

Chapter 11

Indoor Microbial Aerosol and Its Health Effects: Microbial Exposure in Public Buildings – Viruses, Bacteria, and Fungi

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Abstract Mechanisms of aerosolization of microorganisms, composition and dynamics of microbioaerosol are characterized. As well as methods of its detection, incl. modern equipment set-ups and sampling procedures recommended are outlined. Medical impact of (indoor) air dispersed viral, bacterial and fungal propagules (allergies, intoxications, infections), together with the related European legislation is summarized. An overview of real mycoaerosol conditions in our dwellings and their outdoors with different microclimate, settlement and building types, household characteristics and health state of occupants is given, too. Finally, examples of several possible health damages due to exposition to (aerosolized) fungal toxicants *in vitro* and *in vivo* are demonstrated.

Keywords Droplets · spores · hyphal fragments · aerodynamics · respiratory deposition · aeroscope

11.1 Introduction

Bioaerosols comprise microorganisms (viruses, bacteria, fungi), while other propagules may be originated from living organisms (pollen, plant seeds, wooden dust, fragments of animal hair, insects and their excrements etc.). All these components may occur in free forms or are carried by some vehicles, e. g., dust particles or droplets.

Microorganisms are released to the air from their growth sites, or colonized surfaces. Infections, allergies, intoxications, eventually even leading to pre- and cancers can be spread around by the mean of bioaerosolization. Aerosols are discussed also because of their possible misuse (anthrax vs. bioterrorism). The forementioned notices explain the scientific and public health interest in aeromicrobiology increasing over the last years (Piecková, 2013).

During a sneeze, millions of droplets of water and mucus are expelled at about 100 m/s. The droplets initially are about 10–100 μm in diameter, but they dry rapidly

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Table 11.1 Some human diseases transmitted interpersonally by inhaled airborne particles

Virosis (virus agent)	Bacterial disease (bacterial agent)
Chickenpox (Varicella)	Whooping cough (<i>Bordetella pertussis</i>)
Flu (Influenza)	Meningitis (<i>Neisseria</i> sp.)
Measles (Rubeola)	Diphtheria (<i>Corynebacterium diphtheriae</i>)
German measles (Rubella)	Pneumonia (<i>Mycoplasma pneumoniae</i> , <i>Streptococcus</i> sp.)
Mumps - Parotitis (Rubulavirus)	Tuberculosis (<i>Mycobacterium tuberculosis</i>)
Smallpox (Variola)	–

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Table 11.2 Some airborne human diseases dependent on the environmental source

Disease	Source
Psittacosis (<i>Chlamydia psittaci</i>)	Dried, powdery droppings from infected birds (parrots, pigeons, etc.)
Legionnaire's disease (<i>Legionella pneumophila</i>)	Droplets from air-conditioning - HVAC systems, water storage tanks, etc., where the bacterium grows
Acute allergic alveolitis (various fungal and actinomycete spores)	Fungal or actinomycete particles from decomposing organic matter (composts, grain stores, hay, etc.)
Aspergillosis (<i>Aspergillus fumigatus</i> , <i>A. flavus</i> , <i>A. niger</i>)	Fungal particles inhaled from decomposing organic mater
Histoplasmosis (<i>Histoplasma capsulatum</i>)	Spores of the fungus, in old, weathered bat or bird droppings
Coccidioidomycosis (<i>Coccidioides immitis</i>)	Spores in air-blown dust in desert regions (Central, South and North America), where the fungus grows in the soil

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to **droplet nuclei** of 1–4 μm , containing virus particles or bacteria. This is a major means of transmission of several diseases of humans, shown in the Table 11.1.

Several other diseases may be acquired by inhaling aerosolized microbial particles from environmental sources, not directly originated from an infected person (Table 11.2).

Psittacosis is a serious disease due to handling birds or inhaling dust from bird excrements. It is caused by the bacterium *Chlamydia psittaci*, an obligate intracellular parasite. After entering the respiratory tract, the cells are transported to the liver and spleen, multiply there and then invade the lungs, causing inflammation, haemorrhage and pneumonia (<http://archive.bio.ed.ac.uk>).

11.2 Viruses in Air

A virus can multiply only within a host cell. Infected cells can spread viruses directly into the surrounding air (primary aerosolization) or to fluids and surfaces, which can become sources for airborne transmission (secondary aerosolization).

Secondary aerosolization can occur for any virus, predominantly when air displacements or movements around contaminated surfaces or fluids disperse the viruses into the air. It can also occur by liquid splashes, which can aerosolize viruses in liquids or on surfaces. In fact, almost any kind of disturbance of infected organisms or materials, even the bursting of foam in seawater, can produce airborne, virus-laden particles (Aller et al., 2005).

Virus-laden particles are a complex mixture of various components (salts, proteins, and other organic and inorganic matter, including virus particles). The size of the viral particle itself does not rule the airborne particle size. The influence of viruses alone on the granulometric distribution of aerosols is likely negligible compared to that of the remainder of the aerosol. It was demonstrated that the particle size distribution of artificially produced submicrometer and ultrafine aerosols of culture media is not affected by the presence of bacteriophages (Hogan et al., 2005).

Relative humidity (RH) is the most widely studied factor that affects airborne virus infectivity. Depending on the virus, optimal preservation of infectivity may require a low RH (under 30%), an intermediate RH (30–70%), or a high RH (over 70%). Influenza virus, Japanese B encephalitis virus, Newcastle disease virus, and vesicular stomatitis virus, all of which are enveloped, are most stable at low RH, while rhinovirus, poliovirus, rhinotracheitis virus, picornavirus, and viruses of the Columbia SK group, which are nonenveloped (with the exception of the rhinotracheitis virus), are most stable at high RH. Human coronavirus 229E, pseudorabies virus, and rotavirus are most stable at intermediate RH. The first two are enveloped, while mature rotaviruses are usually nonenveloped. RH has no effect on the stability of airborne St. Louis encephalitis virus under the conditions tested (Verreault et al., 2008).

In 2001, a Norwalk-like virus outbreak in a school in the United Kingdom was believed to have been caused by airborne transmission. A similar occurrence has also been reported for a hotel restaurant (Marks et al., 2003). A retrospective cohort study conducted after a severe acute respiratory syndrome (SARS) epidemic in Hong Kong in 2003 suggested that airborne spread may have played an important role in the transmission of the disease. The same mode of transmission was also hypothesized in other studies of SARS (Li et al., 2005, Yu et al., 2005). Middle East Respiratory Syndrome (MERS) is an illness caused by a virus Middle East Respiratory Syndrome Coronavirus (MERS-CoV). Most MERS patients developed severe acute respiratory illness with symptoms of fever, cough and shortness of breath. About 3 to 4 out of every 10 patients reported with MERS have died. The disease was first reported in Saudi Arabia in September 2012. Later retrospective studies identified that the first known cases of MERS occurred in Jordan in April 2012. So far, all cases of MERS have been linked through travel to, or residence in, countries in and near the Arabian Peninsula. The largest known outbreak of MERS outside the Arabian Peninsula occurred in the Republic of Korea in 2015. The outbreak was associated with a traveler returning from the Arabian Peninsula. MERS-CoV, like other coronaviruses, is thought to spread from an infected person's respiratory secretions, such as through coughing. However, the precise ways the virus spreads are not currently well understood (<https://www.cdc.gov/coronavirus/mers/>, (2017)).

Seasonal variations in indoor RH have also been correlated with fluctuations in the morbidity of influenza (low RH) and poliomyelitis (high RH) viruses, with the highest morbidity occurring at the optimal RH for each virus. Seasonal variations have also been observed with measles virus and respiratory syncytial virus (Yusuf et al., 2007).

UV radiation is another factor that influences survivability of microbes. UV germicidal lamps, for instance, can be used to inactivate airborne microorganisms, including viruses, in indoor settings (First et al., 2007). However, in certain cases, RH must be taken into consideration. For example, vaccinia virus is more susceptible to UV radiation at low RH than at high RH (McDevitt et al., 2007).

The gas composition of the air can also have an influence on viruses, as ozone has been shown to inactivate airborne viruses. In fact, virus susceptibility to ozone is much higher than those of bacterial and fungal bioaerosols. However, the ozone efficacy varies from virus to virus. Ions in the air can also reduce the recovery rate of certain viruses, such as aerosolized T1 bacteriophage, with positive ions having the most detrimental effect (Tseng and Li, 2006).

While epidemiological data can help to determine the source of the contamination, direct data obtained from air samples can provide very useful information for risk assessment purposes of (viral) bioaerosols. Many types of samplers have been used over the years, including liquid impingers, solid impactors, filters, electrostatic precipitators, and many others. The efficiencies of these samplers depend on a variety of environmental and methodological factors that can affect the integrity of the virus structure. The aerodynamic size distribution of the aerosol also has a direct effect on sampler efficiency. Viral aerosols can be studied under controlled laboratory conditions, using biological or nonbiological tracers and surrogate viruses (Verreault et al., 2008).

11.3 Indoor-Air-Associated Bacteria

The majority of bacteria that are common indoors belong to the genera of *Micrococcus*, *Staphylococcus*, *Bacillus*, and *Pseudomonas*. They are distributed almost proportionally in the indoor atmosphere as well.

Micrococcus is a sphere-shaped G+, relatively harmless bacterium. It is very common on skin, and it can also be found in soil, water, and meat products. It is generally a saprophyte and can cause spoilage of food. This organism can also be responsible for causing human sweat to smell badly. In immunocompromised patients, it can be an opportunistic pathogen. Some common species include *M. luteus*, *M. roseus*, and *M. varians*.

Staphylococcus is another sphere-shaped G+ bacterium. It is much more known than *Micrococcus*, especially in the context of hospitals. Nosocomial infections (hospital-acquired infections, HAI) are caused mostly by methicillin resistant *S. aureus* (MRSA) strains of this bacteria. However, staphylococci are found almost everywhere, and their presence usually does not result in infection. They

are very common on skin, and can also be found in the nasal cavity, throats, and hair of 50% of healthy individuals. Food poisoning and skin infections, as well as toxic shock syndrome, are among the illnesses caused by *Staphylococcus*. *Staphylococcus* is the facultative anaerobic microbe.

Bacillus is a rod-shaped G+ bacterium. It has the ability to produce endospores – tough structures that can survive adverse environmental conditions. For the most part, bacilli are harmless saprophytes, and can be found in soil, water, dust, and sometimes within the human digestive system. Some species of *Bacillus* can cause food poisoning, and some can cause illness or infection. *B. anthracis* is the etiologic agent of anthrax — a common disease of livestock and, occasionally, of humans — and the only obligate pathogen within the genus *Bacillus*. The symptoms in anthrax depend on the type of infection and can take anywhere from 1 day to more than 2 months to appear. All types of anthrax have the potential, if untreated, to spread throughout the body and cause severe illness and even death. Four forms of human anthrax disease are recognized based on their portal of entry. Among them, inhalation, a rare but highly fatal form, is characterized by flu like symptoms, chest discomfort, diaphoresis, and body aches (Fig. 11.1). There were rather many attempts to misuse spores of *B. anthracis* in bioterroristic attacks reported so far (CDC, 2015).

Pseudomonas is another rod-shaped but G-bacterium commonly present indoors. It can be found in soil and water, and on plants. It is an opportunistic pathogen, and generally considered a nosocomial infection agent as the organism tends only to attack individuals that are immunocompromised. Along with infection, it also has the ability to produce exotoxins.

Some studies revealed also relatively high incidence of *Corynebacterium* in the indoor environment of certain public buildings (schools). These bacteria are

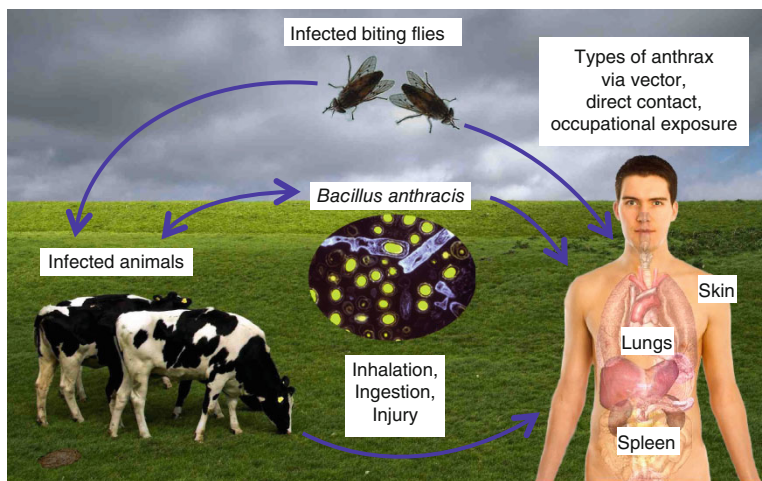


Fig. 11.1 The human exposure scenarios to *Bacillus anthracis* (Author: Renáta Lehotská)

rod-shaped G+ organisms omnipresent in nature. Some are useful in industrial settings, others can cause human disease, including most notably diphtheria, which is caused by *C. diphtheriae*. As with various species of a microbiota, they usually are not pathogenic but can occasionally invade into tissues (via wounds) or break through weakened host defense, that both results in certain infection process (Aydogdu et al., 2010).

While single virus or bacterium particles exist in the air, they tend to aggregate rapidly. Aggregation speed depends on the size distribution of the airborne particles, the concentration of the aerosol, and the thermodynamic conditions. It has been shown that a visually clean environment may be more contaminated by bioaerosols than a visually dirty one. This may be due to the fact that larger particles tend to settle faster than smaller particles do; the settling velocity of 0.001 μm particles is 6.75E⁻⁰⁹ m/s, while 10 μm particles settle at 3.06E⁻⁰³ m/s and 100 μm particles settle at 2.49E⁻⁰¹ m/s. Airborne particles in a “clean” environment are more likely to remain small and inhalable than are particles in a dirty environment, which tend to grow larger by sticking to other airborne particles (Verreault et al., 2008).

11.3.1 Legionnaire’s Disease

This is a common form of bacterial pneumonia in elder or immunocompromised people (Fig. 11.2). It is seldom transmitted directly from person to person. The bacterium *Legionella pneumophila* is an aquatic rod-shaped G-species found naturally in freshwater environments, like lakes and streams. with a temperature

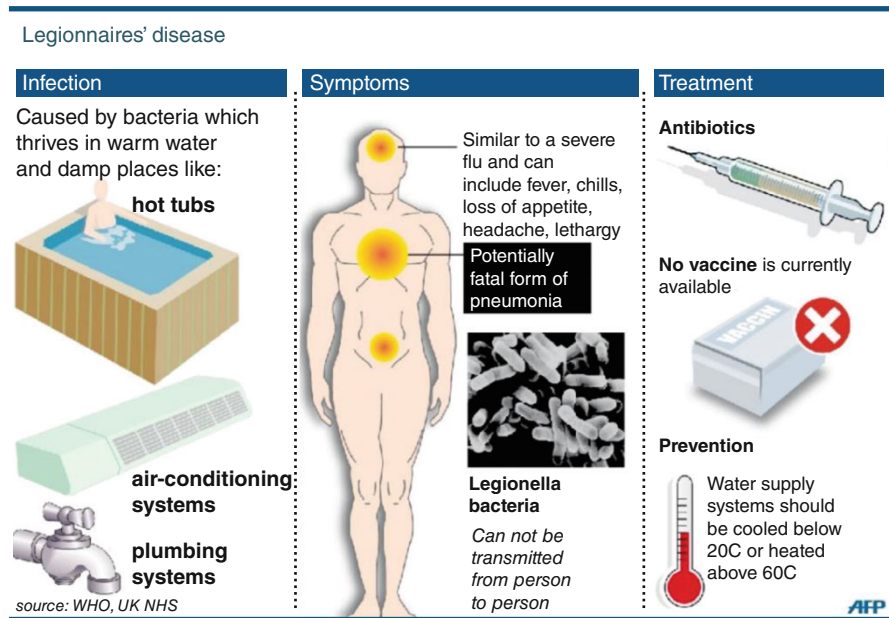


Fig. 11.2 Legionellosis summary (Bunbury, 2015)

optimum of about 36°C, and is a common inhabitant of warm-water systems in buildings like

- hot tubs that aren't drained after each use
- hot water tanks and heaters
- large plumbing systems
- cooling towers (air-conditioning – HVAC units for large buildings)
- decorative fountains

where it can become of a health concern. Infection occurs when people inhale aerosol droplets containing the bacteria. People who get sick after being exposed to *Legionella* can develop two different illnesses: legionnaires' disease and Pontiac fever. Symptoms usually begin 2–10 days after being exposed to the bacteria, but it can take longer, for about 2 weeks after exposure.

Pontiac fever symptoms are primarily fever and muscle aches; it is a milder infection than legionnaires' disease. Symptoms begin between a few hours to 3 days after being exposed to the bacteria and usually last less than a week. Pontiac fever is different from legionnaires' disease because someone with Pontiac fever does not have pneumonia necessarily (Correia et al., 2016).

The key to preventing legionnaires' disease is to prevent *Legionella* colonization (growth) in water systems. Low water volumes combined with high temperatures and heavy bather loads make public hot tub operation challenging. The result can be low disinfectant levels that allow the growth and spread of a variety of germs (e.g. *Pseudomonas* and *Legionella*) that can cause skin and respiratory recreational water illnesses (RWI). Operators that focus on hot tub maintenance and operation to ensure continuous, high water quality are the first line of defense in preventing the spread of RWI. Guidelines for reducing the risk of *Legionella* growth and spread are available for those who maintain and manage building water systems, including systems for potable (water for drinking and showering), non-potable, and recreational water from ASHRAE (ASHRAE Guideline 2000; ASHRAE Standard, 2015).

11.4 Fungal Bioaerosol

When dealing with mouldy (organic) materials, the air concentration of viable fungal propagules can reach the level as high as 10^9 colony forming units (cfu)/m³, incl. a broad spectrum of chemical fungal irritants (mycotoxins, volatile organic compounds). The occupants may suffer from mucous and skin irritations, but some severe acute or chronic damage of their respiratory tract (bronchitis, allergic alveolitis, “farmer lungs,” pulmonary mycotoxicoses) can take place as well. The occupational hygiene must, thus, develop and apply all necessary preventive and protective measures to minimize the bioaerosol hazard in particular plants (Piecková and Jesenská, 1999).

Rather different situation is encountered in mouldy dwellings and/or public buildings like offices, schools, waiting rooms, cultural premises etc., where even the youngest children, elderly people or other vulnerable occupants are staying,

even long time. From the indoor air of such dwellings, 3–450,000 fungal cfu/m³ might be recovered. Though, the quantity of viable and cultivable propagules of aeromicrobiota depends highly on activities carried out in the houses that elevate turbulencies leading to stronger dispersion of spores and hyphal fragments from the fungal bodies (colonies) around. The physiological characteristics of particular fungal species affect their aerosolization, too. Some micromycetes form hygroscopic spores stucked in slimy heads on their aerial mycelium (accremonia, fusaria), while others produce enormous quantity of small conidia in very fragile chains and columns (penicillia, aspergilli, cladosporia). And even other ones, large macrospores (alternariae). There is also a high number of devitalized germs, which can't be entrapped onto cultivation media, but their allergenic and toxic potential remains unchanged (Piecková, 2008).

11.4.1 Methods of Indoor Aeromicrobiota Detection

The indoor air samples in buildings are collected by the mean of different volumetric apparatuses – aeroscopes: germs are immobilized on agar plates or into the liquid medium (impingers). All of them enable qualified quantitative analysis of viable micro(myco)flora present. The dilution method may be employed to characterize the fungi associated with settled dust. And swabs or adhesive tapes are used to sample microbes from the surfaces. These samples first undergo the direct microscopic observation, followed by cultivation evaluation. The modern state-of-art in sampling methods has been broadened by modifications of PCR (microarrays), showing the presence of e. g. stachybotrys directly in the affected material, incl. the air. There are also laboratory setups with propagules' optical size counters coupled to computing of the aerodynamic size of the germs and their depository potential (Sivasubramani et al., 2004).

The Anderson sampler is an example of the impactor aeroscope for selective entrapping different sizes of particles according to their size (momentum). This sampler consists of a stack of 8 metal sections that fit together with ring seals to form an air-tight cylinder. Each metal section has a perforated base, and the number of perforations is the same in each section, but the size of these perforations is progressively reduced from the top of the column to the bottom. To use this sampler, open agar plates are placed between each metal section, resting on three studs. When fully assembled with an open agar plate between each unit an electric motor sucks air from the bottom of the unit, causing spore-laden air to enter at the top and to pass down through the cylinder. The path taken by this air is shown in Fig. 11.3 (<http://archive.bio.ed.ac.uk>).

Figure 11.4 shows the methodology applied indoor of a building (a private gallery) with offending mouldy smell to find its causative agents. Figure 11.5 illustrates the fungal spectrum commonly recovered from the school indoors in Slovakia over last 15 years.

It is also possible to detect the aeroconcentration of fungal ergosterol or betaglukan, that measure total fungal load, i. e. vital and devitalized particles. As there are

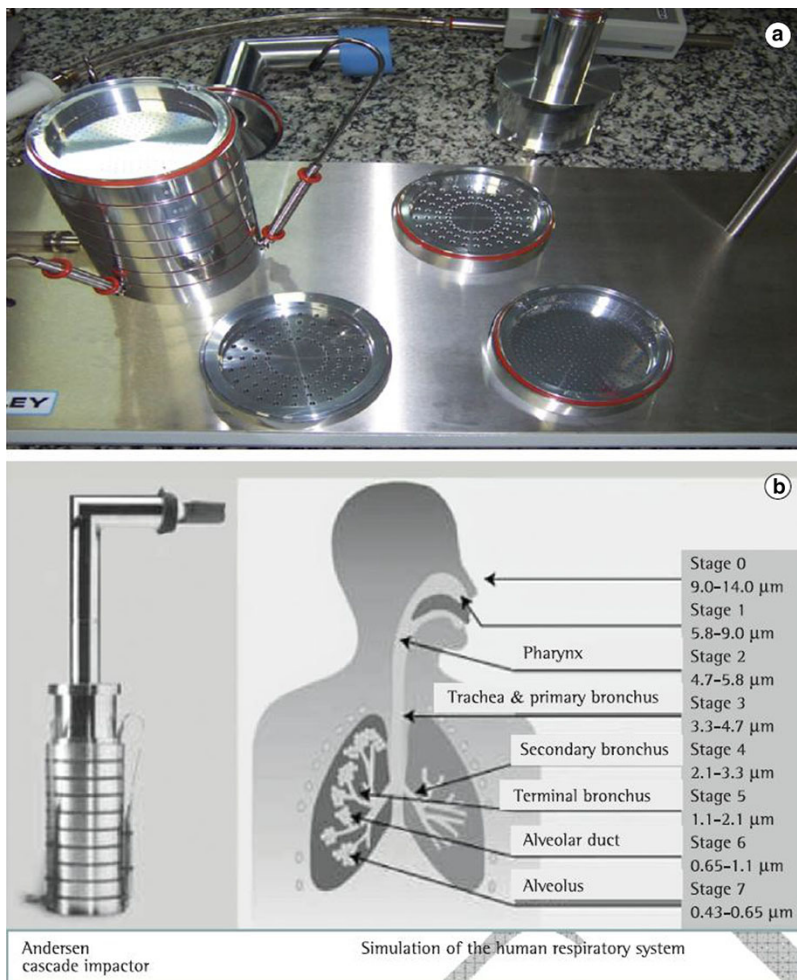


Fig. 11.3 Size distribution of airborne particles entrapped in the Anderson sampler and their airways’ penetrating potential in humans (Andrade-Lima et al., 2012)

remarkable differences in the ergosterol content among fungal species (from 2 up to 14 $\mu\text{g/g}$ of dry biomass), this method is recently ranked as the superficial only (Piecková, 2013).

Very recently, it has become concluded to use the fungal volatile organic compounds (VOC) as a new quantitative marker of undesired mould present in the indoor environment (Piecková, 2010).

The complex study of (fungal) bioaerosol should cover its source, properties and (health) effects.

Mouldy smell

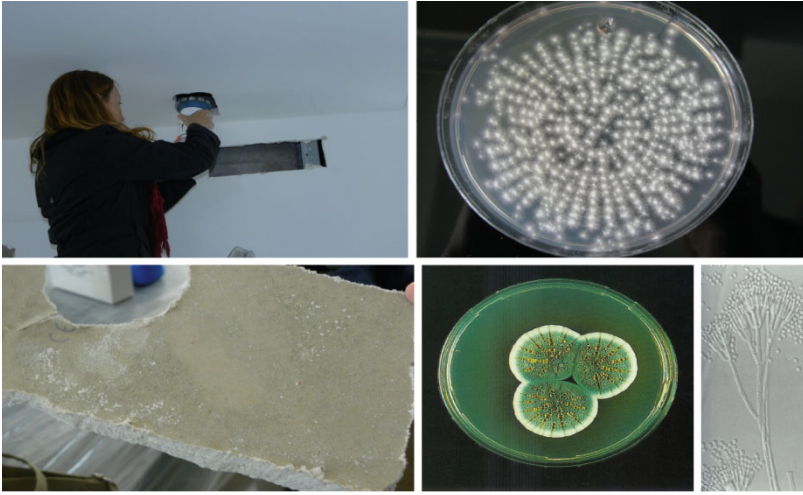


Fig. 11.4 Complex mycological evaluation of the indoor moulds in a private gallery house strongly affected by an excessive malodour

11.4.2 *Bioaerosol Source Characteristics*

The macro- and micromorphology of the micromycete releasing germs or toxicants into the air are usually well known. On the other hand, there is almost an absolute lack of knowledge on the biophysics of fungal growths, e. g. how strong force can cause the propagule release; what is the life dynamics of real natural fungal populations, effects of their biotope, intra- and inter-population relations etc.

11.4.3 *Bioaerosol Characterization*

Microscopic fungi reproduce by vegetative spores (microspores up to 10 μm , macrospores several tenths micrometers) and/or sexual spores (similar size to the microspores). The later ones are produced in fruit bodies (up to hundreds micrometers). The shapes and ornamentations of any spores are species-related. From the aerial dispersion facility point of view, the aerodynamic features of spores (do not copy with their physical size necessarily and are strongly connected with the bioaerosol concentration), their aggregation and conglomeration on its own or on any carriers (dust, pollen particles etc.; ca. 60 % of aerosol particles are present in aggregates) play the crucial role.

Macroscopic appearance of the microfungus body is represented by its colonies (thalus) of different surface structure and coloration. Hyphae of the colony grow superficially and in the substrate. It's become already possible technically to simulate mechanic irritation of the colonies (vibrations, airflow velocity) enabling

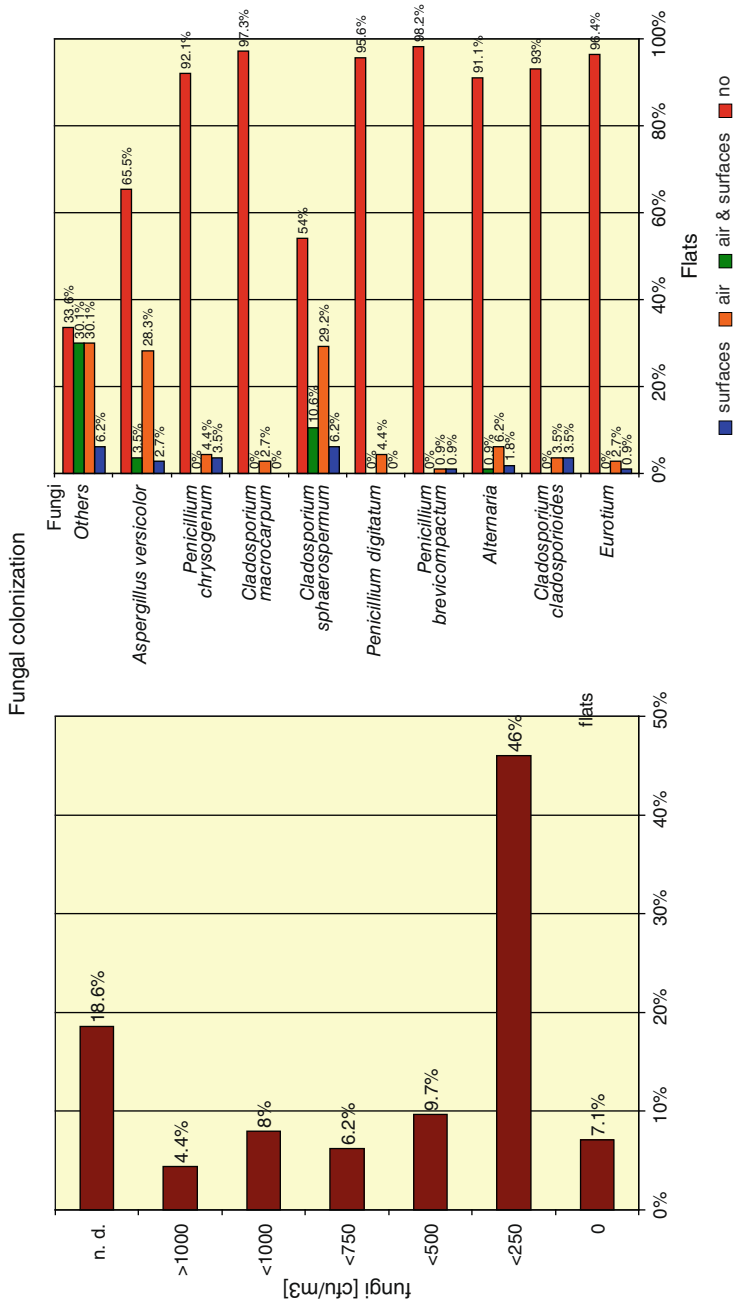


Fig. 11.5 Spectrum of indoor fungi isolated from Slovak schools over last 15 years

effective release of propagules to the air. Regarding that, e. g. the relation between colony texture and formation of aerial microturbulencies is studied recently.

Simultaneously with the spores, also the hyphal fragments are dispersed from the colonies (optical size ca. 1 μm), usually in hundred times higher counts (10^5 cfu/cm²) than the spore ones. Their deliberation depends on the structure of the overgrown substrate as well as on the age of the culture (so called dessication stress) and the concentration of the pre-existing bioaerosol (Górny, 2004).

Settlement of aerosolized germs, their survival and ability to colonize surfaces has their specific rules, too. So, the ability to grow under hygroscopic conditions enables the fungi to colonize/invade human airways.

11.4.4 *Bioaerosol Decay*

The aerosolized fungal propagules are usually aggregated in the units with the diameter of the water vapour (10–20 μm), thus, they form a gentle mist. Its particles then settle in 19–5 min from the level of 3 m (Górny, 2004).

11.4.5 *Inhalatory Exposition to the (Fungal) Bioaerosol*

Its dynamics is not commonly characterized under normal (housing) conditions, neither at the fungal-burst situation. Depository parameters of microbial bioaerosol particles are described only very marginally. While breathing via healthy nose, 30–40% of the particles are entrapped onto the upper airways mucose and other 30–40% pass through to the lower parts of the lungs, incl. alveoli. Mouth breathing, in contrary, enables penetration of ca. 70% of the propagules to the utmost fine pulmonary chambers. The ration of bigger and smaller germs and their aggregates, remains unknown. Asexual fungal macrospores (the biggest fungal germs) finish in the nose, throat and sinuses, microspores (a- and sexual) at the trachea and bronchi, and hyphal fragments may reach alveoli easily as they belong to so called fine particular matters, their depository potential is the highest, even related to other head cavities, incl. the skull ones.

The respiratory tract is highly effective in trapping airborne particles, with sometimes serious consequences for health. The mechanisms involved depend on particle size.

1. Large particles (about 10 μm) have sufficient mass to **impact** onto surfaces, even at low air speeds. They break free from the air as it flows around obstacles. During normal breathing, the airflow in the nose and trachea is about 100 cm per second – sufficient for pollen grains and larger fungal spores, esp. macrospores (*Alternaria* sp. etc.) to be retained on the mucous, where they can cause typical **hay fever symptoms** like (allergic) rhinitis and asthma. These are the types of particle detected on the top plates of the Anderson sampler.
2. Smaller particles do not impact at these air speeds, and the air speed decreases as the respiratory system branches further down. So all the particles of 5 μm or less

are carried deep into the lungs. There they can settle out by **sedimentation** in the brief periods when the air is calm between successive breaths. Particles of 2–4 μm are optimal for alveolar deposition, and this range includes the spores of many *Aspergillus* and *Penicillium* spp. This is how some of the serious fungal infections of humans are initiated – aspergillosis, histoplasmosis, coccidioidomycosis etc.

3. Even smaller particles, such as the spores of actinomycetes along with hyphal fragments (about 1 μm) are less efficient at being deposited in the alveoli, but repeated exposure to aerosol clouds can lead to sensitisation and **extrinsic allergic alveolitis** (farmer’s lungs etc.).
4. Very small particles, less than about 0.5 μm , do not impact but are moved by diffusion (**Brownian motion**) which brings them randomly into contact with surfaces in the lungs, and are able to cross other barriers in human body, e.g. meninges. This is true of the fine dusts that cause many (occupational) diseases.

Do the bacterial and viral pathogens copy this scheme as well?

The nasopharyngeal viroses are associated with large sneeze droplets which impact in the upper airways. Most bacterial diseases also are initiated in the upper airways, when bacteria are carried in large droplets or on “rafts” of skin that impact onto the mucous. However, infections by *Mycobacterium* sp. (**tuberculosis**) and *Bacillus anthracis* (**anthrax**) are initiated in the lungs. These are highly virulent pathogens, and even single cells or spores (about 3 μm for *Bacillus*) can initiate infections after deposition in the alveoli.

The macroorganisms cleans itself of the bioaerosol propagules by the mean of the mucosiliary effect (self-cleaning of the upper respiratory tract involving production of mucous and epithelium cells ciliary beating), or the macrophages. The possible damaging effect occurs at the level of the contact tissues (mucouses of the airways, eyes etc.; Fig. 11.6) up to the (sub-)cellular level (pulmonary alveolar macrophages; Fig. 11.7). The mechanisms might result in respiratory tract

Secondary metabolites

- *in vitro* toxicity

- **tracheal ciliary movement ceased in 24 h**
- **lectin histochemistry – T II lung cells:**

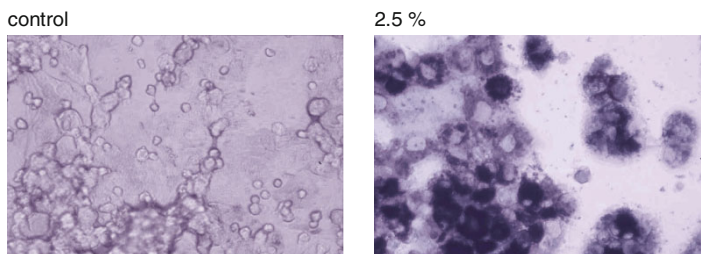


Fig. 11.6 Activity of indoor airborne *Aspergillus versicolor* toxins showing negative *in vitro* effects onto respiratory tract cells



Fig. 11.7 Activity of indoor *Aspergillus versicolor* and *Stachybotrys chartarum* toxins showing negative *in vivo* effects onto respiratory tract cells (arrows indicate elevation/depression of the biochemical or cytological parameters pronouncing the cell damage)

colonization, its inflammatory or cytotoxic debilitation, decreasing of the mucosal immunity (at least) that finally leads to recurred infectious (viral, bacterial, mycotic). The infectious loads of the particular moulds necessary to cause the ill health symptoms in humans are studied by epidemiology and infectology (Piecková, 2008).

To control and sustain so called healthy indoors, there have been adopted some hygienic limits of airborne microbes, incl. fungi – EU legislation and WHO guidelines (WHO, 2009; Reg. Min. Hlth SR, 2008). The basic limit was set down at the level of 500 cfu/m³, qualitatively copying the outdoor mycoflora, without toxic and pathogenic species.

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