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FILAMENTOUS MICROORGANISMS AND THEIR FRAGMENTS IN INDOOR AIR – A REVIEW

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Abstract: The paper summarizes the current state of knowledge regarding the role of filamentous microorganisms (i.e., fungi and actinomycetes) and their submicrometer propagules (fragments) in formation of indoor bioaerosol. It discusses the importance of water damages in buildings and the role of humidity as a cause of fungal and actinomycetal contamination and subsequent deterioration of indoor spaces. The importance of the size of airborne microbial propagules for adverse health effects is broadly commented as well. Regarding the microbial fragments, the method of their release from the contaminated surfaces (including factors influencing their aerosolization, i.e., air velocity, colony structure, moisture conditions, vibration of the surface, time factor), modern measurement techniques and newly obtained results of the immunological reactivity of fragments are discussed. The novel ideas concerning the dynamic description of the release process of microbial propagules from their sources are also presented.

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INDOOR AIR QUALITY – A SERIOUS ENVIRONMENTAL PROBLEM

Indoor air quality has always been within people's scope of interest. Every biohazard, which is unwelcome in the specific environment, can be counted as its contamination. Although there is no indoor space which is sterile and free from microbial contamination (except for special assignments, e.g., in the pharmaceutical or biotechnological industries), the presence of biological contaminants in low concentrations can be treated as a "normal". The contamination problem can be recognized, while the concentration of biological agent(s) arises above background level, permitted for a specific environment. The problem of microbial contamination of indoor spaces connected with biodeterioration of materials and buildings accompanies mankind in the dawn of its history. Probably the first reference about the destructive influence of fungal flora on human dwellings and clothes is found in the 3rd Book of the Bible, Leviticus [13]. Through the centuries, the development of civilization was inseparably connected with an expansion of microbial colonization on places and abodes in which humans had dwelled. Prehistoric times (confirmed by the analyses of rock paintings from Paleolithic caves) [23, 28, 68, 149], archeological investigations [14, 54, 85, 172] and conservation studies [55, 97, 127, 146] have revealed that the destruction of organic and inorganic materials was mainly connected with fungal and actinomycetal biodeterioration activities.

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MICROBIAL CONTAMINATION AS A RESULT OF WATER DAMAGES IN BUILDINGS

Buildings are constantly subjected to microbial exposure. During particular periods of building use, its construction elements undergo an environmental stress created by the presence of different forms of water. Each time when the water appears on the surfaces of construction materials or penetrates them through holes and cavities, it can provoke microbial contamination. Such a situation is particularly visible in the case of water damage. In buildings, this is relatively common and usually associated with mould problems. The scale of this phenomenon is confirmed by numerous studies. American investigations reveal that 27-56% of homes have problems with visible fungal contamination of surfaces, and/or bad quality of indoor air [25, 29, 38, 159]. In Europe, this percentage ranges from 12–80% [1, 11, 18, 20, 77, 84, 117, 123, 137, 139, 148, 173, 176].

Microbial contamination of buildings is very often connected with environmental disasters. One of the latest examples is the flood in Poland in 1997 [116]. In consequence, 20% of civil parishes sustained significant losses, 500,000 hectares of urbanized area with 680,000 dwellings and several thousand factories and institutions were flooded. It could be assumed that in such numbers and scale a long term effect damaging the moist buildings and provoking serious health outcomes may influence most of the family members, whose dwellings had not been rebuilt, drained or protected against moulds. The scale of such problems is usually high. Only in Germany the costs caused by mould damage in buildings are estimated to the amount of more than 200 million Euro per year [150].

HUMIDITY AS A KEY FACTOR INITIATING MICROBIAL CONTAMINATION OF BUILDING

Colonization abilities of microorganisms present in indoor environment are determined by the physical and chemical characteristics of building materials. Among the most important factors, the nutritional substances derived from building materials, together with the moisture content in the substrate, initiate the development of microbial colonies. Therefore, the growth of microorganisms depends to the highest degree on the availability of water freely bounded by adsorption and absorption forces in capillary spaces of a building material.

In microbiology, the moisture availability in hygroscopic and porous materials is described by the parameter of water activity, a_w, which is the ratio of the vapour pressure exerted by water in the material to the vapour pressure of pure water at the same temperature and pressure [45, 76, 112, 187]. Several studies have shown that a_w equal to 0.65 is the lowest value necessary to initiate the microbial growth on the material containing enough nutrition substances [1, 45, 48, 63, 112]. The a_w value below 0.55 causes DNA denaturation [66]. Hence, microbial activity and their ability to conquer the new surfaces increases as the water activity approaches 1, i.e., when water is freely available [101, 131]. Based on a_w parameter, fungal and actinomycetal microorganisms can be categorized according to their ability to initiate the growth on building materials and the order in which they appear on material's surface as primary, secondary, and tertiary colonizers. Such combined classification for several fungal and actinomycetal strains is presented below [2, 19, 48, 63, 64, 125, 147, 184]:

- primary colonizers (a_w<0.85): *Alternaria citri*, *Aspergillus* (*Eurotium*) *amstelodami*, *A. candidus*, *A.* (*E.*) *glaucus*, *A. niger*, *A. penicillioides*, *A.* (*E.*) *repens*, *A. restrictus*, *A. versicolor*, *Paecilomyces variotii*, *Penicillium aurantiogriseum*, *P. brevicompactum*, *P. chrysogenum*, *P. commune*, *P. expansum*, *P. griseofulvum*, *Wallemia sebi*;
- secondary colonizers (a_w=0.85-0.90): *Aspergillus flavus*, *Cladosporium cladosporioides*, *C. herbarum*, *C. sphaerospermum*, *Mucor circinelloides*, *Rhizopus oryzae*;
- tertiary colonizers (a_w>0.90): *Alternaria alternata*, *Aspergillus fumigatus*, *Epicoccum* spp., *Exophiala* spp., *Fusarium moniliforme*, *Mucor plumbeus*, *Phoma herbarum*, *Phialophora* spp., *Rhizopus* spp., *Stachybotrys chartarum* (*S. atra*), *Trichoderma* spp., *Ulocladium consortiale*, *Rhodotorula* spp., *Sporobolomyces* spp., *Actinomycetes*.

SURFACE BIODETERIORATION

Regarding the nutrition requirements, moulds are incredibly elastic and have very broad adaptation possibilities. These microorganisms gain basic nutrients, rich in carbon and nitrogen, due to the decomposition of organic materials [1, 31, 90, 112, 185]. The majority of fungi present in indoor environment is saprophytic, which means that in dwellings they gather nutrients from dead moist materials such as: wood, paper, paints, glues, soil, dust, food chips, etc. However, they are able to successfully grow on surfaces consisting of inorganic moist material (glass, fibreglass, metal or concrete) covered with dust, air contaminants or even finger-marks, creating invisible layer of biofilm [1, 21, 27, 31, 50, 135, 150, 174, 180]. The *Actinomycetes* resembles fungi in the decomposition of many organic compounds, such as lignin, pectin, chitin, keratin, collagen, elastin, and starch [90, 91]. Both groups of microorganisms during the growth process produce and excrete many strong enzymes and acids which can efficiently destroy and/or disintegrate organic materials. Among fungi there are many organisms with strong cellulolytic (e.g., *Trichoderma*, *Botrytis*, *Chaetomium*, *Alternaria*, *Stemphylium*), proteolytic (e.g., *Mucor*, *Chaetomium*, *Aureobasidium*, *Gymnoascus*, *Trichoderma*, *Verticillium* and *Epicoccum*), and lipolytic properties (e.g., fungi from the previous group plus *Paecilomyces*) [9, 36, 49, 52, 64, 93, 164, 165]. Fungi can also produce strong mycotoxins [39, 86, 95, 125, 126, 169, 170]. Among actinomycetes, especially within the genus of *Streptomycetes*, there are several strains with strong proteo- and collagenolytic properties [91, 134].

EPIDEMIOLOGY OF MICROBIAL CONTAMINATION IN A NON-INDUSTRIAL INDOOR ENVIRONMENT

The threat to human health caused by microorganisms is substantial. According to Zyska [187], in Poland 8 million people in 2.7 million dwellings are in danger due to mould allergens and mycotoxins contaminating building materials; 6 million inhabitants in 2 million dwellings are exposed to fungi derived from decaying wood. As stated earlier, fungi and actinomycetes have the ability to evoke biological corrosion of building materials and thereby load the indoor air with numerous harmful substances and structures possessing biological activity. This is particularly important in the case of microbial contamination of indoor air where often exposure to high concentration of biological aerosols initiate immunopathogenic reactions leading to serious health outcomes.

Although there is evidence that damp indoor environment and, in consequence, the growth of filamentous fungi, are strongly connected with symptoms and diseases of the human respiratory tract [15, 51, 78, 104, 107, 133, 159, 175]. The relationship between the amount of inhaled fungal propagules and induction of respiratory health outcomes, however, is still unclear and controversial [24, 51, 53, 79, 81, 82, 120, 136, 144, 166]. While some investigations show that adverse health effects in mouldy buildings are a result of exposure to high concentrations of airborne fungal spores [83, 138, 178], other studies have a problem drawing such a link, mainly due to the lack of difference between the fungal spore concentration in damp and healthy buildings [47, 82, 124, 163]. Adverse health effects, if they are noted, are associated with children [30] as well as with adults [29, 151]. They are observed in different indoor spaces from day-care centres [96] and schools [34, 119, 167], to homes [151].

Much less is known about the causative role of *Actinomycetes* in adverse health outcomes observed in indoor environment. This group of bacteria is relatively numerous indoors [56, 72, 111, 122] and can provoke acute lung diseases and allergic reactions [89, 100, 121, 155, 156, 162]. As has been shown, the spores of *Streptomyces* isolated from mouldy buildings are cytotoxic and able to stimulate mouse and human macrophage cell linings to produce proinflammatory cytokins, tumour necrosis factor, interleukin-6 and to induce the expression of inducible NO synthase with subsequent NO production [73, 80, 87, 88]. They are even more active *in vitro* than fungal spores [80].

PARTICLES IN HUMAN RESPIRATORY TRACT

Biological aerosols can penetrate into the human body through the nose, mouth and conjunctiva epithelium, bronchi and alveoli, as well as the epidermis (mainly on hands) [37]. In the human respiratory tract, the penetration depth and behaviour of bioaerosol particles depends on their size, shape, density, chemical composition and reactivity. Particles, which enter the respiratory system can be deposited by 5 major mechanisms: impaction, sedimentation, diffusion, interception and electrostatic precipitation [10, 16, 109, 142]. The majority of particles with a diameter greater than 10 µm, and up to 80% of particles with diameters between 5-10 µm, is trapped in the nasopharyngeal region due to inertial impaction and centrifugal condensation resulting from anatomic formation of these stages of the respiratory tract (where the air stream gain has the highest velocity) [128, 171]. For particles with an elevated ratio between their length and diameter (e.g, for fungal spore chains), these 2 processes are assisted by the interception mechanism [142]. For particles with diameters above 0.5 µm, the primary deposition mechanisms are sedimentation and impaction, which take place in bronchi, bronchioles, and alveoli, where the air velocity is low [118] and a probability of deposition is directly proportional to the residence time [171]. For particles of less than 0.5 µm, diffusion is the major mechanism for particle separation from the air stream. This process depends (inversely proportional) on particle diameter and is supported by electrostatic precipitation resulting from interaction between surface and particle charges [114, 115, 171]. Submicron particles, especially these below 0.1 µm, penetrate deeper in to the lungs and are deposited almost solely through the diffusion mechanism.

Several field studies show that microbial particles with diameters below 2.5 µm (i.e., actinomycetal spores, majority of fungal spores present in indoor environment), if they are inhaled, are the most dangerous for human health. Having abilities to avoid numerous defence systems in the respiratory tract (e.g, ciliated epithelium, mucus, saliva, etc.), they can load the body with high concentrations of very reactive compounds derived from microorganism propagules [57, 58, 160].

MEASUREMENT OF SUBMICROMETER BIOAEROSOL PARTICLES

There are several sampling instruments, which can be used in submicrometer particle measurements. The majority of them are used for particulate aerosol sampling. The necessity for the preservation of certain features (such as cell integrity, viability, biological activity) characteristic for the particles of biological origin can limit the application of particular samplers for bioaerosol measurements. Nevertheless, a relatively broad spectrum of instruments enables the selection of a proper sampler. Because a bioaerosol measurement is usually a 2-phase procedure, i.e., isolation of particles (e.g., microorganisms) with their subsequent identification, the ideal device should allow the simultaneous performance of quantitative and qualitative analyses.

The first device, for a tempting to perform these 2 tasks is the Ultraviolet Aerodynamic Particle Sizer® (UVAPS, TSI Inc, ST. Paul). This spectrometer measures 3 parameters in real time. It monitors aerodynamic size and

scattered light for particles from 0.5–15 µm, and the fluorescence characteristics of individual particles to distinguish airborne biological propagules (containing NADH, NADPH, and riboflavin) from most inanimate materials [3, 4, 5, 6, 10, 17, 69, 74]. The main advantage of this device is its ability to distinguish particles of biological origin in the cocktail of all environmental particles and to quantify them; detailed genus/species identification, however is not possible. Unfortunately, the UVAPS has the same weakness as many other real time samplers, i.e., physical separation of microorganisms for further analysis to the genus and/or species level is not possible. Therefore, to obtain quantitative and qualitative data, it is still necessary to use a combination of 2 (or more) samplers, for the measurement of bioaerosol concentrations and size selective separation, followed by qualitative macro- and microscopic evaluation. Within the submicrometer size range of particles, both microbial spores and vegetative cells can be found, as well as their own fragments and fragments of structural elements of their colonies. Regarding the fungal fragments, a majority of these small propagules derive from different structural elements of their spores and hyphae. In the case of actinomycetes, particles below 1 µm can represent both intact spores and fragments of spores or hyphae. Based on that, it seems to be of a great importance to select the device which collects with high efficiency all particles with diameters below 1 µm.

There are several instruments on the market today which can descent to a measurement and separation of the particles to the decimal or hundredth part of a micrometer. Among them are: 1. for the concentration measurements – optical particle counters (OPC), condensation nuclei counters (CNC), and aerodynamic particle sizers, 2. for size selective separation of particles – cascade impactors, and 3. combining both the above-mentioned features – electrical low pressure impactor. The optical particle counters, based on light scattering, measure the concentration of particles in the (optical equivalent) size range of 0.25–32 µm (e.g., Grimm 1.109, Grimm Aerosol Technik GmbH, Ainring). In the condensation nuclei counters, upon entering the instrument, particles pass through a saturator tube where they mix with an alcohol vapour and then through a condenser tube where alcohol condenses on the particles causing them to grow into droplets. These can be counted passing through a laser beam and producing flashes of light which are registered by a photodetector. Such types of counters can define particle concentrations within the range of particle diameters between 0.01-2 µm (e.g., P-TRAK™, TSI Inc., St. Paul). Aerodynamic particle sizers (aerosizers) quantify particles based on their aerodynamic diameters and light-scattering intensity. They give high-resolution, real-time aerodynamic measurements in the range from 0.2–200 µm (e.g., Aerosizer DSP, TSI Inc., St. Paul). Impactors are a single or multi-stage, multi-orifice samplers designed to measure the aerodynamic size distribution and mass concentration levels of particles. To detect particles down to hundredth parts of a micrometer, multi-stage impactors are usually used (e.g., Sierra 210 - 10-stage, Andersen Samplers Inc., Atlanta; Mark II - 8- and 7-stage or Marple 290 - 8-stage personal cascade impactor, all from Thermo Electron Corp., Atlanta). Such instruments consist of a series of jets and impaction surfaces. At each stage, an aerosol stream passes through the jets and impacts upon the surface. Particles in the aerosol stream with significant inertia settle upon the impaction area. Smaller particles pass as aerosols on to the next jet stage. By designing the following consecutive stages with higher aerosol jet velocities, smaller diameter particles are collected at each subsequent stage, giving the cascade effect of separation. In such a way, particles with aerodynamic diameters between 0.08-35 µm can be separated. The electrical low pressure impactor (ELPI™, Dekati Ltd., Tampere) enables real time particle size distribution and concentration measurements in the size range 0.03-10 µm. ELPI combines the accuracy of impactor size classification and rapidity of electrical detection for the same device. A precisely known charge given to particles in the charger is measured in real time with highly sensitive multichannel electrometers as the particles impact the collection plates. With ELPI it is possible to measure transient particle size distributions in a wide size range of particles and concentrations.

It should be clearly stated that the possibility to measure such small particles has its negative implications. As a cost of the descent to such small diameters, problems arise connected with the sampling and further analysis of the collected material. The application of the high flow rates during the sampling for a separation of submicrometer particles results in the creation of additional biological stress. Depending on the sampler, it can cause desiccation, bounce and reaerosolization, or even the death of biological particles as an effect of the impaction process or differences in electrical charges between bioaerosol particles and electrical elements of the analyzer [108, 168]. Usually, to have an opportunity to perform further analysis of the colleted microbiological material, it is necessary to apply additional specific separation technique (e.g., covering impaction plates with sticky tape or grease).

THE MICROBIAL SOURCE STRENGTH CONCEPT

Exposure to biological aerosols in the indoor environment is still insufficiently explored. A clear description of the dose-response relationship for the majority of biological agents cannot be established. One of the reasons seems to be the inadequacy of analytical methods applied in bioaerosol exposure assessment. In shortly, traditional methods of fungal and bacterial aerosol sampling and analysis focus on quantitative and qualitative evaluation of microbial spores and vegetative cells, omitting the role of small fragments of their structural elements. The duration of bioaerosol sampling

is usually short, and therefore not adequately represent the real degree of environmental contamination [75, 79, 104]. Moreover, in many situations, the bioaerosol sampling is not the proper way to evaluate the indoor air quality. Reliable measurement results can be biased at the time of measurement, i.e., the moment of microbial propagule release into the air may be sporadic, irregular, dependent on physical factors, sensitive to the specific environmental conditions (e.g., bioaerosol may not be well mixed in the air) and may not correspond well with the sampling time. Such spatial (e.g., far from the source) and temporal variations usually do not permit measurement of the maximum possible concentration of the microbial agent [110]. Hence, the exposure evaluation should contain, as its immanent part, the source identification and, if it is possible, credible measure of microorganisms.

Microorganisms can be sampled from the surfaces by different techniques using transparent sticky tape, swab sampling or contact plates. All these methods can identify the source but cannot evaluate the magnitude of emission of microbial propagules into the surrounding air. On the other hand, bioaerosol sampling documents in a specific way the presence of the source, but the lack of certain types of particles does not eliminate the possibility of their existence in the studied environment [33, 112]. Such a degree of uncertainty impels a new approach to indoor exposure assessment.

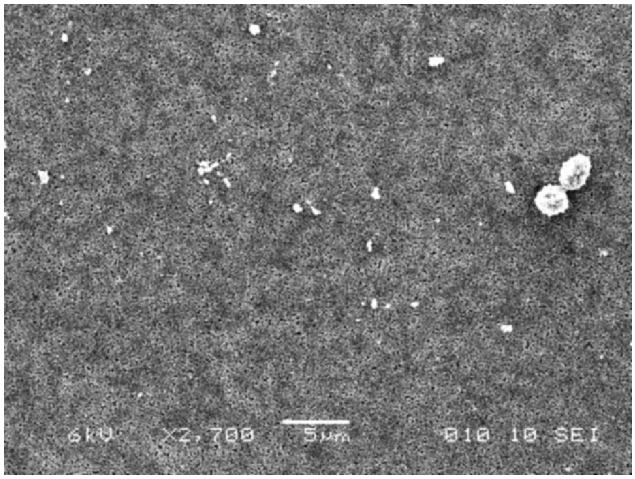
The first attempt, which tries to eliminate all the weak points of the data gathered by traditional bioaerosol sampling, is the microbial source strength concept [67]. This novel idea assumes dynamic description of aerosolization process of microbial propagules from their source (e.g., microbiologically contaminated surface of building material) through the quantification of particle emission rate provoked by the physical and biological factors. According to this concept, the aerosolization potential is not only limited to the emission from the source of fungal or bacterial spores or vegetative cells, but also includes the role of fragments of microbial colony structures. So far, these small propagules, constantly present in the environment, are not measured due to the lack of both proper method and a measurement device. Source strength in such understanding means a total ability of microbial source for maximal emission of propagules into the surrounding air under the most favorable release conditions. The microbial source strength concept combines in a new way the source of contamination (e.g., microbiologically contaminated surface) with its receptor (i.e. man). It allows the assessment of maximal potential exposure not to be dependent on the viability of aerosolized microbial particles as well as on temporal and spatial variations of propagule emission. To overcome all the limitations of conventional sampling methods and to reliably measure the release strength of microbial source, it is necessary to develop a new tool suitable for these types of analyses. In considering the above, in recent years, 2 prototype devices: aerozolization chamber and fungal spore source strength tester have been developed.

AEROSOLIZATION CHAMBER AND FUNGAL SPORE SOURCE STRENGTH TESTER

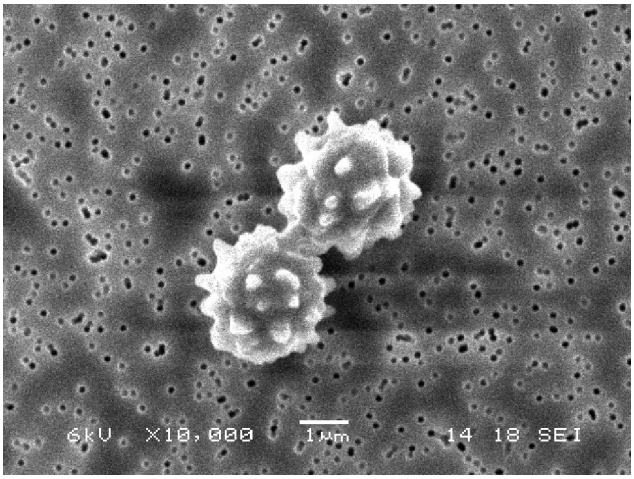
Both devices, i.e., the aerosolization chamber [detailed description in 59-62] and the fungal spore source strength tester [detailed description in 67, 152, 153] operate on the same principal. Fungal or bacterial fragments and spores from the contaminated material are released by passing clean, HEPA-filtered air over the surface with controlled airflow rates. Their concentrations can be controlled in real time both by a direct reading instrument (such as optical particle counter) and collected by particle (e.g., filter) or bioaerosol sampler. To enhance the microbial propagule release, the contaminated surface can be submitted to external vibration, as it takes place in aerosolization chamber. Both devices have the chance to fill the gap in the instrumentation of modern bioaerosol laboratory and become a sampler with broad practical application. So far, there is no instrument which allows for dynamic description of airborne transport of fungal and bacterial propagules from their source into the surrounding environment, based on their maximal emission rate. Each of these devices combines the features of the air and surface sampling, and thus gives a more objective and complete characteristic of exposure. This is especially important when reliable data for epidemiological analysis are required, but it can be successfully used as well in, e.g., efficiency evaluations of remediation actions (both constructions have protection systems preventing contamination of the surrounding air: the aerosolization chamber is tightly sealed within the measurement system [61], fungal spore source strength tester keeps the difference between incoming and outgoing flow rates to the cup to create a negative pressure [153]).

MICROBIAL FRAGMENTS AS A COMPONENT OF INDOOR BIOAEROSOL – WHAT DO WE KNOW TODAY

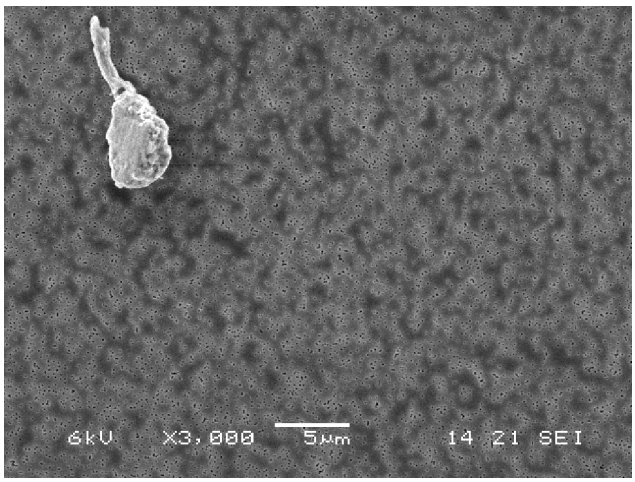
The hitherto obtained results using the aerosolisation chamber show that a significantly higher number of microbial propagules with diameters smaller than those of the spores is released from microbiologically contaminated surfaces [59, 60]. Even with the sensitivity of the device used for the control of fragment concentration (e.g., an optical particle counter, such as Grimm OPC allowing descent to the level of 0.3 µm for the particle diameter), the number of aerosolised fragments can be several hundred times higher than the number of released intact spores from the same surface area. The presence of the submicrometer propagules released in such a way can be documented by scanning electron microscope (SEM) analysis. The particular components of fungal colony structures, i.e., fragments



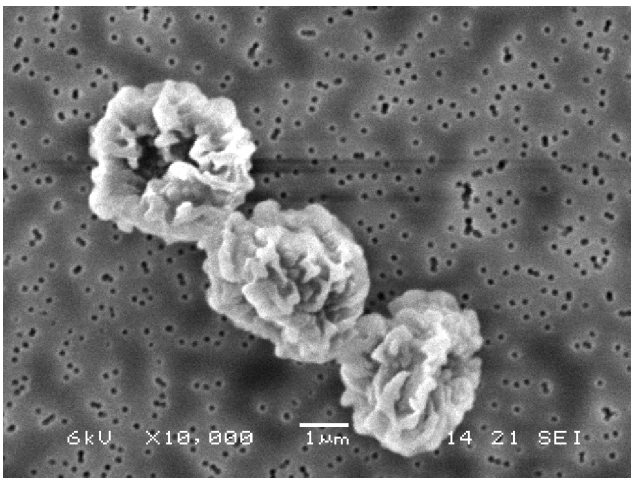
Aspergillus versicolor: Fragments and spores



Spores



Penicillium melinii: Fragment connected with immature spore



Spores

Figure 1. SEM pictures of fungal fragments and spores released from a ceiling tile surface contaminated with *A. versicolor* and *P. melinii* (the pictures were taken in Scientific and Technical Centre Building, Marne-la-Vallée, France).

and spores, aerosolised from contaminated ceiling tile surfaces are shown in Figure 1.

The presence of fragments in the air is well documented with pollen exposure, when seasonal asthma attacks begin several weeks before the exact period of pollen grain dissemination are detected in the air [140, 161]. In contrast, the role of fragments in fungal exposures has not been sufficiently recognized. The reason for this may be that fine and ultrafine fragment propagules cannot be detected with traditional bioaerosol sampling. The conventional sampling methods do not permit the carrying out of this type of analysis. Moreover, the most "popular" measurement procedures are based on bioaerosol incubation, which – taking into account the viability of, e.g., fungal spores at the level of 1-25% – visibly underestimate the real exposure [46, 70, 92]. Despite these difficulties, there are a few studies in which the concentration of fungal fragments derived from mycelium or spores are big enough to be counted by light microscope were measured. Sorenson *et al.* [158], Li and Kendrick [106], and Robertson [145] confirmed the presence of fragments in the air and detected their concentration on an average level of 29-146 particles per m^3, i.e., 6.0–6.3% of the total fungal propagules in the air. Madelin and Madelin [113] revealed that the mycelium fragments are often aerosolized from microbiologically contaminated surfaces, and some of these pieces preserve their viability and are capable of starting a new seat of growth. It is also possible that the fragments are pieces of spores and fruiting bodies, or are formed through nucleation from secondary metabolites of fungi, such as volatile organic compounds (VOCs).

FACTORS INFLUENCING THE RELEASE PROCESS OF FUNGAL AND ACTINOMYCETAL FRAGMENTS AND SPORES FROM MICROBIOLOGICALLY CONTAMINATED SURFACES

Air velocity. The hitherto obtained results confirm that generally the increase of the air velocity above the microbiologically contaminated surfaces augments the emission rate of the fungal and actinomycetal propagules [59, 61, 62, 65, 132, 186]. However, in specific situations,

e.g., as an aerosolization from agar, the release of fungal fragments from smooth surfaces is not affected by the air velocity. The different trend in the release of these submicrometer propagules, compared to that of intact spores, indicates that the fragments are aerosolised through a different process from that for spores. In the case of fungi, it can be assumed that the fragments are already liberated from the mycelium or spores before the air currents carry them away. Thus, all the fragments are aerosolised at low air velocity, and an increase in the velocity does not increase their release. The increased release from rough ceiling tile surfaces appears to be related to the higher air turbulence effect above the surface cavities [62]. The particular components of fungi (hyphae, conidiophores, and spore chains) overgrew almost the entire surface on both materials. Stereomicroscopic observations have revealed that for ceiling tiles, growth occurs not only on the top surface, but the fungal colonies also grow in each of the surface cavities. The fungal mycelium rises vertically upward, creating a mesh-like structure in the recesses of the ceiling tile surface. The higher air velocity with increased turbulence is more likely than the lower air velocity to release fungal propagules from the surface cavities.

Colony structure. Based on microscopic observations [59, 61, 62], it can be concluded that the colonies of fungi such as *A. versicolor* and *P. melinii* have thin, long conidiophores and create long chains of round spores, whereas the conidiophores of *C. cladosporioides* are short and thick and the chains of oval spores are less numerous. During exposure to air currents, elongated *Aspergillus* and *Penicillium* colony parts (conidiophores, metulas, and phialides), as well as other structural elements (e.g., joint areas between the spores), are much more susceptible to desiccation stress (because of the larger exposed area) than the respective *Cladosporium* structures, and probably become much more brittle when subjected to air turbulence created within the surface cavities of the contaminated material, or to vibration (see below). It might be worth while mentioning that in the case of some fungi (such as *Aspergillus niger*) the length of their conidiophores is decided by the amount of the secondary metabolites produced [44], and, through that, influences the reactivity of fragments derived from these structural elements when inhaled.

In the case of actinomycetal fragments, these are most probably neither fragments of the substrate mycelium, which is hard and firm, nor fragments of arthrospores, which are smooth and compact. It appears that these small fragments are pieces of the spiral, spore-bearing hyphae of aerial mycelium, or remnants of the fibrous sheaths surrounding the developing spores [183]. On the contaminated surface, the aerial hyphae originate as simple branches of the substrate mycelium and may cover the entire submerged growth in the form of a cottony mass with a powdery, chalk-like surface. The spores are produced from the aerial mycelium by regular septation of a hyphae enclosed within a fibrous sheath [179]. This sheath is a relatively thin, delicate, extracellular entity [40, 181] and is composed of elongated, hollow or grooved elements, finer fibrillar elements, and amorphous material of nanometer size range [182]. In the *S. albus* experiments, it was observed that the air streams passing over the contaminated surface were probably strong enough to break the integrity of the spore-bearing hyphae of aerial mycelium or the fibrous sheaths surrounding the developing spores of actinomycete colonies, and release substantial amount of fragments into the air [62].

Moisture conditions. The difference in the fragments released between contaminated surfaces can be partially caused by the differences in the material moisture conditions. As was shown in the experiments comparing fungal growth on agar and ceiling tiles, the moisture from agar can penetrate the thick layer of fungal growth and thus can increase the adhesion forces and reduce the release of fungal propagules. It should also be noted that the adhesion forces are higher for fungal fragments than for fungal spores due to the smaller size of the fragments [62].

Vibration. Under normal indoor conditions, building materials are under the constant influence of different vibration sources. Walking, dancing, crowd jumping, children playing, door slamming, listening to music, as well as operating domestic appliances and heating/air-conditioning units have an impact on the floors and walls of buildings. Indoor vibrations can be generated not only by internal but also by external sources such as earthquakes, car traffic, forging hammers, etc. All these mechanical disturbances create vibrations with frequencies between 1-20 Hz [7, 12, 26, 35, 71, 94, 154, 157, 177]. The study performed with vibrated ceiling tiles contaminated with fungi [60] revealed that the highest number of propagules was usually aerosolised by the combination of 1 Hz frequency and 14 W vibration power, compared to other combinations of vibration parameters, i.e., 1 Hz/4 W, 10 Hz/4 W and 10 Hz/14 W. This arrangement of low vibration frequency and relatively high power probably provides a sufficient amount of energy to detach the spores from the mycelium and to break the hyphae structure as well. Besides, if the building material is submitted to the combined effect of vibration and high air velocity (which can be found, e.g., in ventilation ducts), its aerosolisation potential of fungal propagules is much more stronger than this, when the more gentle combination of mechanical disturbance with the lower air velocity simulating typical indoor air currents is applied.

The structure of the microbial colonies probably plays an important role in the release of their propagules when exposed to vibrations. Elongated fungal structures (such as *A. versicolor* or *P. melinii*) seem to be more susceptible to mechanical disturbances than more compact colonies (which can be created by, e.g., *C. cladosporioides* or *S. albus*). The energy supplied by oscillation frequencies can

probably break the connections between the structural elements of the hyphae (conidiophores, metulas, phialides, joint areas between subsequent spores) faster and easier, and thus provoke the aerosolisation of fragments and spores. Moreover, if the colony structure is sufficiently dense, it can prevent additional aerosolization of particles. Even when a strong air stream (e.g., 29.1 m s^{-1}) is applied over the contaminated surface, as observed in the case of *S. albus*, the additional release force in the form of vibration does not affect the number of aerosolised fragments and spores.

Release in time. A significant portion of fungal propagules can be aerosolised from contaminated surfaces during a very short time interval. The study on the release of fungal and actinomycetal fragments and spores from contaminated agar and ceiling tile surfaces revealed that air currents at 29.1 and 0.3 m s^{-1} combined with a surface vibration are able to be aerosolised during the first 10 minutes up to 86%/90% and 53%/76% of the microbial fragments/spores, respectively i.e. during a very short time interval [59, 62]. Such a high number of fungal and bacterial propagules generated during a relatively short time period can significantly contribute to the indoor air quality as well as partially explain the "concentration bursts" and, by that, the spatial and temporal variations in microorganism concentrations observed indoors.

Relationship between aerosolized fragments and spores. In the context of the above-presented findings on the emission potential of microbial propagules, and in the situation where the majority of bioaerosol studies is still carried out using traditional incubation methods, it seems advisable to question how to prognosticate the number of aerosolized fragments based on the known number of fungal or actinomycetal spores in the air. The simplest attempt to answer this is to check the correlation between both microbial propagules. As the hitherto obtained data show, the number of fungal fragments released into the air at high air velocities characteristic, e.g., for ventilation ducts (29.1 m s^{-1}) can be predicted based on the number of aerosolized spores. At the air velocities present in the indoor environment, e.g., 0.3 m s^{-1}, such inference would be burdened with a significant error [62]. In the case of actinomycete propagules, the prediction of the number of fragments, taking into account the measured number of spores, seems to be baseless [59]. These data univocally show that for biologically active submicrometer propagules (see below), their measurements in the indoor environment should be included (apart from the measurements of intact spore concentration) in bioaerosol exposure assessment procedures.

IMMUNOLOGICAL REACTIVITY OF FUNGAL FRAGMENTS AND SPORES

Comparison of allergic responses of spore and *mycelium* extracts revealed that both these structural elements of

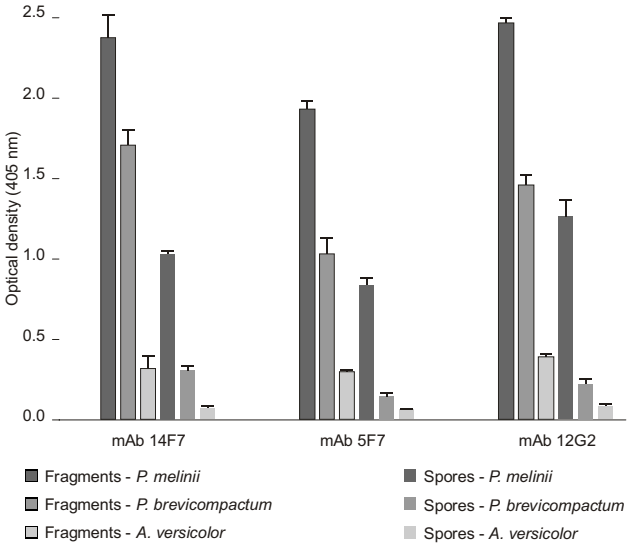


Figure 2. ELISA reactivity (defined as optical density) of fungal fragments and spores with 3 monoclonal antibodies: mAb 14F7, mAb 5F7, and mAb 12G2. The error bars indicate the standard deviation of 3 repeats.

fungal colonies share common allergens but their reactivity varies. Sometimes, the reactivity of *mycelium* extracts exceeded those obtained from spore extracts [8, 42, 43, 129, 130]. The experiments conducted using ELISA test with monoclonal antibodies (mAbs) produced against *Aspergillus* and *Penicllium* fungal species confirm this observation and show that, the doses represent real exposure (i.e., when fungal fragments and spores are released from the same area of contaminated surfaces during the same sampling time), the immunological reactivity of fungal fragments exceed that obtained for the spores (Fig. 2) [62].

The majority of fungal allergens are proteins ranging in molecular weight from 10,000–80,000 Da [98, 102, 105]. Despite this fact, most of today's allergen extracts are a mixture of proteins, glycoproteins, and carbohydrates or other additional substances [41, 139], or can be enzymes, as observed in the case of the most prevalent indoor fungi such as *Aspergillus* and *Penicillium* [32, 99, 139]. The electron microscope observations combined with immunological methods allow the localisation of these molecules in the fungal cell wall, membrane plasma, and cytoplasm [103, 141]. The study of protein content in *P. brevicompactum* extracts obtained by fractional sampling of fungal propagules aerosolised from contaminated agar surface, reveals that the protein concentrations in the extracts contained fragments are higher (up to 14.0 µg ml^{-1}) than those for spore extracts (up to 13.3 µg ml^{-1}) [22]. Hence, the fragments can be treated as a means of transport for dissemination of fungal allergens in the indoor environment.

In the scientific database there is now broad information about the immunological reactivity of fungal fragments extracts estimated by their cytotoxicity and the production of proinflammatory mediators. The preliminary studies carried out with *A. versicolor* extracts obtained by

the air sampling of propagules released from contaminated agar and ceiling tile surfaces, show that the doses of fragments and spores representing the real exposure (i.e., released at the same time from the same area of contaminated surface) result in different cytotoxicity for mouse macrophage cell lining RAW264.7. Whereas the number of fragments (10^6) and spores (10^5) aerosolised from agar surface cause the same percentage of cell deaths (between 11.5–13.5%), the respective doses released from the ceiling tile surface (10^8 and 10^6) kill between 17.9% (spores) and 41% (fragments) of the exposed cells [143]. The production of proinflammatory mediators, i.e., cytokines: tumour necrosis factor a (TNF-a), interleukin 6 (IL-6), and nitric oxide (NO), by the mouse macrophage cell lining RAW264.7 under the influence of fragment and spore extracts obtained by the aerosolisation of *A. versicolor* and *P. brevicompactum* propagules from agar and ceiling tile surfaces, varies. This process strongly depends on the fungal species and type of surface covered by the microbial growth [Reponen, Górny – unpublished data].

CONCLUSIONS

Fungal fragments, so far not measured in indoor air environments, are aerosolised in high numbers and, may thus contribute to adverse health effects. They can, at least partially, be responsible for the symptoms observed among inhabitants with a mould problem and/or water-damage in buildings. The hitherto obtained results on the fragment release from microbiologically contaminated surfaces, and on the immunological reactivity of these submicrometer propagules, show that future exposure assessment studies should contain, as their immanent part, the measurements of the load of fungal and actinomycetal fragments in the indoor air.

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