 1997). These organics represent excellent nutrients for fungal growth, with the result that air filters harbor an abundance of fungal hyphae and spores (Young, 1996). Dust and microorganisms may accumulate in supply air ducts during installation or later from the outdoor air due to leakages between the filter cassette and the assembly frame, or from insufficient efficiency of the filter (Pasanen et al., 1997).

Human infections caused by indoor fungi are very seldom because of the highly efficient defense mechanisms of human cells, such as the cell-mediated response (Perdeli et al., 2006). However, conidia and fragments of hyphae may sometimes cause allergenic responses, and some metabolites produced by fungi may be toxic or have immunomodulating activity in humans (Simons et al., 1997). Also, reduction in the host organism's defensive ability, whether due to cancer, AIDS,

organ transplantation, or any other medical reason, may lead to the uncontrolled multiplication of fungi and consequent onset of infection, sometimes with fatal effects (Perdelli et al., 2006). There are reports that four patients died and 11 contracted the respiratory disease aspergillosis at the Alcalá de Hanares Hospital, near Madrid (Spain). The disease was caused by inhaling the spores of *Aspergillus fumigatus*, which was later detected in the hospital's air-conditioning system (<http://www.acr-news.com/news>)

MATERIALS AND METHODS

Samples studied

Fifteen dust samples were collected from air-conditioning filters from school classrooms and offices. Also, five swab samples were collected from an air conditioner in a hospital surgical ward. None of the analyzed filters from classrooms and offices had been removed or cleaned for two years (Fig. 1a), whereas those from the hospital were cleaned once a month on a regular basis.

Direct examination

The tape lift technique used for direct examination allows for immediate determination of the presence of fungal spores and identification of the types of fungi present. Direct examinations should only be used to sample visible mold growth in contaminated air-conditioning filters. Samples were collected by pulling the tape of the filter surface with slow steady pressure, holding only the tape edges, after which they were put on slides for light microscopy.

Determination of total spore concentrations in dust

Culturable fungal spore concentrations are presented in terms of colony-forming units (CFU)/g of dust. Sub-samples (0.5 g) were taken from each dust sample and suspended in distilled water (0.0425 g/l KH₂PO₄, 0.25 g/l MgSO₄, 0.008 g/l NaOH, 0.02% Tween 80 detergent). Dilution series were prepared and three successive dilutions were plated in triplicate on malt agar medium (MA) with the antibiotic streptomycin, which was added during the preparation process in order to prevent bacterial growth

(Pasanen et al., 1997). The plates were incubated on $22 \pm 2^\circ\text{C}$ and read after 72 to 120 hours. Fungal colonies formed on the medium were identified on the basis of both macroscopic and microscopic characteristics of each isolated colony using identification keys (Ainsworth et al., 1973; Arx, 1974; Ellis and Ellis, 1997; Pitt, 1979; Raper, and Fennel 1965).

RESULTS AND DISCUSSION

The direct examination method revealed the presence of different fungal structures: conidia, conidiophores, chlamidospores and mycelia (Fig. 1b-f). From all dust samples analyzed from classrooms, offices, and hospital air-conditioning systems, six fungal genera with different numbers of species were recorded: *Cladosporium*, *Penicillium*, *Aspergillus*, *Alternaria*, *Epicoccum*, and one Ascomycotyna from the order Sphaeriales (Table 1). The fungal colonies isolated from different sources were characterized not only by the presence of different species, but also by their different abundance (Fig. 2a). The genus *Aspergillus* with five species was the most frequent (Table 1, Fig. 2b, e, f). The abundance of fungal colonies was much higher in classrooms and offices than in the hospital. *Aspergillus* and *Penicillium* species were dominant. *Alternaria alternata* and *Cladosporium cladosporioides* were the most frequent dematiaceous fungi (Fig. 2c, d). Fungal growth was quantified by the number of CFUs. Culturable spore concentrations in 15 dust samples varied from 10^4 to 10^6 CFU/g. In central Finland, culturable and total fungi in dust accumulated in air ducts in single-family houses varied from 10^4 to 10^8 CFU/g (Pasanen et al., 1997).

The results of this research confirmed previous findings that air-conditioning systems are highly linked with fungal pollution of indoor air. The diversity and abundance of fungal species isolated from different air-conditioning systems can be attributed to different ways of maintaining the systems themselves. Greater numbers of fungal genera and species with much higher colony abundance were expected and found in samples isolated from the air-conditioning systems from classrooms and offices since these systems were not cleaned after installa-

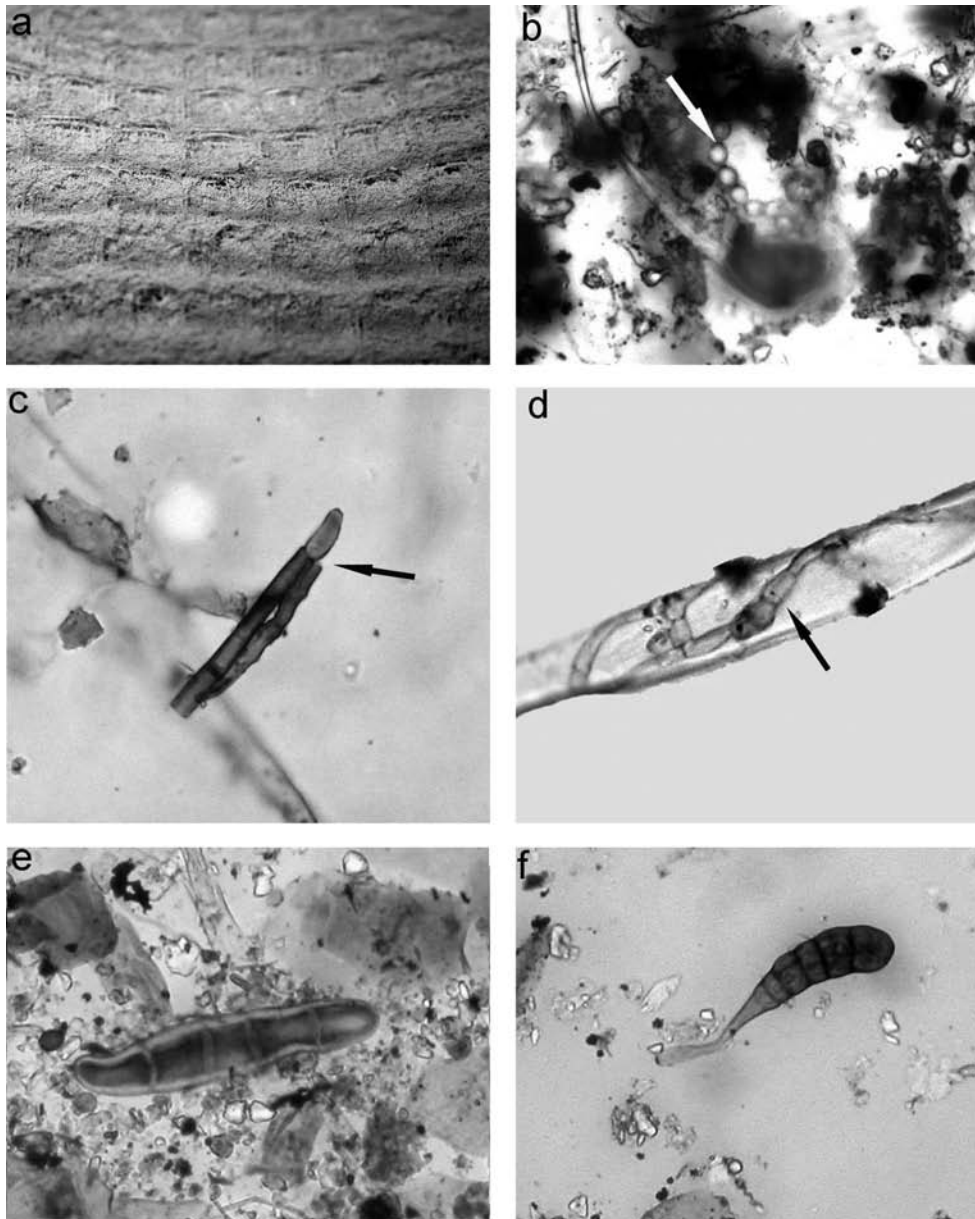


Fig. 1. Fungal structures from air conditioner filter dust: a) two years uncleaned air conditioner filter; b) the conidial chain of *Aspergillus ochraceus*; c) conidiophores with terminal conidium of *Cladosporium sphaeospermum*; d) mycelia with chlamydospores inside filter fiber; e) chlamydospore; f) conidia of *Alternaria* sp.

tion (Fig. 1a). Only two fungal species were found in samples from the hospital units, with very low colony abundance. This result is worrying because one of the two isolated species was *Aspergillus versicolor* (Fig. 2e). *Aspergillus* is a large genus of fungal molds, of which only a few species cause human

infections, most commonly *A. fumigatus*. Spores from these species are widespread in the environment, occurring in soil, in dust, and in outdoor and indoor air. Known as aspergillosis, fungal infection with *Aspergillus* ranges from the benign to the fatal. Healthy individuals usually inhale *Aspergillus* spores

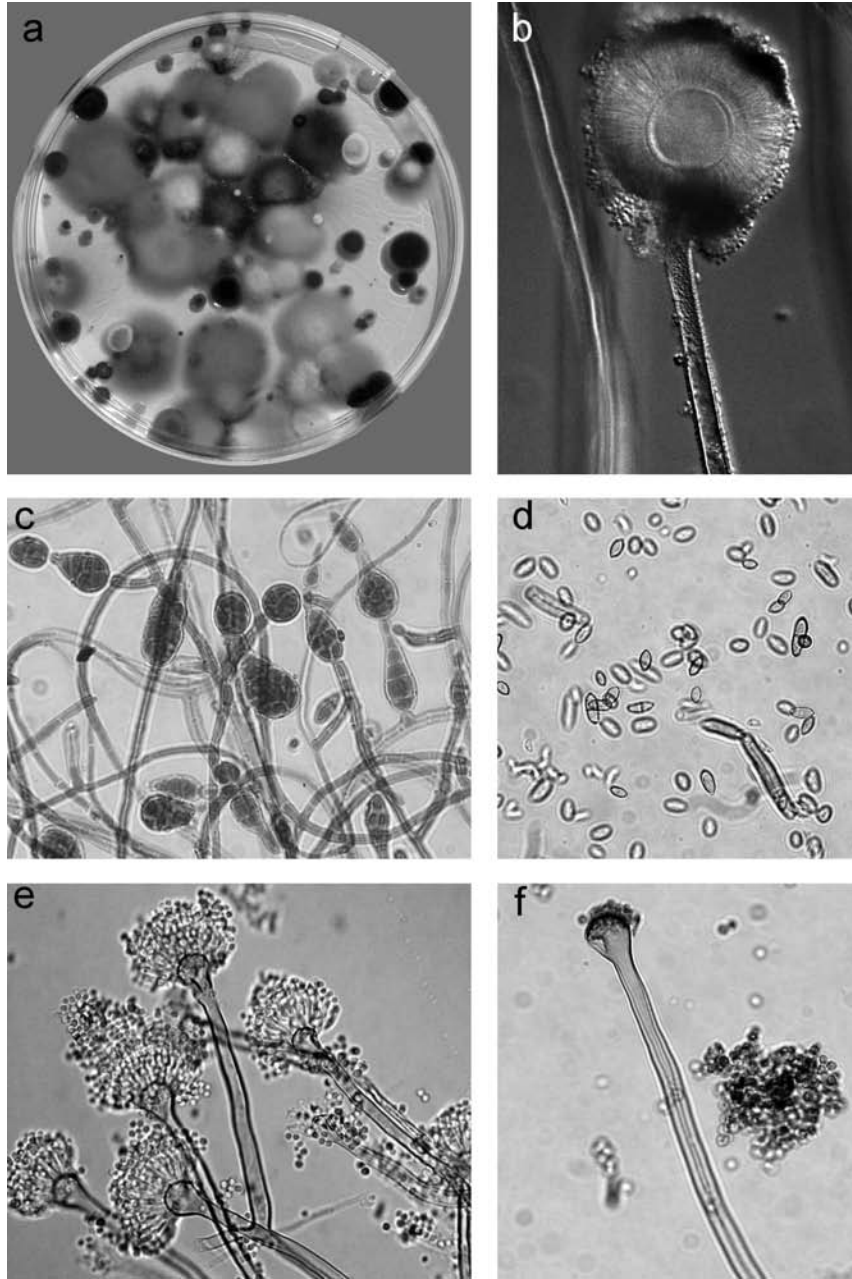


Fig. 2. Micromycetes isolated from air conditioner filter dust: a) fungal colonies on MA; b) *Aspergillus ochraceus*; c) *Alternaria alternata*; d) *Cladosporium cladosporioides*; e) *Aspergillus versicolor*; f) *Aspergillus fumigatus*.

without any untoward effects, but sometimes contract relatively benign infections of the lungs and sinuses. In susceptible compromised patients, however, inhalation leads to multiplication of the fungus in the lungs and subsequent invasive infection that

may spread to any organ of the body. Dissemination to the brain, gastrointestinal tract, and other organs occurs in up to 30% of cases. The disseminated infection is usually fatal, partly because early diagnosis is difficult and treatment often ineffective (French,

Table 1. Micromycetes isolated from analyzed filter dust of air-conditioning systems.

Fungal taxa	Classrooms, offices	Hospital
<i>Alternaria alternata</i>	+	
<i>Aspergillus flavus</i>	+	
<i>Aspergillus fumigatus</i>	+	
<i>Aspergillus niger</i>	+	
<i>Aspergillus ochraceus</i>	+	
<i>Aspergillus versicolor</i>		+
<i>Botrytis cinerea</i>	+	
<i>Cladosporium herbarum</i>	+	
<i>Cladosporium</i> sp.	+	
<i>Cladosporium</i> sp.	+	
<i>Epicoccum purpurascens</i>	+	
<i>Mycelia sterilia</i>	+	
<i>Penicillium verucosum</i> var. <i>cyclopium</i>	+	
<i>Penicillium</i> spp.	+	
Sphaeriales		+

2005). Carpet dust samples ($n = 11$) contained *A. versicolor* in concentrations ranging from $<2.5 \times 10^1$ to 3.6×10^5 (median, 3.1×10^4) CFU/g of dust, and the median proportion of *A. versicolor* in total culturable fungi was 18%. Based on thin-layer chromatography detection of sterigmatocystin, 49 of 50 *A. versicolor* isolates (98%) were found to be toxigenic *in vitro* (Engelhart et al., 2002). Many *Aspergillus* species are well known as potential producers of mycotoxins and other volatile harmful compounds, and many of them can cause aspergillosis in humans. Hospitals and other medical facilities are places where patients with damaged immune systems are commonly found. These patients are very receptive to fungal infections. In order to avoid unwanted fungal infections in hospitals, the air-conditioning systems must be subjected to regular maintenance in order to reduce potential fungal pollution.

Three features of mold biochemistry are of special interest from the standpoint of human health. Molds contain glucan, a compound with inflammatory properties. Spores and mycelial fragments contain allergens (Górny et al., 2002). The spores of some species contain low-molecular-weight chemicals that are cytotoxic or have other toxic properties. Some molds, such as *A. fumigatus*, can cause

opportunistic infection in immunocompromised and healthy individuals and severe allergic diseases, such as asthma or cystic fibrosis (Burge, 2000). In our previous investigations, *Aspergillus* species were recorded in different substrata. Fungal spores can spread from different sources and contaminate air conditioning filters (Kataranovski et al., 2007; Ljaljević and Vukojević, 1997).

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КОЛОНИЗАЦИЈА КЛИМА УРЕЂАЈА МИКРОГЉИВАМА

МИЛИЦА ЉАЉЕВИЋ ГРБИЋ, ЈЕЛЕНА ВУКОЈЕВИЋ И М. СТУПАР

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Микрогљиве, као квантитативно најзначајније биоаеросолне компоненте ваздуха у затвореним просторијама, су чести контаминанти клима уређаја. Оне могу изазвати алергијске реакције типа пнеумонитиса и симптоме сличне астми, а ређе могу бити и изазивачи хуманих инфекција. Из анализираних клима уређаја изоловано је и идентификовано 16 таксона микрогљива. Изо-

ловани *Aspergillus fumigatus* је познат као изазивач леталних инфекција широм света. Филтери клима уређаја који апсорбују влагу и органске материје су погодан супстрат за колонизацију микрогљивама. Веома је значајно напоменути да се колонизација клима уређаја микрогљивама не сме игнорисати и то нарочито у болничким установама.