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THE CAPABILITY OF FUNGI ISOLATED FROM MOLDY DWELLINGS TO PRODUCE TOXINS

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Abstract

Objectives: The main objective was analysis and assessment of toxinogenic capabilities of fungi isolated from moldy surfaces in residential rooms in an urban agglomeration situated far from flooded areas in moderate climate zone. **Material and Methods:** The assessment of environmental exposure to mycotoxins was carried out in samples collected from moldy surfaces in form of scrapings and airborne dust from 22 moldy dwellings in winter season. In each sample 2 mycotoxins were analyzed: sterigmatocystin and roquefortine C produced by *Aspergillus versicolor* and *Penicillium chrysogenum*, respectively. Mycotoxins were analyzed by high-performance liquid chromatography (HPLC) in: scrapings from moldy surfaces, mixture of all species of fungi cultured from scrapings on microbiological medium (malt extract agar), pure cultures of *Aspergillus versicolor* and *Penicillium chrysogenum* cultured from scrapings on microbiological medium; mycotoxins in the indoor air dust were also analyzed. **Results:** The production of sterigmatocystin by individual strains of *Aspergillus versicolor* cultured on medium was confirmed for 8 of 13 isolated strains ranging 2.1–235.9 μg/g and production of roquefortine C by *Penicillium chrysogenum* for 4 of 10 strains ranging 12.9–27.6 μg/g. In 11 of 13 samples of the mixture of fungi cultured from scrapings, in which *Aspergillus versicolor* was found, sterigmatocystin production was at the level of 3.1–1683.2 μg/g, whereas in 3 of 10 samples in which *Penicillium chrysogenum* occurred, the production of roquefortine C was 0.9–618.9 μg/g. The analysis did not show in any of the tested air dust and scrapings samples the presence of analyzed mycotoxins in the amount exceeding the determination limit. **Conclusions:** The capability of synthesis of sterigmatocystin by *Aspergillus versicolor* and roquefortine C by *Penicillium chrysogenum* growing in mixtures of fungi from scrapings and pure cultures in laboratory conditions was confirmed. The absence of mycotoxins in scrapings and air dust samples indicates an insignificant inhalatory exposure to mycotoxins among inhabitants in moldy flats of urban agglomeration situated far from flooded territories. Int J Occup Med Environ Health 2016;29(5):823–836

Key words:
Indoor air, Mycotoxins, Molds, Residential environment, Sterigmatocystin, Roquefortine C

INTRODUCTION

Fungal spores in atmospheric air getting indoors are not capable to grow in housing conditions characterized by intact constructional balance. If, however, there is appropriate indoor temperature, humidity, and organic materials which can be a source of necessary foodstuffs, they settle fast in those premises [1–4]. High temperature in combination with flat flooding incidents and ineffective room ventilation that increase relative humidity of air and cause condensation of steam on surfaces may promote

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the growth of mold [4–7]. Fungi may develop on most of the building materials, carpets, leather, paper, and insulating materials [7–9]. Mold genera found on moldy surfaces usually include *Penicillium*, *Aspergillus*, *Cladosporium*, *Acremonium* and *Alternaria*, as well as genus *Stachybotrys* known for its high pathogenic activity [6,9–13].

Spores of mold or their fragments released into the ambient air from moldy surfaces may be harmful to human health when inhaled or in contact with the skin. The most prevalent adverse health effects of fungal exposure are: allergies, irritation of mucous membranes, respiratory symptoms, or toxic systemic effects [14–19]. The effects of mold on human health vary with the nature of a given species, its amount and products of its metabolism. Besides, the period of exposure to molds or their products as well as individual sensibility to respective species are important [14–19].

Toxic effects of fungi are connected with their products of secondary metabolism, known as mycotoxins. Individual mycotoxins differ in their toxic effects on organisms. Admittedly, they deteriorate the immune system and exhibit carcinogenic, mutagenic, embryotoxic, teratogenic, haemorrhagic, dermatotoxic, cytotoxic, neurotoxic activity, they also impair fertility [19–23]. Toxinogenic activity of individual species of molds is a property that becomes apparent in specific conditions of the substrate's humidity, temperature, availability of nutrients, and the presence of other (competitive) fungi [24,25]. Many fungi produce ≥ 2 mycotoxins which may act synergically, enhancing their toxic effects [26,27].

Bioaerosol containing mycotoxins may be encountered in occupation and private life (in working and living environment). Occupational exposure is connected with processing and storing of crops, feeding of animals, production of foodstuffs, raising of animals and production of animal fodder, composting, biotechnology, work in such buildings as museums, libraries, archives and offices which are affected by moldiness [16,28–32]. Mycotoxins in environments other than occupational usually result from moldiness of damp residential buildings [24,25].

In relevant Polish literature, there are only few reports on toxinogenicity of molds present in domestic environment and attempts to evaluate the exposure of inhabitants of the moldy rooms to mycotoxins produced by molds present in such environments [7,33].

The main objective of the presented research was to analyze and assess toxinogenic capabilities of fungi isolated from moldy surfaces in residential rooms in a big urban agglomeration located in moderate climatic zone far away from flooded areas.

The additional objective of our research is to extend the existing knowledge by providing new information about the species of fungi isolated from moldy surfaces in flats and showing the capability of those microorganisms to produce mycotoxins.

MATERIAL AND METHODS

The studies were carried out in urban agglomeration in central Poland during 2011–2013. This publication presents the results obtained for 22 dwellings selected in a purposeful way from among 754 flats chosen by a simple random-selection method from the National Official Register of Territorial Division (Krajowy Rejestr Urzędowy Podziału Terytorialnego Kraju – TERYT), run by the Central Statistical Office of Poland (Główny Urząd Statystyczny – GUS), after informed written consent to participate in the study had been obtained from the inhabitants of the selected dwellings. The presence of developed mycelium on solid surfaces in the flat, as declared by inhabitants in a questionnaire survey conducted by trained interviewers during their visits to each of the selected flats served as the criterion of the purposeful selection.

To assess environmental exposure to mycotoxins in each of the tested dwellings, samples were collected in 2 ways: from moldy surfaces in form of scrapings, and from airborne dust.

Mycotoxins were analyzed in the following material:
- scrapings from moldy surfaces (22 samples),
- mixture of all species of fungi cultured from scrapings on microbiological medium (22 samples),
- pure cultures of *Aspergillus versicolor* and *Penicillium chrysogenum* cultured from scrapings on microbiological medium (23 samples),
- indoor air dust from moldy rooms (22 samples).

Sampling was conducted in winter.

Scrapings from moldy surfaces

Scrapings in the form of mycelium fragments with a possibly thinnest layer of the base from moldy surfaces (mainly walls in flats) were scraped with a sterile scalpel to disposable sterile containers in each of the examined flats. Each time approx. 1.5 g of scrapings were collected.

Culture and species identification of fungi from scrapings

Culture of a fungi mixture

Scraping samples, 0.5 g each, were placed in disposable sterile containers, and 10 ml of phosphate buffer solution (PBS) (BTL, Poland) were added. The samples were shaken for 60 min with the shaking velocity of 420 rpm. A number of 10-fold dilutions were prepared from the resultant solutions and then, by superficial method, 1 ml of eluates and their dilutions were inoculated on 140 mm plates with malt extract agar (MEA) medium (BTL, Poland) with added chloramphenicol (Galfarm, Poland) and streptomycin (Sigma-Aldrich, Germany) as well as oatmeal agar (OA) medium (Fluka Chemie, Switzerland) for identification of the *Stachybotrys* genus.

The samples were incubated for 14 days at 21°C and 50–55% relative humidity (temperature and humidity selected similarly to the conditions prevalent in flats in winter according to actually measured values). After 7 days' culture, the colonies which grew on the plates were counted to determine the percentage share of identified species in respective samples. The mixtures of fungi which grew on the plates with the medium were subjected to further processing according to the procedure described below.

Fungi species identification

Fungi were identified according to morphologic traits of the colonies grown on diagnostic microbiological media and in microscopic picture, using literature data [34–38]. Yeasts were identified using analytical profile index (API) biochemical tests (bioMérieux, France).

Pure cultures of Aspergillus versicolor and Penicillium chrysogenum

Toxinogenic capabilities of molds isolated in our study were analyzed according to the literature data [39–42]. Two species of molds – *Aspergillus versicolor* and *Penicillium chrysogenum* – were selected for the studies, considering a high incidence of isolated potentially toxinogenic species in samples. The first of the molds specified above was found in 59%, the latter in 45% of the samples collected from the moldy surfaces. Pure cultures of *Aspergillus* and *Penicillium* genera, which can produce the mycotoxins determined by us, were inoculated onto the plates with MEA medium (each strain on 3 plates) and incubated for 14 days at 21°C and approx. 50–55% humidity. After 14 days, the inoculated pure cultures of mold with the medium were subjected to further processing according to the procedure described below.

Sampling of airborne dust

Airborne dust samples inside residential rooms were collected using the "aspirator and head with filter" sets. The set consisted of GilAir 5 (Sensidine, USA) aspirator, elastic hose and open measuring head (Two-Met, Poland), with 37 mm diameter and 0.7 μm pore diameter GF/F glass fiber filter (Whatman, UK). The set was operated continuously at 2 l/min air flow for 24 h. Air dust samples were collected in a stationary way, at a height of approx. 1–1.2 m from the floor level. Filters with the collected dust were transferred to the laboratory and stored at –20°C until analysis.

Analysis of mycotoxins

Due to the limited budget for the study, only 2 types of mycotoxins were analyzed. Based on literature survey, mycotoxins most often synthesized by the above mentioned species of fungi were selected for analysis: sterigmatocystin (*Aspergillus versicolor*) and roquefortine C (*Penicillium chrysogenum*) [33,40,42,43].

Preparation of samples

Media with culture of molds

Malt extract agar media (BTL, Poland) with mycelium which grew thereon (initial mixture of fungi and pure cultures of *Aspergillus versicolor* and *Penicillium chrysogenum*) were subjected to pasteurization (85°C, 30 min) and comminution with a sterile scalpel. The samples were dried to a solid mass at 50°C for 40 h, and 79:20:1 acetonitrile-water-acetic acid extraction mixture (POCH S.A., Poland) was added at 1 g sample: 4 ml mixture ratio, subsequently the samples were extracted for 90 min with a KL-2 Multi-Purpose Shaker (Edmund Bühler GmbH, Germany). Then the samples were centrifuged at 1000 g for 15 min (Laboratory Centrifuge 2-16K, Sigma, Germany); the resultant supernatant was subjected to chromatographic analysis. Dry mass of pure cultures of molds of *Aspergillus versicolor* and *Penicillium chrysogenum* species with the substrate ranged 1.2–1.7 g, whereas the mass of fungal mixtures together with substrate was 1.1–1.7 g.

Scrapings

Samples of scrapings in the amount of 1 g from each flat were subjected to pasteurization (85°C, 30 min), extraction, centrifuging and chromatographic analysis in a similar way as the fungal samples described above.

Airborne dust

All filters were flushed with identical volume of the extraction mixture described above (4 ml) and then the procedure followed exactly that applied for fungal samples as described above (see above: "Media with culture of molds").

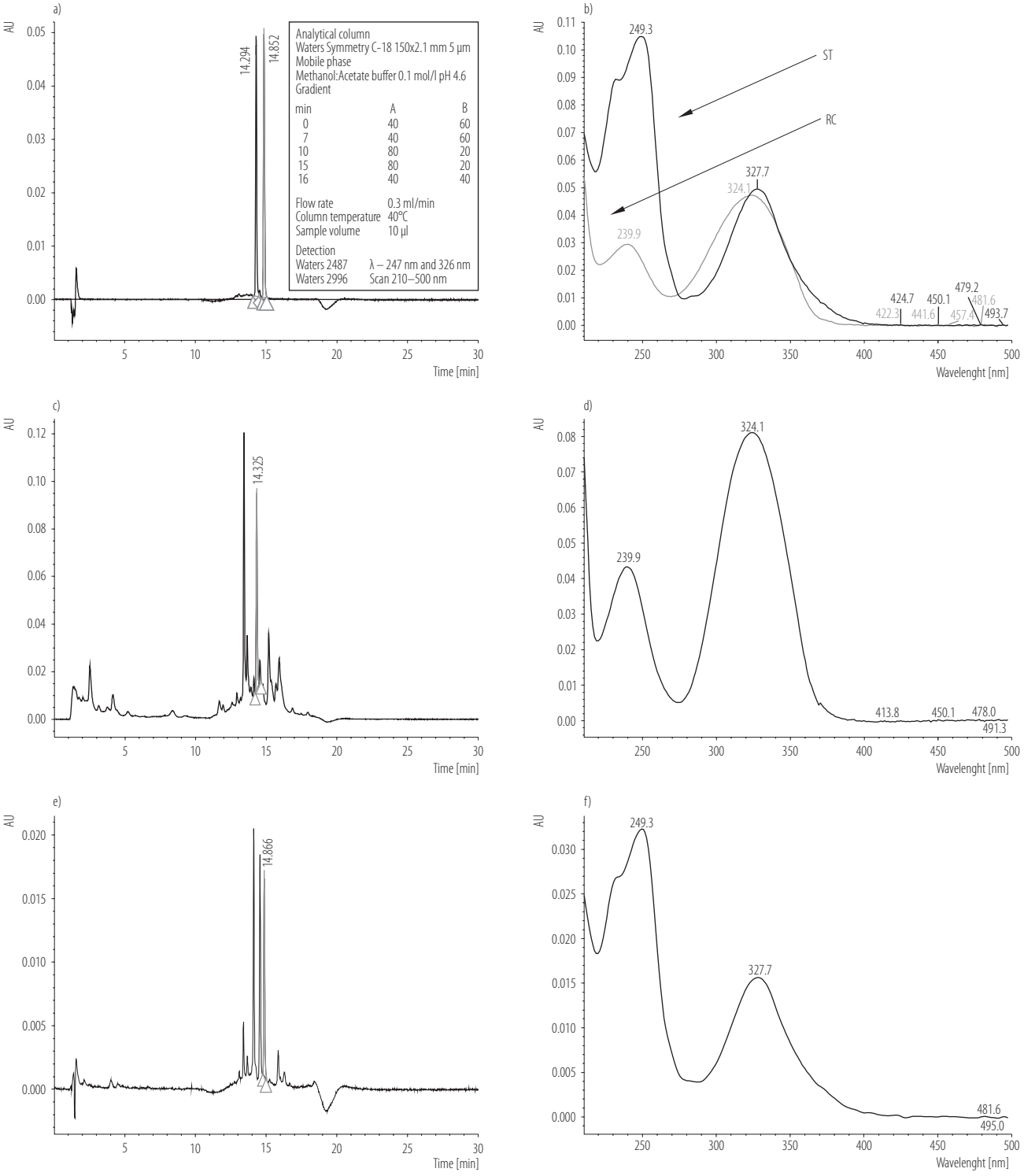
Chemicals and apparatus

The following reagents were used for the analysis: standard sterigmatocystin, high-performance liquid chromatography (HPLC) grade acetonitrile (Sigma-Aldrich, USA), standard roquefortine C (LCG Standards, Germany), sodium acetate, acetic acid (POCH S.A., Poland), HPLC grade methanol (JT Baker, Netherlands) and water purified by using Milli-Q Plus Purification System (Merck Millipore, Germany).

Qualitative and quantitative analyses were conducted using a Waters Alliance HPLC system equipped with Waters 2695 separation module, Waters 2996 PAD detector, Jet Stream column thermostat and Empower 2 software or Waters Breeze HPLC system equipped with Waters 1525 binary pump, Waters 717 plus autosampler, Waters 2487 dual absorbance detector, Jet Stream column thermostat and Breeze software. Chromatographic separations were carried out using C-18 Waters Symmetry (150×2.1 mm, 5 μm) analytical column eluted (gradient elution) with methanol:acetate buffer mobile phase.

HPLC analysis

Extracts of cultures of molds and scrapings were evaporated to dryness with gentle stream of nitrogen at 40°C. Dry residue was dissolved in 1 ml of acetonitrile and analyzed by means of HPLC method. Stock solutions of sterigmatocystin (ST) and roquefortine C (RC) were prepared in acetonitrile at concentrations 1 mg/ml and 0.1 mg/ml, respectively. Calibration standards were prepared by subsequent dilution of ST and RC mixture (10 μg/ml each). At least 6-point 0.2–10 μg/ml calibration curve was prepared. Determinations were conducted simultaneously at 247 nm and 326 nm. Identifications of ST and RC were made by comparison of UV spectra (200–500 nm, Waters 2996) of standards and corresponding (retention time – RT) peaks of analyzed samples (Figure 1). In the case of samples for which spectral identification was not possible (low concentration of analyzed compounds), calculations of area under the curve (AUC) ratio of ST and RC standards



AU – absorbance units; ST – sterigmatocystin; RC – roquefortine C.

Fig. 1. Chromatogram and UV spectra of a–b) sterigmatocystin and roquefortine C standards and c–f) chromatograms of 2 samples with identified c–d) roquefortine C and e–f) sterigmatocystin

Table 1. Area under the curve (AUC) ratio of roquefortine C and sterigmatocystin standards

Compound concentration [μg/ml]	AUC		Ratio		AM±SD (RSD)
	247 nm	326 nm	AUC_{326}/AUC_{247}	AUC_{247}/AUC_{326}	
Roquefortine C					1.7±0.1 (7.4)
0.2	3 844	6 231	1.6		
0.5	8 655	12 829	1.5		
1	16 686	26 880	1.6		
2	30 276	52 641	1.7		
5	72 463	131 099	1.8		
10	145 813	259 899	1.8		
Sterigmatocystin					2.1±0.2 (7.2)
0.2	11 646	6 634		1.8	
0.5	26 266	12 680		2.1	
1	58 632	28 153		2.1	
2	116 040	55 465		2.1	
5	285 156	135 258		2.1	
10	584 378	268 999		2.2	

AM – arithmetic mean; SD – standard deviation; RSD – relative standard deviation.

and corresponding (RT) peaks measured at 247 nm and 326 nm were made. Concentration of ST or RC was calculated only for samples for which $AUC_{247/326}$ ratio was within ±10% of the mean value calculated for standards (Table 1).

Statistics

The results for isolated fungi are presented as a percentage which they constitute in the total concentration of fungi determined in the tested samples. The results of mycotoxin analysis were expressed in terms of micrograms per gram of the tested sample (μg/g) (scrapings, dry mass of cultured molds with substrate) and micrograms per cubic meter of air (μg/m^3) (airborne dust).

RESULTS

Mycological analysis of the samples collected from moldy surfaces demonstrated the presence of molds and yeasts. Fourteen species of molds belonging to 8 genera and 3 species of yeasts belonging to 2 genera were detected. Considering the incidence of particular species of fungi, most frequently isolated on infected surfaces were: *Aspergillus versicolor*, *Cladosporium cladosporioides*, *Penicillium chrysogenum*, *Ulocladium chartarum* and *Acremonium charticola*. Among the cultured fungi, 4 genera of molds which allergize humans were found (*Aspergillus*, *Penicillium*, *Cladosporium* and *Phoma*), as well as 2 species capable to synthesize the analyzed mycotoxins hazardous for humans (*Aspergillus versicolor*, *Penicillium chrysogenum*) (Table 2).

Table 3 shows concentration of mycotoxins in various types of samples. While assessing the toxinogenicity of individual strains of *Aspergillus versicolor* cultured on the medium, the production of sterigmatocystin ranging 2.1–235.9 μg/g was confirmed in conditions similar to the conditions prevailing in the tested flats for 8 of 13 isolated strains, while for *Penicillium chrysogenum* the production of roquefortine C ranging 12.9–27.6 μg/g was confirmed

Table 2. Genera/species of fungi isolated from samples of scrapings and composition

Genus/species of fungi	Composition of samples [%]
Dwelling 1	
Ulocladium chartarum	38.9
Rhodotorula mucilaginosa 2	34.7
Cladosporium herbarum	20.8
Aspergillus versicolor	3.5
Penicillium chrysogenum	1.4
Penicillium brevicompactum	0.7
Dwelling 2	
Aspergillus versicolor	41.2
Cladosporium cladosporioides	36.3
Acremonium charticola	22.5
Dwelling 3	
Cladosporium sphaerospermum	82.3
Cladosporium cladosporioides	17.7
Dwelling 4	
Aspergillus versicolor	27.3
Acremonium charticola	27.3
Cladosporium cladosporioides	18.2
Cladosporium herbarum	9.1
Tritirachium sp.	9.1
other fungi	9.0
Dwelling 5	
Aspergillus versicolor	96.1
Cladosporium cladosporioides	3.4
other fungi	0.5
Dwelling 6	
Cladosporium cladosporioides	80.9
Penicillium chrysogenum	19.0
Dwelling 7	
Cladosporium cladosporioides	75.5
Aspergillus versicolor	20.4
Candida famata	4.1
Dwelling 8	
Cladosporium cladosporioides	76.9
Aspergillus versicolor	11.5
Penicillium chrysogenum	7.7
Candida famata	3.9

Table 2. Genera/species of fungi isolated from samples of scrapings and composition – cont.

Genus/species of fungi	Composition of samples [%]
Dwelling 9	
Cladosporium cladosporioides	95.2
Ulocladium chartarum	2.4
Penicillium chrysogenum	2.4
Dwelling 10	
Cladosporium cladosporioides	99.0
Acremonium charticola	1.0
Dwelling 11	
Cladosporium cladosporioides	92.4
Aspergillus versicolor	0.7
other fungi	6.9
Dwelling 12	
Geomyces pannorum	97.7
Candida famata	2.3
Dwelling 13	
Acremonium charticola	86.3
Cladosporium sphaerospermum	13.7
Dwelling 14	
Phoma sp.	56.3
Ulocladium chartarum	35.0
Aspergillus versicolor	2.9
Penicillium brevicompactum	1.9
Penicillium variabile	1.0
other fungi	2.9
Dwelling 15	
Candida famata	99.7
Penicillium chrysogenum	0.3
Dwelling 16	
Cladosporium cladosporioides	74.0
Aspergillus versicolor	26.0
Dwelling 17	
Candida guilliermondi	60.6
Aspergillus versicolor	29.5
Penicillium chrysogenum	5.3
Acremonium butyri	4.5
Dwelling 18	
Penicillium chrysogenum	65.3

Table 2. Genera/species of fungi isolated from samples of scrapings and composition – cont.

Genus/species of fungi	Composition of samples [%]
Dwelling 18 – cont.	
Ulocladium chartarum	34.7
Dwelling 19	
Penicillium chrysogenum	85.2
Aspergillus versicolor	5.6
Cladosporium cladosporioides	3.7
Candida guilliermondi	1.8
other fungi	3.7
Dwelling 20	
Penicillium chrysogenum	81.8
Aspergillus versicolor	9.1
Ulocladium chartarum	6.1
Phoma sp.	3.0
Dwelling 21	
Cladosporium cladosporioides	92.5
Cladosporium sphaerospermum	5.0
Acremonium charticola	2.5
Dwelling 22	
Cladosporium sphaerospermum	47.9
Penicillium chrysogenum	22.6
Aspergillus versicolor	16.8
Rhodotorula mucilaginosa 2	5.8
Acremonium sp.	3.2
Ulocladium chartarum	0.5
other fungi	3.2

for 4 of 10 isolated strains. Analysis of the mixture of fungi cultured from scrapings indicated that the number of molds coexisting on one infected surface varied from 1 to 6 species. In 11 of 13 samples of the mixture of fungi cultured from scrapings in which *Aspergillus versicolor* was found, the presence of sterigmatocystin was found at the level of 3.1–1683.2 μg/g, whereas in 3 of 10 samples in which mold of *Penicillium chrysogenum* species occurred, the production of roquefortine C was confirmed to vary from 0.9 μg/g to 618.9 μg/g.

Table 3. Concentration of mycotoxins in various types of samples from studied moldy dwellings

Mycotoxin	Pure cultures of *Aspergillus versicolor* and *Penicillium chrysogenum*			Mixture of fungi cultured from scrapings			Scrapings			Indoor air dust		
	positive samples [n]	concentration		positive samples [n]	concentration		positive samples [n]	concentration		positive samples [n]	concentration	
		μg/ml	μg/g		μg/ml	μg/g		μg/ml	μg/g		μg/ml	μg/m³
Sterigmatocystin[a]	8/13	3.6–357.3	2.1–235.9	11/13	3.6–1 901.2	3.1–1 683.2	0/22	bdl	bdl	0/22	bdl	bdl
Roquefortine C[a]	4/10	18.1–38.8	12.9–27.6	3/10	1.3–699.1	0.9–618.9	0/22	bdl	bdl	0/22	bdl	bdl

[a] Determination limit: 0.2 μg/ml.
bdl – below determination limit.

Air samples and scrapings from moldy surfaces collected in flats where isolated was any of the 2 potentially toxinogenic (*Aspergillus versicolor* or *Penicillium chrysogenum*) species of fungi, were analyzed for the presence of mycotoxins (sterigmatocystin and roquefortine C) using the HPLC method. The analysis did not show in any of the tested samples the presence of analyzed mycotoxins at concentration exceeding the limit of determination of the method (0.2 μg/ml).

DISCUSSION

The surfaces of moldy residential rooms may be infected with a number of species of molds and yeasts. Microbiological analysis in the study flats revealed 17 species of fungi which belong to 10 genera. Fungi of 5 genera: *Cladosporium*, *Aspergillus*, *Penicillium*, *Ulocladium* and *Acremonium* were isolated most frequently. Similar results were obtained by other authors [6,9–13,44].

The analysis showed that sterigmatocystin was confirmed to be present in 11 of 13 samples of mixtures of fungi cultured from the scrapings. Similar results were obtained by other authors. Gravesen et al. found sterigmatocystin in 19 of 23 samples of building materials infected in laboratory conditions with fungi collected from moldy Danish buildings [13]. Piontek isolated from building materials and other moldy surfaces 22 strains of *Aspergillus versicolor*, of which 19 in laboratory conditions synthesized sterigmatocystin at 0.03–534.38 mg/kg [33].

Gutarowska et al. examined the toxicity of fungi isolated from 34 tenement houses with visible signs of moldiness on different surfaces [7]. To check the toxinogenic activity of the collected *Aspergillus* and *Penicillium* genera, the authors cultured them on microbiological media and on the building materials inoculated with the mycelium in the laboratory. The molds cultured both on the media and on the building materials were the mycotoxin-producing ones. Molds of *Aspergillus versicolor* species produced sterigmatocystin on MEA medium and on building materials at 34 000–94 000 μg/kg and 82–480 μg/kg, respectively. Molds belonging to *Penicillium chrysogenum* produced roquefortine C on MEA medium and on building materials at 2600 μg/kg and 100–1100 μg/kg, respectively [7].

Analysis of scrapings and air samples did not indicate presence of studied mycotoxins at quantities exceeding the limit of determination of the method. Several other researchers determined the tested mycotoxins in samples of moldy materials or in the air of moldy buildings.

Vishwanath et al. tested the content of a number of different mycotoxins, including sterigmatocystin and roquefortine C in samples of different building materials collected from moldy buildings from Slovakia and Austria [45]. They demonstrated that of 14 samples of scrapings, in 10 samples sterigmatocystin was present at concentrations ranging 1.8–3900 μg/kg, while in 5 samples roquefortine C was found at concentrations 4.2–1000 μg/kg [45].

Tuomi et al. found sterigmatocystin in 19 of 79 samples of moldy materials collected from buildings in Finland [12].

Taubel et al. tested the content of mycotoxins in building materials collected from moldy residential buildings and public utility buildings. Similar to our results, they also found sterigmatocystin and roquefortine C (sterigmatocystin was found in 14 and roquefortine C in 5 of the total 42 samples) [46].

Polizzi et al. investigated the presence of mycotoxins in air and in scrapings collected from various types of surfaces infected with fungi in moldy residential buildings [30]. Of the investigated mycotoxins, sterigmatocystin and roquefortine C were determined at highest amounts. The analysis indicated that of 20 collected air samples, in 3 the presence of sterigmatocystin was determined in the amounts ranging 0.003–1.767 ng/m^3, whereas in 1 sample roquefortine C was found at 4 ng/m^3, while of 52 samples collected from moldy surfaces, in 19 samples sterigmatocystin was found at concentrations 0.06–778.4 ng/cm^2 and 14 samples contained roquefortine C at concentrations ranging 0.106–7.2 ng/cm^2 [30].

The results presented above indicate that molds growing on various types of surfaces inside the buildings may constitute a source of mycotoxins in domestic environments. The studies conducted in buildings have not explained if airborne mycotoxins may be as dangerous for inhabitants as those getting into the organism with food.

However, it is important to note that exposure by inhalation of some mycotoxins can be more toxic than by ingestion, especially in the occupational environment [31,47,48]. The fungi species which synthesize mycotoxins were shown to locate these compounds in spores and hyphae [49–51], creating a risk to the people who inhale these morphological structures [52]. Each spore or hypha inhaled into the organism may constitute a source of one or more mycotoxins [43,53]. In the case of a high concentration of airborne spores and hyphae of fungi, especially those of the smallest size, they may be deposited in respirable area and systemic effects can be expected such as local respiratory lesions, absorption to circulatory and lymphatic systems and distribution to other organs [52].

Most of the quoted results obtained by other authors refer to the studies carried out in largely damp flats but, similarly as in our research, those not affected by flood. All studies, similarly to ours, were performed using the HPLC technique, therefore it is not clear why, in our research, we could not demonstrate the presence of mycotoxins in the air dust and scraping samples from flats. Perhaps this is due to climatic differences. The study which was most similar to ours and was carried out in the same agglomeration indicated a synthesis of mycotoxins by fungi collected from walls but, as in our study, only in cultures grown in laboratory conditions [7].

Sterigmatocystin, produced by molds of *Aspergillus* genus (mainly *Aspergillus versicolor* species) is a toxic secondary metabolite whose structure resembles that of aflatoxin, a precursor of its biosynthesis [21,54]. Similarly to aflatoxin and ochratoxin A, it inhibits protein synthesis at the level of transcription and translation. Changes in DNA resulting from the effects of sterigmatocystin lead to the immune system deficiency, and thereby to increased incidence of infectious diseases. Furthermore, this mycotoxin exhibits carcinogenic properties [52]. The other mycotoxin analyzed in this article, roquefortine C, produced by a number of species belonging to genus *Penicillium*, exhibits neurotoxic effects [40].

Although the analysis did not show the presence of sterigmatocystin or roquefortine C in the air or in scrapings from infected surfaces, they were shown to be present in the mixture of fungi cultured from the scrapings. This confirms that the strains of *Aspergillus versicolor* and *Penicillium chrysogenum* classified as toxinogenic exhibit this property in laboratory conditions when cultured on MEA medium.

Literature indicates that toxinogenic activity of fungi is to some extent connected with the presence of other species in the environment, which compete for access to foodstuffs [55,56]. Besides, indispensable for synthesis of mycotoxins by fungi are appropriate climatic conditions (temperature, humidity) and, first of all, access to medium rich in organic substances [24,25,56].

Please note that the results of our studies indicated the presence of mycotoxins in the culture grown in laboratory conditions, i.e., when optimum conditions for fungi growth were provided. The same mixture of fungi species was present on the walls in the examined flats (scrapings) but the laboratory analysis did not show any mycotoxins in them, which could result from the lack of conditions proper for mycotoxin synthesis. According to literature, there are, though scarcely, strains which produce far fewer mycotoxins when growing on building materials, as compared to growing on microbiological media. There are also potentially toxinogenic strains which have lost their capability to produce mycotoxins in natural conditions and these are capable to produce them only in specific laboratory conditions [53,56].

The results of our research demonstrate that a change in the conditions prevailing in the flat that affect fungal metabolism may result in production of mycotoxins. To avoid contamination of indoor air and surfaces by mycotoxins, first of all, it is necessary to prevent the growth of fungi; therefore, the conditions such as relative humidity, substrate humidity, temperature, availability of nutrients should be permanently monitored.

The presented results enrich the existing knowledge about the diversity of fungi species isolated from moldy surfaces in the flats not affected by flood and confirm the practical capabilities of some of these microorganisms to produce mycotoxins.

Although the research is limited by a low number of collected and analyzed samples, yet the results, consistent with similar studies reported by other authors, allow to formulate the following conclusions.

CONCLUSIONS

Fungi representing 17 species belonging to 10 genera were identified in moldy flats. Molds of the following genera were predominant on the infected surfaces: *Aspergillus*, *Cladosporium*, *Penicillium*, *Ulocladium* and *Acremonium*.

Among the identified fungi, there were genera which may exhibit the capability to produce mycotoxins harmful to human health (*Aspergillus* and *Penicillium*). The research confirmed the occurrence, on the tested surfaces, of strains of *Aspergillus versicolor* genus which can produce sterigmatocystin and strains of *Penicillium chrysogenum* genus capable of producing roquefortine C.

The capability for synthesis of sterigmatocystin by molds of genus *Aspergillus versicolor* and roquefortine C by *Penicillium chrysogenum* growing in mixtures of fungi from scrapings and pure cultures in laboratory conditions was confirmed.

The absence of mycotoxins in scrapings and air dust samples indicates an insignificant inhalatory exposure to mycotoxins in case of people who live in moldy flats of urban agglomerations situated far from flooded territories, but in which some specific conditions (such as substrate humidity, temperature, availability of nutrients) can promote higher production of mycotoxins. To avoid synthesis of, and contamination by mycotoxins, the conditions specified above should be monitored.

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