

## **SPERGILLI ON BUILDING PARTITIONS INFESTED WITH MOULDS IN RESIDENTIAL HOUSING AND PUBLIC UTILITY PREMISES**

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### Abstract

*Aspergilli* constitute a serious risk to the health of the inhabitants of infested rooms. Mycological analysis conducted in buildings infected with moulds in the area of the Lubuskie province (Poland) demonstrated the presence of 9 species of *Aspergillus* moulds: *A. carbonarius*, *A. clavatus*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. ochraceus*, *A. terreus*, *A. ustus* and *A. versicolor*. The highest frequency (4 - frequently) was observed in the case of *A. versicolor*, while frequency 3 (fairly frequently) was characteristic of such species as *A. flavus* and *A. niger*. *A. ustus* was encountered with frequency 2 (individually), while frequency 1 (sporadically) referred to four species: *A. carbonarius*, *A. clavatus*, *A. fumigatus* and *A. terreus*. Because *Aspergillus versicolor* occurs with the highest frequency in buildings, and as a consequence of this, synthesizes toxic and carcinogenic sterigmatocystin (ST), it constitutes the greatest risk to the inhabitants of the infested premises. All species of *Aspergillus* present on building partitions are able to synthesise mycotoxins, are pathogens and may cause allergies.

Keywords: *Aspergillus* species, mycotoxins, residential housing

### 1. INTRODUCTION

The occurrence of moulds on building partitions is a problem whose severity has been increasing in many countries, not just in Poland. This

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negative phenomenon in buildings contributes to the bio-deterioration of building materials and reduces the sanitary quality of the internal air [1-3]. Analyses regarding the internal environment, which have been conducted in the recent years, indicate the two most dangerous species: *Stachybotrys chartarum* and *Aspergillus versicolor* [4-7, 3]. These moulds are able to synthesise such mycotoxins dangerous to the health of inhabitants as: macrocyclic trichothecenes, trichodermin and satratoxin G (*S. chartarum*) or sterigmatocystins (*A. versicolor*). The above-mentioned compounds can be present in the majority of samples coming from the building materials of damp flats and from samples of settled dust [8].

Regardless of whether the microorganisms grow on brick walls, concrete walls, plasterboard walls, wall paper, wood or silicone joints, they still constitute the source of spores and fragments of mycelium as well as air-borne mould from the building materials inside the building, which are all dangerous to the health of its inhabitants [9-11, 7]. New technologies applied in the construction industry are conducive to the development of mould on building partitions. Therefore, it is important to demonstrate the mycological risks, which these microorganisms may pose for the health of the inhabitants, as well as any loss of the utility properties of the materials which the walls are made of [7].

The basis for the evaluation of the risks caused by an infestation with mould in residential housing is mycological analysis. The area of the Lubuskie Province (Poland) has been selected as a sufficiently large and representative research area for many buildings found in Europe in the temperate climate zone. The aim of the research was to determine the frequency of moulds species occurrence on infected building partitions. The species which were most frequently present in residential housing were identified and the conclusion was drawn that thermal-upgrading renovations caused an increase in the number of premises infested with moulds, as well as a negative quality change, since the less toxic moulds which most frequently colonise building partitions were replaced by the *Aspergillus* species which are capable of synthesizing, among others: AFB<sub>1</sub>, ST, 5-ST, OTA or PAT.

A factor which always determines the growth of moulds on building partitions is the soil humidity. It is important to know the specific humidity requirements for moulds isolated in flats, with particular consideration given to the Equilibrium Relative Humidity  $a_w$  - *water activity*. The humidity required for the growth of *Aspergillus* is presented in table 1. The humidity level also determines the division into the 3 succession groups (primary, secondary and tertiary colonisers).

Table 1. Required humidity for the growth of selected microorganisms which belong to the genus *Aspergillus*, in buildings, on finishing materials and interior equipment [12-15]

Humidity level	Category of microorganisms
<b>High</b> ( $a_w > 0.90$ ; ERH > 90%)	<b>Tertiary colonisers (hydrophils)</b> <i>Aspergillus fumigatus</i>
<b>Medium</b> ( $a_w$ 0.80-0.90; ERH 80-90%)	<b>Secondary colonisers</b> <i>Aspergillus clavatus</i> <i>Aspergillus flavus</i> <i>Aspergillus ochraceus</i> <i>Aspergillus terreus</i> <i>Aspergillus versicolor</i> <sup>a</sup>
<b>Low</b> ( $a_w < 0.80$ ; ERH < 80%)	<b>Primary colonisers (xerophils)</b> <i>Aspergillus niger</i> <i>Aspergillus versicolor</i> <sup>b</sup>

$a_w$ , - water activity; ERH - equilibrium relative humidity; <sup>a</sup> at 12 °C; <sup>b</sup> at 25 °C

Moulds which represent the *Aspergillus* genus can grow on building partitions already at a low humidity level ( $a_w < 0.80$ ), which causes them to appear on walls with higher and higher frequency.

## 2. MATERIALS AND METHODS

Mycological analyses were carried out in more than 280 buildings. Samples were collected from the inner surfaces of building partitions with visible mould. The partitions were made of plasterboard, bricks, breeze blocks and concrete slabs, and the finishing materials included: plaster, acrylic paint, wallpaper etc. The samples were collected directly at the site of occurrence and transferred onto Petri dishes, which contained both the natural media such as Malt Extract Agar (MEA), (Merck), Potato Dextrose Agar (PDA), (Merck) and synthetic media such as Czapek - Dox Agar (Merck) and Seawater Nutrient Agar (SNA), (Merck). The analysis was performed in accordance with the methodology established by CBS (Centraalbureau voor Schimmelcultures), [15]. The direct plating method was used. According to this method, small fragments of the material infected with moulds are transferred or spread onto the Petri dishes containing the culture media [16]. The samples were covered with white linen and incubated in a cultivation room at a room temperature between 18° and 22° C, maintaining the circadian rhythm of day and night. Pure (axenic) cultures were isolated from mixed starter cultures by their passaging on the media: Czapek-Dox and MEA. The time of plating, cultivation and observation for an isolated single species was 21 days [17,18]. The isolated strains were

subjected to identification tests using keys for taxonomic identifiers: 19-32, 15.

The frequency with which the isolated mould species was present in the tested residential premises was determined. According to Piontek [33], their percentage share was expressed using the five-grade scale (1-5), (table 2).

Table 2. Frequency of occurrence of moulds in residential housing according to Piontek [33]

Frequency (%)	Scale
0-5 %	1 - sporadically
>5-10 %	2 - individually
>10-15 %	3 - fairly frequently
15-20 %	4 - frequently
>20 %	5 - very frequently

### 3. RESULTS AND DISCUSSION

The mycological analyses conducted in the residential housing in the area of the Lubuskie province suggest that there are 83 species of moulds present on building partitions. The following species were observed with the highest frequency: *Cladosporium herbarum* and *Penicillium chrysogenum* - frequency 5, (table 3).

Table 3. Frequency of moulds observed on building partitions in the area of the Lubuskie Province [3, supplemented]

Types and species of moulds		Frequency in flats *
<i>Absidia</i>	<i>corymbifera</i> Sacc. et Trotter	1
	<i>glauca</i> Hagem	1
<i>Acremonium</i>	<i>bacillisporum</i> W. Gams	1
	<i>charticola</i> W. Gams	1
	<i>murorum</i> W. Gams	1
	<i>strictum</i> W. Gams	3
<i>Alternaria</i>	<i>alternata</i> Keissler	2
	<i>tenuissima</i> Wiltshire	1
<i>Arthrinium</i>	<i>phaeospermum</i> M.B. Ellis	1
<i>Aspergillus</i>	<i>carbonarius</i> Thom	1
	<i>clavatus</i> Desmazieres	1
	<i>flavus</i> Link	3
	<i>fumigatus</i> Fressenius	1
	<i>niger</i> van Tieghem	3
	<i>ochraceus</i> Wilhelm	1
	<i>terreus</i> Thom	1

	<i>ustus</i> Thom et Church	2
	<i>versicolor</i> Tiraboschi	4
<i>Aureobasidium</i>	<i>pullulans</i> Arnaud	1
<i>Beauveria</i>	<i>bassiana</i> Vuillemin	1
<i>Botrytis</i>	<i>cinerea</i> Persoon ex Fries	1
<i>Botryotrichum</i>	<i>piluliferum</i> Sacc. et Marchal	1
<i>Chaetomium</i>	<i>elongatum</i> Czerepanova	2
	<i>torulosum</i> Bainier	1
<i>Chromelosporium</i>	<i>sp.</i>	1
<i>Cladosporium</i>	<i>cladosporioides</i> de Vries	2
	<i>herbarum</i> Link ex Gray	5
	<i>macrocarpum</i> Preuss	3
	<i>sphaerospermum</i> Penzig	1
<i>Doratomyces</i>	<i>stemonitis</i> Pers.	1
<i>Epicoccum</i>	<i>nigrum</i> Link	1
<i>Fusarium</i>	<i>aquaeductum</i> Lagerheim	1
	<i>culmorum</i> Saccardo	1
	<i>equiseti</i> Saccardo	1
	<i>oxysporum</i> Schlechtendal ex Fries	1
	<i>sambucinum</i> Fuckel	1
	<i>solani</i> Saccardo	1
	<i>sporotrichioides</i> Sherbakoff	1
	<i>verticillioides</i> Nirenberg	1
<i>Geotrichum</i>	<i>candidum</i> Link	1
<i>Gilmaniella</i>	<i>humicola</i> Barron	1
<i>Humicola</i>	<i>brevis</i> Gilman et Abbott	1
	<i>fuscoatra</i> Traaen	1
<i>Monocillium</i>	<i>indicum</i> S. B. Saksena	1
<i>Moniliella</i>	<i>acetobutens</i> Stolk & Dakin	1
<i>Mucor</i>	<i>circinelloides</i> van Tieghem	1
	<i>globosus</i> Fischer	1
	<i>hiemalis</i> Wehmer	1
	<i>mucedo</i> Fresenius	1
	<i>piriformis</i> Fischer	1
	<i>plumbeus</i> Bonorden	1
	<i>racemosus</i> Fresenius	3
<i>Paecilomyces</i>	<i>farinosus</i> Brown et Smith	1
	<i>marquandii</i> Hughes	1
	<i>variotii</i> Bainier	1
<i>Penicillium</i>	<i>aurantiogriseum</i> Dierckx	1
	<i>brevicompactum</i> Dierckx	1
	<i>chrysogenum</i> Thom	5

	<i>expansum</i> Link ex Gray	1
	<i>funiculosum</i> Thom	1
	<i>glabrum</i> Westling	1
	<i>janthinellum</i> Biourge	1
	<i>lanosum</i> Westling	3
	<i>thomii</i> Maire	1
	<i>viridicatum</i> Westling	1
	<i>vulpinum</i> Seifert et Samson	1
	<i>waksmanii</i> Zaleski	1
<i>Phoma</i>	<i>glomerata</i> (Corda) Wollenw.	1
<i>Rhizomucor</i>	<i>pusillus</i> Schipper	1
<i>Rhizopus</i>	<i>stolonifer</i> Lind.	2
<i>Scopulariopsis</i>	<i>brevicaulis</i> Bainier	1
	<i>candida</i> Vuill.	1
<i>Stachybotrys</i>	<i>chartarum</i> Hughes	1
<i>Thamnidium</i>	<i>elegans</i> Link	2
<i>Trichoderma</i>	<i>koningii</i> Oudemans	1
	<i>viride</i> Persoon ex Gray	2
<i>Trichothecium</i>	<i>roseum</i> Link ex Gray	2
<i>Tritirachium</i>	<i>oryzae</i> (Vincens) de Hoog	1
<i>Ulocladium</i>	<i>botrytis</i> Preuss	2
	<i>chartarum</i> Simmons	4
	<i>consortiale</i> Simmons	1
<i>Verticillium</i>	<i>lecanii</i> Viégas	1
	<i>luteoalbum</i> Subramanian	2

\*Frequency in flats (table 2); shaded moulds with a frequency higher than 1

Mycological analysis demonstrated a significant qualitative change in the mould species in relation to the studies conducted by Piontek during the preceding years [33, 3]. A significantly higher frequency on building partitions was observed in the case of such species as *Aspergillus* in lieu of such moulds as e.g. *Mucor racemosus* (the frequency of occurrence on partitions had decreased from 4 to 3).

The genus *Aspergillus* and its teleomorphs currently contain 254 accepted species [34]. Studies have shown that *Aspergilli* on the walls are represented by 9 species. They constitute 11% of all the species isolated from the partitions in the Lubuskie province. The highest frequency among them - 4 (frequently) was demonstrated by *Aspergillus versicolor*. Such species as: *A. flavus*, *A. figer* occurred with a frequency of 3. One species - *A. ustus* was observed individually (frequency 2), while such species' as *A. carbonarius*, *A. clavatus*, *A. fumigatus*, *A. ochraceus* and *A. terreus* were present only sporadically. Despite the low frequency of occurrence, the presence of these species on building partitions cannot be assumed to

remain without any effect on the health of the inhabitants, as they have biochemical abilities to synthesize mycotoxins and cause allergies and mycosis.

All the identified species are widespread in nature, and can also colonise building materials. Each of the 9 species of *Aspergillus* has the ability to synthesize secondary metabolites and mycotoxins. The mycotoxins synthesized by *Aspergilli* are listed in table 4.

Table 4. List of mycotoxins and secondary metabolites synthesized by moulds of the genus *Aspergillus*

Species	Formed mycotoxins and secondary metabolites* [35, 36, 15, 7]*
<i>A. carbonarius</i> Thom	<b>ochratoxin A</b>
<i>A. clavatus</i> Desmazieres	<b>cytochalasin E, patulin</b> , ascladiol, tryptocuivalin, tryptocuivalon, kojic acid
<i>A. flavus</i> Link	<b>aflatoxin B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub>, cyclopiaconic acid, 3-nitropropionic acid, sterigmatocystin</b> , aflatrem, aspertoxin, flavicidin, aspergillic acid, kojic acid, $\beta$ -nitropionic acid, o-methyl sterigmatocystin
<i>A. fumigatus</i> Fressenius	<b>fumitremorgin A, B, C, gliotoxin, verruculogen</b> , fumigaclavin A, B, C, fumitoxins (fumigacin, fumagillin), tryptocuivalins, kojic acid
<i>A. niger</i> van Tieghem	malformins A, B, C, naphtho- $\gamma$ -pyrones, <b>ochratoxin A</b> , nigragillin
<i>A. ochraceus</i> Wilhelm	<b>ochratoxin A, penicillic acid</b> , xahthomegnin, viomellein, vioxantin
<i>A. terreus</i> Thom	<b>citreoviridin, citrinin, gliotoxin, patulin</b> , kojic acid, usitin acid, terreic acid
<i>A. ustus</i> Thom et Church	<b>austamide, austdiol, austocystins A and B</b>
<i>A. versicolor</i> Tiraboschi	<b>cyclopiaconic acid, sterigmatocystin, 5-methoxy-sterigmatocystin</b> , dihydroxy-sterigmatocystin, nidulotoxin, averufin, versiconol, versicolorin A, B, C

\* important toxic metabolites are listed **in bold**

The *International Agency for Research of Cancer* (IARC) published the list of carcinogenic substances, using the following grading scale: 1, 2A, 2B, 3, 4. The greatest danger is caused by such toxic and carcinogenic mycotoxins as aflatoxin B<sub>1</sub> synthesized by *A. flavus* (1 - an epidemiologically confirmed relationship between the exposure and the incidence of a disease), ochratoxin A (*A. carbonarius*, *A. niger*, *A. ochraceus*) and sterigmatocystin (*A. flavus*, *A. versicolor*), which were qualified into category 2 B (lesser probability of carcinogenic effect), [37].

Table 5 presents the data regarding the mortality of selected test organisms after subjecting them to acute toxicity tests for AFB<sub>1</sub>, OTA and ST.

Table 5. Comparison of toxicity of aflatoxin B<sub>1</sub>, ochratoxin and sterigmatocystin for test animals [35, 38, 7, 18, 3]

Test organism	Mortality rate	AFB <sub>1</sub>	OTA	ST
Ducklings	LD 50 (mg kg <sup>-1</sup> )	0.012	3.0	1.0
Rats	LD 50 (mg kg <sup>-1</sup> )	5.5 - 17.9	3.9-22	65 - 166
Mice	LD 50 (mg kg <sup>-1</sup> )	3-9	500	800
Chicken embryos	LD 50 (mg kg <sup>-1</sup> )	0.05 µg/embryo	0.4-2.0 µg/embryo	15 µg/ embryo
Chickens	LD 50 (mg kg <sup>-1</sup> )	2	3 - 4	10-14
Shrimp larvae ( <i>Artemia salina</i> )	LC 50 (mg dm <sup>-3</sup> )	1.3 - 39.0	10.1 -26.4	0.54 - 5.95
Planarians ( <i>Dugesia tigrina</i> )	LC 50 (mg dm <sup>-3</sup> )	Not tested	Not tested	8.2 - 603

The combined data regarding the test animals indicates that the moulds of the genus *Aspergillus* synthesize toxins which are dangerous to the health of the inhabitants. The obtained results show that sterigmatocystin is about 10 times less toxic than aflatoxin B<sub>1</sub> for rats and 100 times less toxic for mice and chicken embryos, but it is a hepatocarcinogenic substance and tests conducted on animals prove that *A. versicolor* consequently synthesizes ST on building materials. Toxicological studies conducted at the laboratory of the Institute of Environmental Engineering of the Zielona Góra University, using planarians, demonstrated the varied toxicity of strains of *A. versicolor* coming from buildings. The LC 50 values ranged between 8.2 - 603 mg dm<sup>-3</sup>, which means that strains ranging between low-productive (non-poisonous) and very strongly poisonous may occur in buildings [18, 3]. All the toxins synthesized by *Aspergilli* create a potential risk in buildings, therefore, their presence on building partitions must not be tolerated.

Moulds are pathogens. In 1996, under the auspices of the European Confederation of Medical Mycology, a list which sets out the BSL classification was drawn up for the respective species of moulds (BSL 1 - 3), [29]. On this basis it is possible to determine the potential risk to people who live in infested rooms. Table 6 contains the BSL classification for isolated species of the genus *Aspergillus*.

Table 6. Biosafety levels of selected moulds potentially pathogenic for man and animals (BSL), [29]

Species of moulds of the genus <i>Aspergillus</i>		*BSL, indicative risk assessment to people and animals
<i>Aspergillus</i>	<i>carbonarius</i> Thom	-
	<i>clavatus</i> Desmazieres	1
	<i>flavus</i> Link	2
	<i>fumigatus</i> Fressenius	2
	<i>niger</i> van Tieghem	1
	<i>ochraceus</i> Wilhelm	1
	<i>terreus</i> Thom	2
	<i>ustus</i> Thom et Church	1
	<i>versicolor</i> Tiraboschi	1

\*BSL1—infections are superficial, non-invasive or benign,

BSL2—in patients with severe immunological disorders, moulds can cause deep opportunistic infections,

BSL3—pathogens potentially capable of inducing severe deep fungal infections in apparently healthy people.

The BSL2 category comprises as many as 3 species: *A. flavus*, *A. fumigatus* and *A. terreus*. These are the species which can cause aspergillosis. The other species from the genus *Aspergillus* are categorised as BSL 1 - they can cause coincidental, superficial, non-invasive and benign infections. The most dangerous pathogens (BSL3), which are potentially able to cause severe and deep fungal infections in persons with overall good health did not occur in the analysed flats in the Lubuskie province.

#### 4. CONCLUSIONS

The mycological tests in buildings in the temperate climate zone demonstrated the qualitative change in the mould species that infest the building partitions. 9 species of moulds that represent the genus *Aspergillus* (Photo 1- 9 [27]) were isolated from and identified on infested building partitions. All the species are able to synthesize mycotoxins, are pathogens and can cause allergies. It was concluded that the frequency of the moulds of the genus *Aspergillus*, which can constitute the potential risk to the health of the inhabitants of the infested premises had increased, therefore mycological analyses in buildings are important and must be continued.



Photo 1. *Aspergillus versicolor* (Vuillemin) Tiraboschi on Czapek Dox medium



Photo 2. *Aspergillus versicolor* (Vuillemin) Tiraboschi on MEA medium

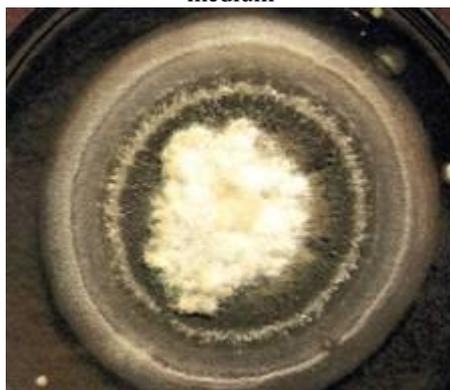


Photo 3. *Aspergillus ustus* (Bainier) Thom et Church

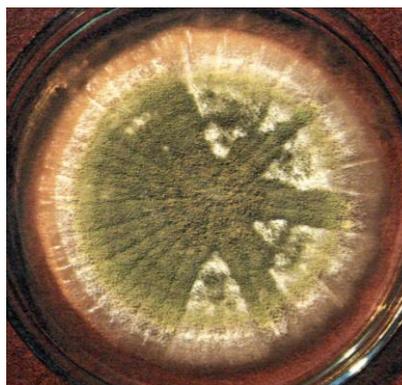


Photo 4. *Aspergillus flavus* Link ex F. Gray



Photo 5. *Aspergillus clavatus* Desmazieres

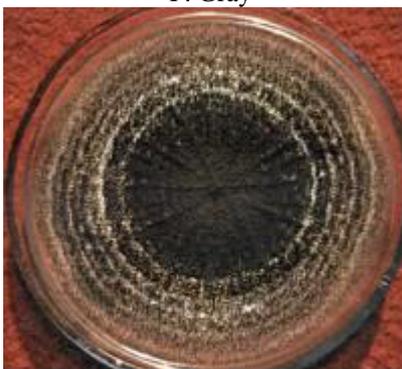


Photo 6. *Aspergillus niger* van Tieghem

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ASPERGILLI NA PRZEGRODACH BUDOWLANYCH ZAINFEKOWANYCH  
GRZYBAMI PLEŚNIOWYMI POMIESZCZEŃ MIESZKALNYCH  
I UŻYTECZNOŚCI PUBLICZNEJ

Streszczenie

Grzyby pleśniowe z rodzaju *Aspergillus* stanowią poważne zagrożenie dla zdrowia mieszkańców zainfekowanych pomieszczeń. Badania mikologiczne przeprowadzone

w porażonych grzybami obiektach na terenie województwa lubuskiego (Polska) wykazały występowanie 9 gatunków grzybów z rodzaju *Aspergillus*: *A. carbonarius*, *A. clavatus*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. ochraceus*, *A. terreus*, *A. ustus*, *A. versicolor*. Z największą frekwencją (4 - często) wystąpił *A. versicolor*, natomiast z frekwencją 3 (dość często) wystąpiły takie gatunki jak *A. flavus* oraz *A. niger*. *A. ustus* wystąpił z frekwencją 2 (pojedynczo), natomiast z frekwencją 1 (sporadycznie) wystąpiły cztery gatunki: *A. carbonarius*, *A. clavatus*, *A. fumigatus* i *A. terreus*. Stwierdzono, że gatunek *Aspergillus versicolor* przez to, że występuje z największą frekwencją w budownictwie oraz konsekwentnie syntetyzuje toksyczną i kancerogenną sterygmatocystynę ST stanowi największe niebezpieczeństwo dla mieszkańców zainfekowanych pomieszczeń. Wszystkie gatunki z rodzaju *Aspergillus* posiadają zdolność do syntezy mikotoksyn, są patogenami oraz mogą powodować alergie.

Słowa kluczowe: *Aspergillus*, mikotoksyny, budownictwo

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