MICROSCOPIC FUNGI IN DWELLINGS AND THEIR HEALTH IMPLICATIONS IN HUMANS

Elena Piecková, Zdenka Jesenská

Institute of Preventive and Clinical Medicine, Bratislava, Slovakia


Abstract: The article reviews the quantitative and qualitative incidence of microscopic filamentous fungi in dwellings, methods for their detection, mycotoxins, glucans and volatile organic compounds produced by microscopic fungi in the indoor air of homes. Characteristics and properties of the most important species of fungi in dwellings (Alternaria spp., Aspergillus spp., Cladosporium spp., Fusarium spp., Penicillium spp., Stachybotrys spp., and Wallemia spp.) and the health problems of occupants of the "moldy" homes are also discussed.

Address for correspondence: Ing. Elena Piecková, MPH, PhD, Institute of Preventive and Clinical Medicine, Limbová 14, 833 01 Bratislava, Slovak Republic.
E-mail: pieckova@upkm.sk

Key words: fungi, dwellings, Alternaria, Aspergillus, Cladosporium, Fusarium, Penicillium, Stachybotrys, Wallemia.

Nobody can be content living in a dwelling with mold growing on walls, damp indoor air and the bad smell of mold. In such a situation people are often frustrated because of subjective discomfort, and in some cases their health conditions have been adversely affected, though the origin and real cause of all their complaints are difficult to define.

The problems connected with the presence of microscopic fungi (commonly called molds) in the working environment and their effects on the health status of employees have been studied in many papers and it has been found that health conditions of people working with moldy materials can be affected. The air of working environments may contain as much as 10⁹ viable germs of microscopic filamentous fungi (MFF) and yeasts per m³ and a great amount of various mycotoxins. Regarding the character of the substrates with which people work, irritation of eye, nose and mouth mucous membranes were found, as well as serious acute and chronic damage of respiratory organs, i.e. bronchitis, allergic alveolitis “farmer’s lung”, lung mycotoxicoses and similar disorders. Some mycotoxins can also possess cancerogenic properties in lung tissue [14, 15, 36, 86].

While the work with moldy materials is mostly sporadic and the possible adverse effects of the microscopic fungi and their toxins can be foreseen and thus the exposed persons can be protected using respiratory filters, families including infants living in homes with moldy walls are exposed to these noxae for a long time.

In Canada - an extensive questionnaire study was carried out and the relations between the damp and moldy dwellings and the occurrence of respiratory symptoms in infants and adults, was clearly confirmed. In Belgium, in connection with the worldwide oil crisis in 1970, many occupants of the homes reduced room temperatures when heating, and the houses were also poorly aired. Consequently, a sharp rise in the amount of MFF in dwellings was found and the incidence of patients with allergic diseases increased [90]. Similar conditions in home heatings were found by hygienists in Germany in 1992–1994 and the claims of home occupants to treat the problems of moldy dwellings increased [85].

The aim of this review was to present a survey of literature - as complete as possible - on the present problems of microscopic fungi in dwellings from the view point of
filamentous fungi in dwellings. For this review we have studied the findings and results found in our collection of over 13,000 papers dealing with the problem of microscopic fungi, published in journals worldwide. In our literature references we present only those authors who have presented - in our opinion - the most important knowledge. In addition, we also include our own results obtained during scientific studies and expert examinations.

QUANTITATIVE AND QUALITATIVE REPRESENTATION OF MICROSCOPIC FILAMENTOUS FUNGI IN DWELLINGS

The amount of colony forming units of MFF (cfu/m$^3$) in indoor air of dwellings differs in the reports of various authors: 3-6000, average 654 cfu/m$^3$ [45], <12-450000 cfu/m$^3$ [33], 165-850 cfu/m$^3$ [60], 100-2300 cfu/m$^3$ in "moldy" dwellings [60], 500-1000 cfu/m$^3$ - current standard [56].

The amount of viable and culturable microscopic fungi in indoor air is very variable. Spores from the growth on substrates are liberated into the air in places with higher turbulence, during home maintenance, cleaning, dusting, vacuum cleaning and vegetables peeling, when a door is opened, when a dog or cat enters or leaves, etc. At these times, the number of isolated colonies can be increased, in some cases even 3,000 times [33, 48, 94, 95].

The liberation of the spores into the indoor air depends on the physiological properties of individual species of microscopic fungi colonizing the relevant home: spores of some fungi are formed on the air mycelium in small drops of slime and are thus liberated into the air only in a low amount, e.g. Acremonium spp., some Fusarium species. Other species form tiny spores in high amounts making some fungi are formed on the air mycelium in small drops of slime and are thus liberated into the air only in a low amount, e.g. Acremonium spp., some Fusarium species. Other species form tiny spores in high amounts making

Methods for detection of microscopic fungi in dwellings

Isolation techniques. Samples from the indoor air can be taken with various samplers. The gravitation method is simple, but we obtain only orientation data and the results can be misleading [45, 56]. It is also possible to examine the dust from vacuum cleaners with the dilution plate method, but it is preferable to take samples directly from the moldy localities or substrates, and to inoculate them onto media aiming to prepare direct preparations on slides for the microscopic evaluation of the microstructures of fungi.

Isolation media. Individual species finding matching conditions for colonization and reproduction in some homes have in vitro various physiologic demands, it is therefore recommended when examining the samples to use more kinds of culture media: Sabouraud’s agar, Sabouraud’s agar with higher NaCl content, or with higher glucose or sucrose content. DG 18 agar and others, all media with incorporated antibacterial antibiotics [75].

Stachybotrys chartarum colonies grow on agar media slowly, therefore their germs in dwellings may be not detected. Therefore, their pronounced cellulolytic activity is used and thus a 1 ml sample of dust diluted in saline is inoculated into a Petri dish with sterile paper [56]. The growth of this species is observed with the naked eye or with a magnifier.

Ergosterol. Some professionals take for advantageous to determine the ergosterol amount in the examined air or dust samples since this analytic method can also detect
the devitalized germs of fungi. In the examined dust samples from households 4–45 µg ergosterol/g were found. However, it was found that there are great differences among the individual fungal species regarding the ergosterol content in their biomasses, e.g. *A. fumigatus* comprises 14 µg/g, while other fungi, e.g. *A. niger, Cladosporium* sp. and *Penicillium* sp. far less, only 2–8 µg ergosterol/g of dry culture mass [2, 5]. Therefore, the method for ergosterol determination in a sample is not always considered suitable.

**MYCOTOXINS**

Mycotoxins are natural products - secondary metabolites of molds able to initiate a toxic response in vertebrates, when introduced in small concentrations by a natural route (mouth, respiratory system or the skin). The toxic properties and degree of their toxicity vary depending on the administration, chemical structure and concentration [27]. The toxic effects of ingested moldy foodstuffs or feedstuffs, usually of plant origin (crops, seeds, nuts, dried fruits and vegetable, beans, etc.), are very different, comprising acute or chronic damage to the liver, kidneys, gastrointestinal tract, the heart, central nervous system and the immune system.

*Aspergillus flavus* and *A. parasiticus* strains are able to produce the mycotoxins aflatoxins; the hepatocarcinogenic aflatoxin B1 is considered to be the most potent carcinogenic substance of biological origin. The carcinogenic ochratoxin A produced by *A. ochraceus* and *Penicillium verrucosum* strains on stored crops can cause nephropathies.

Species of *Fusarium* and *Stachybotrys* produce macrocyclic trichothecenes with potential effects on the immune system, and proteosynthesis. *S. chartarum* strains produce dermatotoxic and cytotoxic trichothecenes stachybotryotoxins and satratoxins. The fusarium trichothecenes (e.g. T-2 toxin, deoxynivalenol, diacetoxy-scarpenol etc.) are able to cause haemorrhagic syndromes. Some fusaria can also produce zearealenone - the mycotoxin with estrogenic effect [6]. *Fusarium moniliforme, F. subglutinans* and *F. proliferatum*, important spoilers of maize, produce fumonisins, etc. These mycotoxins are responsible for several animal mycotoxicoses (leuкоencephalomalacia in horses and pulmonary edema in swines) and possibly also for esophageal cancer in humans [54].

Production of mycotoxins is dependent on water activity of substrate and heat conditions. The mycotoxins with various chemical structures (e.g. kumarine compounds like aflatoxins and sterigmatocystin, small lactons like patulin, etc.) are relatively small organic compounds (less than 1000 daltons). Detection of mycotoxins is usually performed by thin-layer chromatography, high-performed liquid chromatography, and by some immunochemical, or biological analytical methods [7].

**Mycotoxins in indoor air.** Recent research has concentrated on the alimentary intake and direct skin contact with these toxic fungal products. Mycotoxins located inside as well as on the surface of the spores have been demonstrated. It has been found that the potency of the respiratory route is higher than the alimentary. The dose of mycotoxin required to cause particular effects is typically one order of magnitude less when administered by the respiratory tract than by ingestion [29]. Experimental growth on wallpaper glue agar demonstrated that *Aspergillus versicolor* produced the carcinogenic sterigmatocystin, and *Penicillium expansum* released the nephrotoxic citrinin and patulin with modulatory effects on phagocytosis [27]. Macrocyclic trichotheccenes produced by *Stachybotrys chartarum* have been reported as etiological agents of chronic health disabilities of people living in moldy dwellings [21].

**GLUCANS**

Glucans are polyglucose compounds which represent the major structural components of fungal cell walls. The potential role of fungal glucans, especially (1→3)-B-D-glucans, as inducers of chronic pulmonary disease has been reported. Glucans could be associated with signs of a non-specific inflammation. The water insoluble form causes a delayed response in term of decrease in macrophages and lymphocytes in the lung wall, 1–7 days after exposure [17]. Glucan levels above 1 ng/m³ caused symptoms of chronic bronchitis, joint pains, itchy nose, chest tightness and heaviness in the head [77, 93]. Studies demonstrated that glucan can be used as a marker for risk of airway inflammation.

**VOLATILE ORGANIC COMPOUNDS RELEASED BY MICROSCOPIC FUNGI INTO THE INDOOR AIR OF DWELLINGS**

Volatile organic compounds (alcohols, aldehydes, esters, hydrocarbons, terpenes, ketones, acids and other low molecular substances) can be the cause of disagreeable odour in damp homes. The present intensive investigation of these metabolites released by microscopic fungi revealed that there are great numbers of such volatile compounds (VOCs). Their qualities and amounts are often due to environmental conditions and biological properties of the producers. It was found that some of them belong to the known carcinogens [91, 98], Ethylhexanol, e.g., produced by *A. versicolor*, has disagreeable, offensive odor [8]. In some well known toxinoigenous strains a direct relation was found between the mycotoxin production and production of volatile products, e.g. ketones [67]. It is proposed that volatile products of microscopic fungi be used as a new quantitative characteristics in the examination of damp homes [12]. For this purpose suitable determination methods have been studied [47].
CHARACTERISTICS AND PROPERTIES OF THE MOST IMPORTANT SPECIES OF MFF IN INDOOR AIR OF DWELLINGS

**Alternaria (Nees) Wiltshire**

Fungi of *Alternaria* genus are characteristic and striking due to their dark large crosswise and lengthwise septated conidia.

**Allergic diseases.** In men professionally exposed to wood and sawdust during paper production in paper mills, sensitization to the antigen of *Alternaria* sp. and interstitial hypersensitivity pneumonitis and bronchitis were found [81]. From a group of 292 patients tested for sensitivity against *Alternaria iridis* extract, in nine patients allergy to that antigen was recorded, nevertheless, most patients were found to be “multiallergic” suffering from conjunctivitis, asthma and rhinitis [19]. It was also found that young atopic humans with respiratory disorders when aged 14–19 years react in the skin test to *Alternaria* allergens more often (20% positive) than when aged over 30 years, and also more often than to *Aspergillus*, *Penicillium* and *Botrytis* spp. allergens (7–11%) [43]. *Alternaria* spp. are usually estimated as the most important fungal allergens [44].

**Indoor air in dwellings.** *Alternaria* spp. were found in the indoor air of homes in the USA (24–32% of examined places), in Canada (in 87% of examined households, 0-282 cfu/m³, on the average 30 cfu/m³), in Ontario, Canada (on the average 52 cfu/m³), in the Netherlands (in 8% of homes and in 25% of schools).

The most commonly known *Alternaria* spp. metabolite is tenuazonic acid, but these fungi are known to produce over 70 various mycotoxins and phytotoxins [59].

*Alternaria alternata* is regarded as the main cause of allergic and asthma in children aged 6–11 years [28] and to be a xerophilic, xerotolerant and acidophilic fungus. The spores germinate at 25°C at aₚ 0.85, minimum aₚ for mycelium growth is 0.88, minimum temperature 0°C, optimum 20–25°C, minimum air humidity 85%, optimum 98%, for conidia formation the optimum aₚ is 0.90, minimum air humidity 90%, optimum up to 99%.

**Aspergillus Michel**

Regarding the health conditions of the occupants whose dwellings have moldy walls, it is important to pay special attention to the species *A. clavatus* and *A. fumigatus*, to the species from the groups *A. niger* and *A. versicolor*, but rarely to other fungi of that genus.

Another group of the *Aspergillus* fungi found in raw food materials and victuals forms an important and large part of microbial population in dwellings. Till now it is not known whether they could invade the human organ tissues as parasites, or whether they could be harmful to human health as producers of certain important mycotoxins consumed with contaminated food. They are mainly various xerophilic and osmophilic species, and for most of them the name of teleomorphic stage is preferred.

**Indoor air in dwellings.** In the USA, the *Aspergillus* spp. germs were found in 9–29% and in Scotland in 74% of the examined homes. In Ontario, Canada, their concentration in the air of dwellings was 22 cfu/m³.

In general, it is thought that the amount of *Aspergillus* spp. germs is higher in indoor air than outdoors at the same time. In a home, the amount of germs is vehemently increased when the cleaning is carried out mechanically, for example, when carpets are vacuum cleaned.

*Aspergillus clavatus* is often associated with allergic alveolitis (hypersensitivity pneumonitis) in workers of malthouses [26, 76]. Minimum aₚ value for its growth is 0.85.

*Aspergillus fumigatus* is the most important and well known potential pathogen for humans with affected immunity.

**Allergic diseases.** As much as 10.8% out of asthma patients had positive reactions to the extract from *A. fumigatus* strain. Nevertheless, *A. fumigatus* possesses similar biochemical structure and antigenic properties to *Penicillium glabrum* (frequentans), *P. verrucosum* var. *verrucosum* and *A. versicolor*. *A. fumigatus* spores have the ability to be bound on lung epithelium in asthma patients, causing complications in the health status of the patients [9]. In that case, the course of the patient’s disease assumes a very destructive character [43].

This pathogen was found in the indoor air of dwellings, e.g. in South California in 2.9% and in the Netherlands in 4.5% of examined dwellings. In wallpapers of a hotel in Singapore its incidence was as high as 18–88%. In an air conditioning system in Saudi Arabia this pathogen formed 11% of all fungal isolates and its concentration was 15700 cfu/g dust.

When examining materials from indoor work environment it was found that about 70% of *A. fumigatus* spores are able - due to their size - to penetrate into the trachea and primary bronchi and less than 1% into alveoli [57].

The best known toxic metabolites of *A. fumigatus* are mainly fumigaclavin A, B, C, and D, spinulosin and tremorgenous toxins, e.g. verruculogen. Some rarely isolated strains produce kojic acid, sterigmatocystin and various unknown toxins affecting *Artemia salina* and *Bacillus megaterium* NRRL 1366, chicken embryos and other organisms. From 10⁶ spores of the *A. fumigatus* SRRC – 2006 strain an amount of 9.89 ng of fumigaclavin A was isolated [55]. In five out of eight *A. fumigatus* strains isolated from the indoor work environment in a sawmill, fumigaclavin C and verruculogen were found [46]. The investigation was...
conducted in connection with the assumed lung mycosis in workers. In the filtrate from mycelium and medium the presence of the hemolytic toxin was demonstrated, containing large protein amount and traces of saccharides [100].

Minimum temperature for mycelium growth is 10–12°C, optimum 37º to 43°C, minimum relative air humidity 85%, optimum 98% and for conidia formation minimum 90%, optimum 98–99%. Minimum aw value for A. fumigatus growth is 0.94 and for sporulation 0.95. No spore germination at 4–8°C was observed, optimum germination temperature is 25°C and optimum water activity is 0.94. Optimum pH value of the environment for A. fumigatus ranges from 3.0–8.0.

Aspergillus niger. Aspergillus species, characteristic with their striking black pigmentation of the colonies, are classified within a large A. niger group. In most papers describing the isolation of such strains they are presented simply as A. niger. However, in that case it is difficult to differentiate which species were involved in the activity described below in the indoor environment and in the pathologic process.

Indoor air in dwellings. The A. niger isolates were described in homes in London and Central Scotland, in Pízech (Czech Republic) (0.71–1.88% of all isolates) and in Egypt (15% of all isolates). They were found in Saudi Arabia in the dust from an air conditioning system (10600 cfu/g and 7.93% of all isolates) and in dust from an air conditioning system in the indoor environment and in the Netherlands (in 9 of 11 examined homes, in 32% of all examined homes and in 50% of examined schools). It formed 16–27% of isolates in homes in Turin and 11.5% of isolates from moldy wallpapers. A. versicolor was found also in dust in dwellings in Saudi Arabia (15000 cfu/g and 7.66% of all isolates). This fungus is placed among the primary wall colonizers at 25°C in dwellings [25] and was also found in dust from mattresses.

A. versicolor spores germinate in 12–20 days in dependence on the temperature and the pH values of substrates and at the aw values ranging from 0.75–0.81. The aw values reported for this species are within the range 0.78–0.98, minimum growth temperature is 6–9°C, and optimum temperature is 25–27°C.

Most of the relevant papers deal with the production of toxic and carcinogenic sterigmatocystin by A. versicolor [96]. The amount of toxigenic strains is rather high. We counted arithmetically the number of tested strains and strains producing sterigmatocystin described in 14 papers and found that there were 256 strains tested of which 191 (74%) produced this toxin under various laboratory conditions. From the 16 strains of A. versicolor isolated in the Czech and Slovak Republics sterigmatocystin was produced by 11 (68.7%) strains [96].

Aspergillus versicolor has an antigenic structure similar to that of Penicillium glabrum (frequentans) [20]. After an inhalation of A. versicolor spores, laboratory rats after one month exposure showed granulomatous lesions in lung tissue, localized mainly near to the bronchi [89].

Indoor air in the dwellings. A. versicolor was found in the indoor air of homes in London, in Central Scotland, and in the Netherlands (in 9 of 11 examined homes, in 32% of all examined homes and in 50% of examined schools). It formed 16–27% of isolates in homes in Turin and 11.5% of isolates from moldy wallpapers. A. versicolor was found also in dust in dwellings in Saudi Arabia (15000 cfu/g and 7.66% of all isolates). This fungus is placed among the primary wall colonizers at 25°C in dwellings [25] and was also found in dust from mattresses.

A. versicolor spores germinate in 12–20 days in dependence on the temperature and the pH values of substrates and at the aw values ranging from 0.75–0.81. The aw values reported for this species are within the range 0.78–0.98, minimum growth temperature is 6–9°C, and optimum temperature is 25–27°C.

Most of the relevant papers deal with the production of toxic and carcinogenic sterigmatocystin by A. versicolor [96]. The amount of toxigenic strains is rather high. We counted arithmetically the number of tested strains and strains producing sterigmatocystin described in 14 papers and found that there were 256 strains tested of which 191 (74%) produced this toxin under various laboratory conditions. From the 16 strains of A. versicolor isolated in the Czech and Slovak Republics sterigmatocystin was produced by 11 (68.7%) strains [96].

Cladosporium Link ex Fries

Cladosporium species (their older, but no more correct name is Hormodendrum species) are often found in various materials by laboratories engaged in environmental mycology. Colonies are not formed on media with a low pH value 3.5.

Allergic diseases. It has been reported that 0.7% out of 292 or 2.9% out of 2916 examined patients were allergic to C. herbarum allergens, but most of the patients were multiallergic [10, 19]. Atopic infants are the most sensitive patients against Cladosporium spp. allergens, in skin tests positive reactions were found even in 42% children aged up to four years. With increasing age, the positive reactions showed decreasing tendency [43].

Indoor air in dwellings. In general it is thought that Cladosporium spp. occur more often in the outdoor air than indoors. Cladosporium spp. were found within homes in the USA (70% of examined sites) [51], in Canada (in all homes in an amount ranging from 12–4637, on an average 437–456 cfu/m³) [18], in Saudi
Arabia (in dust in the concentration of 10000 cfu/g, forming 10% of all isolates) [78]. *Cladosporium* spp. in the air incidence in the indoor air of dwellings in Scotland (15% of all isolates on average) was dependent on the mechanical carpet cleaning. During vacuum cleaning, in a distance of 0.3 m from the vacuum cleaner, the concentration of *Cladosporium* spp. in the air increased from 12 cfu to 79000 cfu/m³ and after 90 minutes dropped to 789 cfu/m³. At a distance of 2 m from the vacuum cleaner, the original amount of 24 cfu/m³ increased to 18000 cfu/m³, and after 90 minutes it dropped to 506 cfu/m³ [33].

In dwellings *Cladosporium* spp. are regarded as secondary wall colonizers after the primary ones such as the *Penicillium* species and *A. versicolor* [25]. It was found that only 0.6% of airborne spores of *Cladosporium* are of such a size, that they can penetrate into the terminal bronchi and alveoli in humans [74].

*Cladosporium cladosporioides* is a producer of many enzymes [23]. Since it was isolated also from glycerol water solution used for photographic purposes, it was found in an experiment that it can grow even in a medium containing as much as 45% glycerol, but in higher concentrations no growth was observed [55]. This species is considered to be xerophilic, xerotolerant, but also psychrophilic. It is able to reproduce even on frozen meat [22]. Minimum aw value for spore germination is 0.86 (but also 0.815), for linear mycelium growth 0.88 and for sporulation 0.90 at 25°C, but the aw values are influenced by the pH value of the environment [32, 53]. Colonies of this species can grow at room temperature and also at 30–35°C.

*Cladosporium herbarum* is a world-wide known fungus occurring mainly on plants and plant materials and present also in soil, food and other environments. The species was found in dwellings in the Netherlands (in 11% of all examined dwellings), in London, Ontario, Canada (in the average concentration of 96 cfu/m³ in air and 1.9 × 10⁷ cfu/g in dust), and in dust of dwellings in Saudi Arabia (10200 cfu/g and 5.4% of all isolates). *C. herbarum* has the ability to reproduce on frozen meat. At -2°C it grows as early as after one month in the form of colonies with a diameter of 1 mm, at -5°C after four months [22]. This is a xerophilic and xerotolerant fungus.

Minimum air humidity for mycelium growth is 85–86%, optimum 95–96%, minimum for conidia formation 88-89%, optimum 96–98%, for conidia germination minimum 88%. The fungus belongs to the fenol degradating micromycetes. At 25°C the aw value for germination is 0.85, whereas for linear mycelium growth and sporulation 0.90, but also 0.75 [24, 53].

**Fusarium Link et Fries**

*Fusarium* species were known mainly as phytopathogenic fungi which is why not enough attention has been paid to these species from the medical point of view. However, the *Fusarium* species belong to important agens causing mycoses in humans [37], and to important producers of mycotoxins harmful to health. Trichotheecenes, zearalenon, fumonisins, belong to the best known toxins.

**Indoor air in the dwellings.** Positive findings of *Fusarium* spp. were made in US homes (in 2–6% of examined sites) [51], in South California (in 25% from 68 examined dwellings with a maximum of 47 cfu/m³ and minimum 4.5 cfu/m³) [45], in homes in Scotland (in 10% of all examined homes) [33] and in the Netherlands (in 9% of homes) [75].

The *Fusarium* incidence in a dwelling is proof of humidity in the environment [87].

**Penicillium Link**

Fungi of the *Penicillium* genus are a very important group of micromycetes regarding the economy and health of humans. They produce mycotoxins whose presence and activities spoil food raw materials and victuals, as well as other components of human environment, some of them being also potential pathogens [38]. Identification of isolated strains is very complicated, thus the mycologists in practice - when identifying the isolated colonies - must be satisfied with a simple classification of the isolates into the *Penicillium* genus, when a more detailed identification is not necessary for special monitoring or research.

**Indoor air in the dwellings.** Germs of *Penicillium* spp. were isolated in homes in the USA (in 26–51% out of examined places), in South California (in 91% of 68 examined dwellings, at the maximum concentration in the air 4737 spores/m³, on an average 168 spores/m³), in Canada (the concentration in indoor air was on an average 108 cfu/m³, in dust 8.1 × 10⁷ cfu/g), in homes in central Scotland (in 95% of all examined homes, 34% of all isolates), in dust from dwellings in Saudi Arabia (17% of all isolates, at the concentration of 23500 cfu/g), and in homes in the Netherlands (in 11% of all examined homes).

In indoor work environment at maize storing, 11% of viable *Penicillium* spp. spores were of such a size that they were able to form a deposit only in the upper part of the respiratory tracts, 13.2% in the pharynx, 18.9% in trachea and primary bronchi, 33.5% in secondary bronchi, 17% in terminal bronchi and 3.6% in alveoli [30].

In Germany, 1481 *Penicillium* spp. strains [49] isolated from victuals and fodder were tested for the ability of mycotoxin production. Of these, 829 (55.9%) produced the following mycotoxins on malt wort agar: cyclopiazonic acid (226 isolates), S-toxin (164), penicillic acid (140), patulin (82), brevinamid A (63), citrinin (63), penitrem A (62), xantomegnin (61), PR toxin (55), griseofulvin (43), ochratoxin A (39), rugulozin (30), verrukulogen TR₁ (19), roquefortin (15), fumitremorgen...
B (14), citreoviridin (7), viridicatumtoxin (3), erythroskyrin (1), islandotoxin (1) and luteoskyrin (1).

Penicillium spp. strains grow in the $a_w$ values ranging from 0.80–0.90. Minimum $a_w$ value for Penicillium expansum growth at 25°C is 0.85. Minimum growth temperature for this species is -3ºC, optimum 25–26ºC, maximum 33–35ºC. Optimum air humidity for conidia formation is 95–97%, optimum pH value is 4.4–7.5. On the bread crust surface the fungus can grow only when the crust has 22% humidity.

**Stachybotrys chartarum**

In the scientific literature there exist some synonyms for this species. For instance, some mycologists use the name S. alternans and others S. atra. It is interesting that the first strain of this species was isolated from paper and described in Prague, in the Czech Republic.

In workers handling contaminated substrates such as hay and straw, conjunctivitis, inflammation of mucous membranes of respiratory ways, skin irritation, bleeding from the nose, and in some more serious cases even leukopenia [64] were found. Chronic intoxication also suffered by the occupants of a house where the air conditioning system and wet ceiling were contaminated with S. chartarum, and in indoor air there were found spores of that species [11]. A study was carried out on unusual incidence of acute lung hemosideroses in infants aged six weeks to six months in Cleveland, Ohio, USA. One child died, and the only common sign was that all those families lived in dwellings contaminated with S. chartarum germs.

Among others Memnoniella echinata strains were also isolated from the environment, and it was found, that their metabolites were highly cytotoxic. It was also proved that the M. echinata strains produce trichotheccene mycotoxins: trichodermol and trichodermin. The authors [35] reported that the germs of that species can sometimes grow together with the S. chartarum strains. Massive incidence of spores of that species was also found in the indoor work environment which had been accidentally flooded. The employed staff (53 persons) suffered from symptoms of toxic damage to lower part of lung tissue, skin and eyes irritations and chronic weakness. Also, a statistically significant damage of the immune system, especially of T-lymphocytes was found [41].

**Allergic diseases.** Asthma attacks of a four year old boy were caused by S. chartarum, scattered from a moldy carpet [45].

**Indoor air in dwellings.** Positive isolation findings were recorded in 3% of 68 examined homes in South California, USA (maximum 52 spores/m$^2$, on average 0.3 spores/m$^3$), in 13% of 47 examined homes in London, in 4% samples from Central Scotland, and in one out of 50 examined dust samples in Canadian homes.

In dwellings, S. chartarum is classified as a tertiary wall colonizer that follows after the primary colonizers Penicillium species and A. versicolor and after secondary Cladosporium species. The fungus grows well on all materials rich in cellulose and decomposes them. In libraries it can cause a partial decay of paper, and has been found also on paintings.

The experts recommend that during the cleaning off of growths of this fungus in homes and workplaces the workers should wear breathing masks and protective clothing, since the arising spore dust irritates aggressively the skin and respiratory organs [11].

S. chartarum strains produce toxic trichotheccenes, e.g. satratoxins, verukarins, roridins and others. The toxic metabolites stachybotryotoxins-trichotheccenes are concentrated in the cells of the fungus [68], they are produced in phialides, conidia, conidiophores and the toxin diffuses into the medium. Strains start producing the toxins on straw as early as after four days, with a maximum after 2–3 weeks. S. chartarum grows at pH 3.0–9.8, with optimum at pH 5.6–6.0, and at temperature 2.5–40ºC, with optimum at 20–25°C. At 60°C the spores die in 10 minutes. Minimum $a_w$ value for growth and toxin production is 0.94. On 2% malt agar the minimum $a_w$ value at 12ºC is 0.91 and at 25ºC 0.93. The spores are highly resistant against drying. The fungus abundantly multiplies and produces satratoxins G and H on wallpapers and plasters with 84–100% humidity [61].

Based on a great number of studies it was concluded that the trichotheccene toxins are produced by nearly two thirds of all strains, and that strains from various countries produce the same toxins. However, sometimes it is enough to enrich the culture medium with glucose or other components for the non-toxic strain to be converted into a toxic one [3, 4, 31, 34].

**Wallemia sebi (Fr.) v. Arx**

This fungus was described for the first time by Cordon in 1829 as Torula epizoa. Later on, it was described as Sprendonema epizoon and also as Wallemia ichthyphaga.

**Allergic diseases.** 18% of 74 examined asthma patients had allergic reactions against the extract prepared from W. sebi [79].

**Indoor air in dwellings.** Positive findings were reported from the indoor air of dwellings in the Netherlands. The source of the germs in the indoor air can be the dust from mattresses which contained these fungi in concentration of 0.5 × 10^5 cfu/g [52].

Since W. sebi is a very strong xerophilic species, the presence of its germs cannot always be proved when using mycologic media. Special media like malt agar with 64% sucrose are recommended for its isolation.

The toxicity of W. sebi strains was comparable in bioexperiments with that of citrinin and penicillic acid [99].
At 20°C and a_w value 0.69 the spores germinate on a glucose or fructose medium in 46 days, at 30°C and a_w value 0.79 in 9 days. Optimum growth is at 20°C and a_w value 0.93–0.99, at 30°C and a_w value 0.85–0.95, and at 34°C and a_w value 0.87. Minimum a_w value at 22°C is 0.75 [97].

HEALTH PROBLEMS IN OCCUPANTS OF “MOLDY” DWELLINGS

A. Allergy. Vital and devitalized spores of fungi in the indoor air of houses are important aeroallergens in genetically determined humans. In dwellings occupied by children suffering from asthma significantly greater spore counts of Cladosporium species and Penicillium species were found [50]. In some case studies an evident relationship between the presence of the fungus in dwellings and allergic reactions of their occupants was found. After cleaning the contaminated sites, or when the occupants moved into a new home, the symptoms disappeared [82].

It was found that allergic alveolitis (hypersensitivity pneumonia) was caused by Penicillium expansum [66], and Rhodotorula rubra [83]. Kanny et al. [42] found that the cause of eczema and respiratory disorders in a 25 year old female student was her hypersensitivity against the antigens of micromycetes growing on the walls of her home, i.e. Fusarium sp., Cladosporium sp., Pullularia sp., Rhizopus sp. and Penicillium species. An explicit relation was found in Japan between the summer hypersensitivity pneumonia and the presence of Trichosporon spp. in homes of the patients [1, 58, 88, 101, 102]. Kauffman et al. [43] revealed that atopic humans suffer from acute symptoms of fungal asthma when they inspire a great number of spores. An adverse situation appears when mucous membrane of the epithelium of the respiratory tract of sensitive person is systematically colonized with fungi, whose cells possess the ability to penetrate into the deeper parts of lung tissue and to produce protease in a depository niche. These authors also found that sensitization with fungal antigens is dependent on the person’s age, as most humans with positive skin tests to the antigens of micromycetes are persons in the younger age categories. An increasing mortality among asthmatic patients was observed at the time when a statistically significant higher number of fungal spores was found in the air [92].

We have presented only a few out of a large amount of data published in the scientific literature on allergy to fungi. The data show that there is a close relationship between the presence of germs of microscopic fungi in the indoor air of dwellings and allergic symptoms in some patients. Nevertheless, most of the patients suffer from “multiallergy”, with allergic reactions also against other environmental components. Evaluation of this problem is an important task for clinical allergology.

The relationship between the fungal antigens and the allergic patients is so complicated and considerably influenced by the personality of the patient and because in some groups of atopic humans a clear relation between the spore count in the air and the reaction of the patients could not be proved [13, 43]. From the mycologists’ point of view there are problems in preparing a high quality antigen test set to prove allergy in the patient.

B. Health Problems with Unclear Etiology. Some authors revealed that in occupants of “moldy” dwellings there is a higher incidence of bronchitis, sore throat, concentration difficulties, back-aches, irritation of eyes and mouth cavity, feeling of weakness, etc. [73, 90]. Ill health symptoms were also associated with increased amounts of Epicoccum spp., Aureobasidium spp. and yeasts in dwellings [87]. Health problems in occupants of homes where spores of Stachybotrys chartarum were found have been described above.

An occupant of a “moldy” dwelling is exposed in that environment not only to microscopic fungi but also to a number of various volatile compounds released from furniture, carpets, various paints and other materials. The mechanism of the potential pathological effects of MFF and their toxins on lung tissue in healthy humans has not been yet been made sufficiently clear.

Some pathologic symptoms or subjective difficulties in occupants of homes or people working in moldy contaminated environment have been described above, however, for estimating the micromycete effects in the indoor home environments, it is necessary to take into consideration the results of the experiments conducted in vitro or on laboratory animals. Thus, it was found that 47% of the examined strains isolated from the dwellings were cytotoxic against human embryonal diploide fibroblasts from lung tissue [84]. The known toxic metabolites of MFF, e.g. trichotheccenes and sterigmatocystin, and extracts from some strains, cease the moving of cilia of tracheal mucous membranes in organ cultures from one day old chickens and thus affect the self-cleaning ability of these membranes [39, 69, 70, 71, 72]. Intranasal application of 1 × 10^6 spores of satratoxin producing Stachybotrys chartarum strain causes inflammatory reactions in the lung tissue of experimental mice together with exudative processes in alveoli and bronchi. These disorders could be classified as lung mycotoxicosis [62]. The damage in the lung tissue after inspirings A. versicolor spores in rats has been presented above.

Other experiments were carried out in connection with indoor work environment [36], however, the identified strains and mycotoxins are only rarely found in large amounts in homes: aflatoxins and D-secalonic acid.

CONCLUSIONS

Nearly all studies presented in the literature point to the fact that there are certain relations between the presence of germs of filamentous fungi in the indoor home
environment and the health status and comfort of the occupants of the dwellings. There are allergic reactions in the form of differing intensity of rhinitis, asthma, hypersensitivity pneumonitis in adults, but especially in children of lower age categories, as well as various ill health problems of mostly unknown etiology, such as frequent bronchitis, chronic cough, mucous membrane irritations, and others. The latter especially disorders are at present the subjects of intensive studies and regarded as caused by intracellular and extracellular toxic metabolites of some MFF.

As presented in our survey, various species of microscopic fungi colonize the walls and objects in dwellings and are liberated into the air depending on their physiologic properties. The fungi may be xerophilic, xerotolerant, osmophilic and hydrophilic. Some of their spores are easily liberated into the indoor air, some less so; some are tiny while others are large and can be caught on the mucous membranes of the upper respiratory tracts. There are species of microscopic fungi whose toxicity after intake together with contaminated food is well known and intensive, nevertheless the toxicity against various structures of respiratory organs has not as yet been cleared. Toxicity of further metabolites and strains colonizing dwellings against the respiratory organs has also not yet been sufficiently cleared, but all found data point to the fact that the tissue of human respiratory organs can be seriously affected by the microscopic fungi and their toxins in the indoor environment of dwellings.

REFERENCES


