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Original Research

An Overview of the Species of Fungi Occurring in School Rooms – a Four-Year Study

Elżbieta Ejdys*, Maria Dynowska, Anna Biedunkiewicz, Ewa Sucharzewska

Department of Mycology, University of Warmia and Mazury in Olsztyn,
Oczapowskiego 1A, 10-957 Olsztyn-Kortowo, Poland

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Abstract

The objective of this research was to determine the species composition of school rooms of various functionalities, the selection of typical species, and the development of assumptions of school environment monitoring. Out of the 151 species identified in the studied school rooms, 52 were grown at a temperature above 25ºC, which constituted 34.4%. Seven species were isolated by incubating the samples at 40ºC, yet it was indispensable only for *Acremonium alabamense*. Thermophilic fungi constituted 55.3% of the species with the above-indicated Bio-Safety Level status. Out of the identified fungi, one was classified to bio-safety class (*Blastomyces dermatitidis*), whereas 22 species were claccified as BSL2 (most were: *Aspergillus funigatus*, *A. flavus*, *A. terreus*) and 61 species to class 1. So far, investigations of the indoor bio-aerosol of rooms in this part of Europe have not demonstrated such a wide species spectrum of fungi, as is the case in this study.

Keywords: indoor, school, air, fungi, children

Introduction

The commonness and ubiquity of fungi are the reasons behind the fact that they are detected in all parts of the biosphere. The key factor in the dissemination of their diaspores is air. Due to the microscopic size of fungi spores, the most important role in their transmission is attributed to anemochory. Even the slightest air shifts may transfer fungal spores, and their small mass allows them to float for a long time in the air we breath. Those characteristics of fungal spores determine their distinct predominance over other bio-components of air, constituting a specific bio-colloid with gas as a dispersing phase. The quality of air bio-aerosol depends on the number of condensation sites, adsorption of extrinsic bodies in aerosol molecules, or on the change in their electric charge. The more spherical the shape of their molecules the more stable of the dispersion phase of the aerosol. It is of great significance to fungi whose spores are over 10 μm in size and are falling down with the speed described approximately with Stoke's law [1]. The smaller the spores, the slower the speed they are falling down and the greater their tendency to be drifted with Brownian motions, and thus to diffuse in different directions. The duration of fungal spore retention in the atmospheric aerosol also is affected by meteorological factors, including: atmospheric pressure, temperature, humidity, or air shifts [1, 2].

In a moderate climate, the mycobiota of atmospheric air is determined by other biotic and abiotic factors as well as by phenological changes [2]. The indoor bio-aerosol is subject to fluctuations linked to a great extent with the heating of rooms or lack thereof [3]. In some countries of Eastern Europe, the heating season may span for 7 months.

In autumn and winter Polish citizens spend most of their time in closed rooms, with children and adolescents spending 8 hours a day in school facilities [4]. School complexes are public buildings that are simultaneously used for many different purposes, including education, play, consumption of meals, and exercising sports.

*e-mail: elzbieta.ejdys@uwm.edu.pl

Results of a previous study [5] addressing the presence of fungi in the indoor bio-aerosol demonstrated that intensively-utilized school facilities, including hallways and dressing rooms, had the widest spectrum of fungi species and thus constituted a potential threat to the health of children and school staff. Fungi inhaled with air are increasingly often indicated not only as the causative agent of mycoses but also of allergic reactions and/or mycotoxicoses induced by secondary metabolites of fungi that tend to accumulate in different parts of the ontosphere, especially in parenchymatous organs [6]. According to Kurnatowska [7], the ontosphere should be understood as the body of man together with all organisms inhabiting it. A preliminary/fragmentary research [8] showed that school rooms were posing a greater risk of fungal infections than the air outside the buildings. This conclusion was drawn based on the count of yeast-like fungi isolated from the bioaerosol of a school building with the appropriate parameters of humidity and temperature, and that of yeast-like fungi isolated from atmospheric air. The first case identified as many as 28 species from 15 genera (all noted in clinical specimens originating from man), whereas the other indicated 24 species belonging to 14 genera [3, 8].

The identification of as complete as possible a taxonomic spectrum of the mycobiota of rooms is important from cognitive, epidemiological, and prophylactic concerns. The objective of this research was, therefore, to determine the species composition of school rooms of various functionalities, the selection of typical species, and the development of assumptions of school environment monitoring.

Material and Methods

The experimental material were fungi isolated from rooms of two school buildings: one in Olsztyn (building I) and one in Dobre Miasto (building II). Olsztyn (53º46'26"N 20º29'30"E) and Dobre Miasto (53º59'15″N 20º23'45″E) are located in the Olsztyn Lakeland area. Climatic conditions occurring therein are characterized by lower temperatures, higher air humidity, and a shorter vegetative season when compared to the other regions of Poland [9].

Both buildings under study were built in the 1970s using prefabricated concrete technology. Currently, they are utilized by a similar number of people. The following types of school rooms were selected for mycological analyses: a classroom, a hallway, toilets (women's and men's), sports locker room and shower (ground floor), and school dressing room (basement). The heating season spanned from October to April. In September, May, and June, the heating of school buildings was switched off.

Samples were collected from air with the sedimentation method [10]. Research stations were established in two corners of each type of room, diagonally. Fungi spores were collected from walls with the method of surface swabbing [11] used successfully to study the walls of buildings standing on flood areas [12], as well as assessment of frescoes [13]. At three measurement site of each room facility surfaces painted with acrylic or emulsion paint that were further referred to as rough were selected, and walls painted with oil paint or glaze further referred to as smooth. At each station (in total 8 per each type of room), samples were collected for each of the three planned incubation temperatures onto four different culture media. No macroscopic changes were observed on the walls that would indicate the development of mycelium.

Analyses were carried out in May (with heating off) and in November (with heating on), for four consecutive years. Temperature and humidity of the air of school rooms were measured during sample collection.

The mould fungi were grown on the Sabouraud and Czapek-Dox [14] medium in three parallel cultures run at: 25, 37, and 40ºC, for at least three to maximally five weeks. The cultures of yeast and yeast-like fungi were run for one week also at three temperatures on: Sabouraud medium with chloramphenicol and on culture medium with rose Bengal [15]. The addition of rose Bengal slightly delays the growth of filamentous fungi and enables the isolation of more slowly growing saccharomycetes. Isolation and identification was determined with standard methods applied in mycological laboratories [16-18].

Fungi of the genus *Aspergillus* and *Penicillium* were identified to species based on the keys by: Raper and Fennell [19] as well as Raper, Thom, and Fennell [20]. The other species were identified using the atlas of fungi noted in clinical materials [21]. The yeast-like fungi and yeast were identified based on keys by Kreger-van Rij [22], Barnett, Payne and Yarrow [23], as well as Kurtzman and Fell [24].

For practical reasons, use was made of species names according to Kurtzman and Fell [24] and of the nomenclature used in manuscripts describing results of the preliminary studies [3, 5]. Teleomorphic and anamorphic stages were discriminated. Isolates growing at 37 and 40 degrees were considered thermophilic [25]. Fungi has been assigned the status of BSL [26].

Results

In total, 772 isolates of fungi belonging to 151 species from 48 genera and 70 sterile isolates were obtained in the study (Table 1). The most frequently isolated fungi included those belonging to the genera (Table 2): *Aspergillus* (36 species), *Penicillium* (26 species), and *Candida* (14 species+2 anamorphs). The predominating species turned out to be *Aspergillus fumigatus* (126 isolates), followed by *Penicillium citrinum* (80) and *P. chrysogenum* (76). A threefold lower number of isolates (44), compared to the prevailing species, was obtained for *Cladosporium herbarum*. Slightly more than 50% of the species detected in the experiment were isolated only once (Table 2).

Out of the identified fungi, one was classified to BSL3 (*Blastomyces dermatitidis*), whereas 22 species were classified BSL2, and 61 species BSL1. The other species have so far not been granted any status in this classification.

The greatest species diversity was observed in toilet rooms (73 species). The school dressing rooms and hall-

Table 1. Number of fungal species isolated in different categories: spring and autumn, school premises, and incubation temperature (quantitative summary).

Number of fungal species	Sampling time		Type of room*						Incubation temperature [ºC]		
	spring	autumn	cl	h	sd	sl	t	sr	20	37	40
Only in this category	77	40	10	27	27	6	38	13	83	50	2
Total	112	75	44	51	55	37	73	19	98	66	7

* cl – classroom, h – hallway, sd – school dressing, sl – sports locker room, t – toilet, sr – shower

Table 2. Fungi isolated in spring and autumn of school premises and the identity of their incubation temperature and BSL status (qualitative summary).

No.	Species Name	Number of isolates			BSL	Type of room						The incubation temperature [ºC]		
		S	A	S		cl	h	sd	sl	t	sr	20	37	40
1.	*Ac. blochii* (Matruchot) W. Gams	0	2	2	1	√	√					x	x	
2.	*Alternaria alternata* (Fr.) Keissl.	4	1	5	1	√	√	√	√	√		x		
3.	*Al. chlamydospora* Mouchacca	4	0	4	1						√	x		
4.	*A. caesiellus* Saito	5	0	5	1	√	√	√		√			x	
5.	*A. candidus* Link	3	2	5	1	√		√	√	√		x	x	
6.	*A. clavatonanicus* Batista, Maia & Alecrim	6	0	6	1		√			√	√	x		
7.	*A. clavatus* Desmazieres	1	5	6	1	√		√		√		x		
8.	*A. duricaulis* Raper & Fennel	6	0	6	-	√	√					x		
9.	*A. ficuum* (Reich.) Hennigs	1	1	2	-					√		x		
10.	*A. flavipes* (Bain.& Sant.) Thom & Church	2	0	2	1	√	√					x		
11.	*A. flavus* Link	17	0	17	2	√	√		√		√	x	x	
12.	*A. fumigatus* Frasenius	46	80	126	2	√	√	√	√	√	√	x	x	x
13.	*A. granulosus* Raper & Thom	2	1	3	1			√		√	√	x	x	
14.	*A. janus* Raper & Thom	10	4	14	1	√	√			√		x	x	
15.	*A. nidulans* (Eidam) Wint	2	0	2	1						√	x		x
16.	*A. niger* Van Tieghem	5	7	12	1	√	√	√		√	√	x	x	x
17.	*A. phoenicis* (Cda.) Thom	2	3	5	-	√	√	√		√		x		
18.	*A. quadrilineatus* Thom & Raper	2	0	2	-	√			√			x		
19.	*A. sclerotiorum* Huber	3	0	3	1			√	√		√	x		
20.	*A. sydowii* (Bain.& Sart.) Thom & Church	11	4	15	1		√	√	√	√	√	x	x	
21.	*A. terreus* Thom	5	6	11	2	√		√	√	√		x		
22.	*A. unguis* (Emile-Weil & Gaud.) Thom & Raper	2	3	5	1		√	√	√			x		
23.	*A. versicolor* Tiraboschi	15	6	21	1	√	√	√	√	√		x	x	
24.	*Aureobasidium pullulans* (de Bary) Arnaud	2	0	2	1			√		√		x		
25.	*Bipolaris spicifera* (Bain.) Subram.	2	0	2	1						√	x		
26.	*Bjerkandera adusta* (Willd.: Fr.) Karst	4	1	5	1	√		√	√			x	x	
27.	*Botrytis cinerea* Pers. ex Pers.	2	0	2	-	√						x	x	

Table 2. Continued.

No.	Species Name	Number of isolates			BSL	Type of room						Incubation temperature [ºC]		
		S	A	S		cl	h	sd	sl	t	sr	20	37	40
28.	*C. dubliniensis* Sullivan et al.	0	2	2	2			√	√				x	
29.	*C. glabrata* (Anderson) S.A Meyer & Yarrow	1	2	3	-			√		√			x	
30.	*C. gloebosa* Yarrow & S.A. Meyer	5	4	9	-	√		√	√	√			x	x
31.	*C. guilliermondii* (Castellani) Berkhout	1	4	5	1		√		√	√			x	
32.	*C. versatilis* (Etchells & T.A. Bell) S.A.Meyer & Yarrow	2	0	2	-	√	√						x	
33.	*Chaetomium atrobrunneum* Ames	9	0	9	1	√	√	√		√			x	
34.	*Chrysosporium queenslandicum* Apinis & Rees	1	1	2	1					√		x		
35.	*Cladosporium cladosporoides* (Fres.) de Vries	5	0	5	1				√	√		x		
36.	*Cl. herbarum* (Pers.) Link: Fr.	44	0	44	1	√	√					x		
37.	*Cl. oxysporum* Berk. & Curt.	9	0	9	1			√	√	√		x		
38.	*Cl. sphaerospermum* Penz.	4	0	4	1			√		√		x		
39.	*Cystofilobasidium informominiatum* (Fell, I.L. Hunter & Tallman) Hamamoto, Sugiyama & Komagata	3	1	4	-		√	√	√		√		x	
40.	*Debaryomyces hansenii* (Zopf) Lodder & Kreger-van Rij	1	4	5	1	√		√		√			x	
40a.	*C. famata* (Harrison) S.A. Meyer & Yarrow	0	2	2	-			√	√				x	
41.	*D. polymorphus* (Klőcker) Price & Phaff	0	5	5	-	√			√	√			x	
42.	*Epidermophyton floccosum* (Harz) Langer. & Milochevitch	3	0	3	2			√				x		
43.	*Fusarium incarnatum* (Rob.) Sacc.	3	0	3	1	√				√		x		
44.	*Geotrichum candidum* Link: Fr.	12	0	12	1	√		√	√				x	
45.	*G. clavatum* de Hoog et al.	4	0	4	2					√			x	
46.	*Gymnoascus dancaliensis* (Castell.) v. Arx	2	0	2	2		√					x	x	
47.	*Hormographiella aspregillata* Guarro et al.	2	0	2	1					√			x	
48.	*H. verticillata* Guarro et al.	2	0	2	1					√			x	
49.	*Kluyveromyces lactis (*Dombrowski) van der Walt	0	12	12	-		√	√	√	√			x	
50.	*K. marxianus* (E.C. Hansen) van der Walt	0	3	3	1	√				√			x	
51.	*K. thermotolerans* (Filippov) Yarrow	0	2	2	-	√				√			x	
51a.	*C. dattila* (Kluyver) S.A. Meyer & Yarrow	1	0	1	-			√					x	
52.	*K. wickerhamii* (Phaff, M.W. Miller & Shifrine) van der Walt	1	1	2	-			√					x	
53.	*Mrakia frigida* (Fell, Statzell, I.L. Hunter & Phaff) Y. Yamada & Komagata	2	0	2	-		√			√			x	

Table 2. Continued.

No.	Species Name	Number of isolates			BSL	Type of room						Incubation temperature [ºC]		
		S	A	S		cl	h	sd	sl	t	sr	20	37	40
54.	*Nadsonia commutata* Goluber	2	0	2	-		√	√					x	
55.	*Penicillium chrysogenum* Thom	71	5	76	1	√		√	√	√	√	x	x	
56.	*P. citrinum* Thom	59	21	80	1	√	√	√	√	√		x		
57.	*P. marneffei* Segretain	5	2	7	2		√	√	√	√		x		
58.	*P. nigricans* (Bain.) Thom	6	1	7	-		√					x		
59.	*P. notatum* Westling	0	2	2	-		√			√		x		
60.	*P. piceum* Raper & Fennel	7	3	10	-	√	√		√	√		x		
61.	*P. solitum* Westling	2	0	2	-	√			√			x		
62.	*P. spinulosum* Thom	0	2	2	1		√			√		x		
63.	*P. steckii* Zaleski	4	1	5	-			√				x		
64.	*P. verruculosum* Dierckx	0	2	2	1	√				√		x		
65.	*P. waksmanii* Zaleski	2	2	4	-		√	√				x		
66.	*Pichia farinosa* (Lindner) E.C. Hansen	4	2	6	-		√	√	√	√			x	
67.	*P. membranifaciens* (E.C. Hansen) E.C. Hansen	1	3	4	-	√			√	√			x	
68.	*Rhizopus microsporus* v. Tiegh.	0	3	3	-	√	√			√		x		
69.	*Rhodotorula glutinis* (Fresenius) F.C. Harrison	13	1	14	1	√	√	√		√		x	x	x
70.	*Scopulariopsis brumptii* Salvanet-Duval	3	0	3	2			√			√	x		
71.	*Sc. flava* (Sopp) Morton & G. Smith	2	0	2	1		√					x	x	
72.	*Saccharomyces cerevisiae* Meyen ex E.C. Hansen	5	3	8	-	√		√	√	√			x	x
73.	*Saccharomycopsis capsularis* Schiőnning	2	1	3	-	√	√		√	√			x	
74.	*Trichophyton verrucosum* Bodin	2	0	2	2	√						x		
75.	*Yarrowia lipolytica* (Wickerham, Kurtzman & Herman) van der Walt & von Arx	0	2	2	1				√	√			x	
76.	Species isolated only once[3)]	46	30	76		6	16	17	7	26	6	48	27	1
77.	Sterile isolates	51	19	70										
78.	Total isolates	568	274	842										
79.	Number of fungal species	112 77[1)]	75 40[1)]			44	51	55	37	73	19	98 83[2)]	66 50[2)]	7 2[2)]

S – spring; A – autumn; BSL – Bio-Safety Level (De Hoog 1996); cl – class room, h – hallway, sd – school dressing, sl – sports locker room, t – toilet, sr – shower; a. – anamorphic stage; [1)] number of fungal species isolated only in this time of year; [2)] number of species of fungi isolated only in the incubation temperature, [3)] Alphabetical list of species isolated only once:
Acremonium alabamense Morgan-Jones, S, BSL1, sd, 40ºC; *Ac. hyalinulum* (Sacc.) W. Gams, S, BSL1, sd, 20; *Ac. kiliense* Grütz, S, BSL2, cl, 20; *Ac. roseogriseum* (S.B. Saksena) W. Gams, S, BSL1, t, 37; *Ac. spinosum* (Negroni) W.Gams, A, BSL1, t, 20; *Aternaria infectoria* Simmons, S, BSL1, h, 20; *Artrographis kalrae* (Tewari&Macpherson) Singler & Carmichael, S, BSL2, t, 37; *Arxula adeninivarans* (Middelhoven, Hoogkamer-Te Niet & Kreger-van Rij) van der Walt, M. Th. Smith & Y. Yamada, A, -, t, 37; *Aspergillus allahabadi* Mehrotra & Agnihotri, A, -, sl, 20; *A. asperescens* Stolk, S, -, h, 20; *A. aureolus* Fennell & Raper, S, -, t, 20; *A. auricomus* (Gueguen) Saito, S, -, sd, 20; *A. crystallinus* Kwon & Fennel, S, -, h, 20; *A. funiculosus* Smith, A, -, sd, 20; *A. glaucus* Link, S, BSL1, h, 37; *A. longobasidia* (Bain.) Moseray, S, -, sd, 20; *A. malodoratus* Kwon & Fennell, S, -, sd, 20; *A. niveus* Blochwitz, S, BSL1, sl, 20; *A. ochraceus* Wilhelm, S, BSL1, sd, 37; *A. olivaceofuscus* Mosseray, S, -, h, 20; *A. repens* De Bary, S, -, t, 20; *A. rugulosus* Thom & Raper, S, -, sl, 20; *A. ustus* (Bain.) Thom & Raper, S, BSL1, sd, 20; *A. viridenutans* Ducker & Thrower, S, -, t, 20; *Blastomyces der-*

Table 2. Continued.

matitidis Dilchrist & StokeS, A, BSL3, sr, 37; *Candida albicans* (Robin) Berkhout, S, BSL2, h, 37; *C. holmi* (Jorgensen) S.A Meyer & Yarrow, A, -, sr, 37; *C. intermedia* (Citerri & Ashford) Langeron & Guerra, A, -, t, 37; *C. krusei* (Castellani) Berkhout, S, BSL2, h, 37; *C. parapsilosis* (Ashford) Langeron & Talice, A, -, t, 37; *C. rhagii* Jurzitza, Kühlwein & Kreger-van Rij, A, -, t, 37; *C. solani* Lodder & Kreger-van Rij, S, -, t, 37; *C. utilis* (Henneberg) Lodder & Kreger-van Rij, S, BSL1, t, 37; *Chaetomium zonatum* Al.-Musallan & Tan, A, BSL1, t, 20; *Cladophiarophora boppi* (Borelli) De Hoog et al., A, BSL2, sr, 20; *Corynespora cassiicola* (Berk. & Curt.) Wei, S, BSL1, cl, 20; *Debaryomyces occidentalis* (Klöcker) Kurtzman & Robnett, A, -, h, 37; *D. vanrijiae* (van der Walt & Tscheuschner) Abadie, Pignal & J.L. Jacob, A, -, t, 37; *Emericella quadrilineata* (Thom & Raper) C.R. Benjamin, S, BSL1, sr, 20; *Emmonsia crescens* Emmons & Jellison, S, BSL2, t, 20; *Kluyveromyces yarrowii* van der Walt, E. Johannsen, Opperman & Halland, A, -, t, 37; *Lipomyces starkeyi* Lodder & Kreger-van Rij, S, -, sd, 37; *Madurella grisea* MacKinnon et al., S, BSL2, t, 20; *Microsporum fulvum* Uriburu, S, BSL1, sd, 20; *Mucor amphibiorum* Schipper, S, BSL2, sl, 20; *M. ramosissimus* Samutsevich, S, BSL1, sl, 37; *Oosporidium margaritiferum* Stautz, A, -, sl, 37; *Paecilomyces marquandii* (Masse) HugneS, A, BSL1, sr, 20; *P. variotii* Bain, A, BSL2, t, 20; *P. viridis* Segretain at al. Ex Samsen, A, BSL1, t, 20; *Penicillium baarnense* van Beyma, A, -, t, 20; *P. brevicompactum* Thom, A, -, sd, 20; *P. camemberti* Thom, S, -, h, 37; *P. claviforme* Bainier, S, -, h, 20; *P. commune* Thom, S, BSL1, h, 20; *P. corylophilum* Dierckx, A, -, sd, 20; *P. cyaneo-fulvum* Biourge, A, -, sd, 20; *P. cyaneum* (B.& S.) Biourge, S, -, sd, 20; *P. egyptiacum* van Beyma, A, -, cl, 20; *P. frequentans* Westling, A, -, t, 20; *P. jenseni* Zaleski, A, -, h, 20; *P. kapuscinskii* Zaleski, A, -, t, 20; *P. psittacinum* Thom, A, -, t, 20; *P. purpurrescens* (Scopp) n. comb., S, -, h, 20; *P. urticae* Bainier, A, -, h, 20; *Phialophora bubakii* (Laxa) Schol-Schwarz, S, -, Öcl, 20; *Phoma cruris-hominis* Punithalingam, S, BSL2, t, 20; *Pichia anomala* (E.C. Hansen) Kurtzman, A, BSL1, t, 20; *Rhizomucor pusillus* (Lindt) Schipper, A, -, sd, 20; *Rhodosporidium dacryoideum* Fell, I.L. Hunter & Tallman, S, -, t, 37; *Rh. toruloides* Banno, S, BSL1, h, 37; *Scopulariopsis brevicaulis* (Sacc.) Bain., S, BSL2, h, 37; *Saccharomyces fructuum* Lodder & Kreger van Rij, A, -, sd, 37; *Scytalidum lignicola* Pesante, S, BSL1, cl, 37; *Trichohyton schoenleinii* (Lebert) Nannizzi, S, BSL2, cl, 20; *T. tonsurans* Malmsten, S, BSL1, sd, 20.

ways were characterized by an alike diversity of fungi, i.e. 55 and 51 species, respectively. A slightly lower number of taxa was determined in classrooms and sports locker rooms (44 and 37, respectively). A dependency was noted between the prevalence of specific species and the total number of fungi species identified in a select type of room. Two types of room facilities were distinguished: group A – a classroom and a sports locker room, and group B – the other types of room facilities (Fig. 1). In the A group rooms, the percentage of specific species did not exceed 19%, whereas in the B group rooms it exceeded 33% (Table 3).

No yeast-like fungi being typical of classrooms were detected in the study. In the dressing rooms (both the sports and the general ones) and on the hallway they did not exceed 8% of the species spectrum of fungi of those room facilities. In the toilet and the shower, the prevalence of yeast-like fungi reached 15%. In the classroom, no fungi of the genus *Aspergillus* were noted among the specific fungi (Table 3).

Out of the 151 species identified in the studied school rooms, 52 were grown at a temperature over 25ºC, which constituted 34.4%. Seven species were isolated by incubating the samples at 40ºC, yet it was indispensable only for *Acremonium alabamense*. Thermophilic fungi constituted 55.3% of the species with the above-indicated BSL status.

In the investigated school rooms, more fungi were occurring in the spring time than in the autumn. Three groups of fungi were distinguished in respect to the term of occurrence (Fig. 2). The first included fungi occurring in the rooms after the terminated heating season (77 species). The other group included species isolated in school building in the heating season (40 species). The other 33 species occurred in both heating seasons (group III). The latter group constituted 21.7% (489) of all isolates. The predominating genus of the third group was *Aspergillus*, in the case of which 225 isolates from 12 species were achieved in the study. An insignificantly lower number of isolates (189) was achieved for 7 species of *Penicillium*. The yeast-like fungi and yeast constituted 1/3 of the total taxonomic spectrum of fungi occurring in the school rooms with the heating on and off. The described group of fungi was addition-

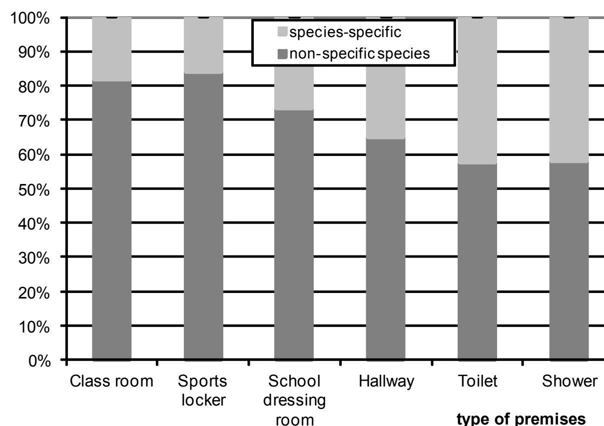

Fig. 1. The incidence of specific species in the studied rooms in relation to total isolated fungi.

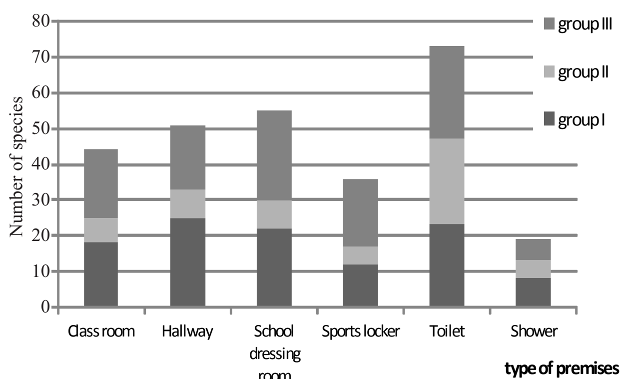

Fig. 2. Groups of fungi in terms of incidence of the season (I – included fungi occurring in the rooms after the terminated heating season, II – in the heating season, III – in both heating seasons).

Table 3. The specificity of the rooms in terms of occurrence of fungi.

Type of room	Number of isolated species	Species of fungi isolated from the studied rooms			
		Number	%	Species name	
Class room	44	8	18.2	*Acremonium kiliense* *Botrytis cinerea* *Corynespora cassiicola* *Penicillium egyptiacum*	*Phialophora pubakii* *Scytalidum lignicola* *Trichohyton schoenleinii* *T. verrucosum*
Sports locker	37	6	16.2	*Aspergillus allahabadi* *A. niveus* *A. rugulosus* *Mucor amphibiorum*	*M. ramosissimus* ***Oosporidium margaritiferum***
Hallway	51	19	37.3	*Alternaria infectoria* *Aspergillus asperescens* *A. crystallinus* *A. glaucus* *A. olivaceofuscus* ***Candida albicans*** ***C. krusei*** ***Debaryomyces occidentalis*** *Gymnoascus dancaliensis* *Penicillium camamberti*	*P. claviforme* *P. commune* *P. jenseni* *P. nigricans* *P. purpurrescens* *P. urticae* ***Rhodotorula toruloides*** *Scopulariopsis brevicaulis* *Sc. flava*
School dressing room	55	20	33.4	*Acremonium alabamense* *Ac. hyalinulum* *Aspergillus auricomus* *A. funiculosus* *A. longobasidia* *A. malodoratus* *A. ochraceus* *A. ustus* ***Candida dattila*** *Epidermophyton floccosum*	***Kluyveromyces wickerhamii*** ***Lipomyces starkeyi*** *Microsporum fulvum* *Penilillium brevicompactum* *P. corylophilum* *P. cyaneum* *P. steckii* *Rhizomucor pusillus* ***Saccharomyces fructuum*** *Trichophyton tonsurans*
Toilet	73	31	42.5	*Acremonium roseogriseum* *Ac. spinosum* *Artrographis kalrae* ***Arxula adeninivarans*** *Aspergillus aureolus* *A. ficuum* *A. repens* *A. viridenutans* ***Candida intermedia*** ***C. parapsilosis*** ***C. rhagii*** ***C. solani*** ***C. utilis*** *Chrysosporium queenslandicum* *Ch. zonatum* ***Debaryomyces vanrijiae***	*Emmonsia crescens* ***Geotrichum clavatum*** *Hormographiella aspregillata* *H. verticillata* ***Kluyveromyces yarrowii*** *Madurella grisea* *Penicillium baarnense* *P. frequentans* *P. kapuscinskii* *P. psittacinum* *P. variotii* *P. viridis* *Phoma cruris-hominis* ***Pichia anomala*** ***Rhodosporidium dacryoideum***
Shower	19	8	42.1	*Alternaria chlamydospora* *Aspergillus nidulans* *Bipolaris spicifera* ***Blastomyces dermatitidis***	***Candida holmi*** *Cladophiarophora boppi* *Emericella quadrilineata* *Paecilomyces marquandi*

bold name: yeasts and yeast-like fungi
underlined name: species-specific spaces

ally characterized by the highest percentage of thermophilic species – 54.5%. In the heating season (group II), only 69 isolates were determined, 52.1% of which were thermophilic saccharomycetes. In turn, 13 *Penicillium* species were noted, but only one species of *Aspergillus*. The latter was represented by as many as 23 species in group I (fungi isolated off the heating season), and *Penicillium* by as few as 6 species. The lowest percentage of that group was constituted by yeast-like fungi – 28 isolates. Out of the 214 isolates from the spring season, there were 8 species of all dermatophytes identified in the study.

In both analytical seasons, *Aspergillus fumigatus* was occurring and developing in each type of room facility and at all three incubation temperatures applied.

In the investigated school rooms, values of the relative air humidity ranged from 48 to 52% in the spring (heating off) and from 39 to 46% in the autumn (heating on). The temperature of non-heated rooms was diversified and ranged from 17.0 to 23.5ºC. Once the heating had been switched on, the amplitude reached 2.3ºC, and temperature range was 18.2-20.5ºC. No dependencies were observed between the occurrence of fungi and the relative air humidity or temperature of rooms.

Discussion

Fungi spores and other elements of mycelium are the major constituents of both atmospheric air and indoor bio-aerosol [27]. While the composition of the first may be affected by citizens only to an insignificant extent, some actions may be undertaken to reduce the concentration of fungi spores in buildings. The greater the isolation of buildings from the external environment (enforced by e.g. climate), the easier the regulation of spores concentration in rooms. The taxonomic structure and population numbers of fungi in rooms may be affected by two groups of factors. The first is related to the construction of a building, while the second one is of no less importance to the users of buildings, their number, and behavior [28]. The mode of school building utilization is a consequence of behaviors of children and adolescents and of the presence of all key links of the epidemiological chain of mycoses in the school environment [4]. A large population of school children, being at a similar age, is closed within the restricted area of a school complex for 1/3 of the day, five days a week, 10 months a year. It constitutes a group of individuals especially predisposed to infections with fungi and their migrating reservoir. The high density of school pupils significantly shortens the routes of infection or colonization of their bodies by microbes suspended in the bio-aerosol of rooms. The high percentage of thermophilic isolates obtained from the investigated rooms poses a real threat to school pupils. The quality of indoor bio-aerosol may considerably affect the health and development of children and adolescents because most of the individual traits of man are being developed over the 12-year period of staying in the school environment.

The observed correlation/dependency between the number of users and the number of fungi species in particular school room facilities may be attributed to the various functions of those rooms. A classroom and a sports locker room are used by pupils from one class (ca. 25 pupils), whereas a hallway, a school dressing room and toilets are available to all persons staying in the building. Surprising may be the very high frequency of fungi in the toilets, but at school they serve a slightly different function than in other public buildings. As they are not monitored, they serve children also as a site for meetings and sometimes even for meal consumption (!). The specific character of toilets is additionally emphasized by a high contribution of thermophilic saccharomycetes isolated only in those rooms. In the case of the thermophilic strains, staying outside a warm-blooded organism is so unfavorable that they occur therein only at increased humidity, for instance on surfaces capable of fast condensation of water vapor – e.g. on glaze covering walls of toilets. The survivability of cells of the genus *Candida* spp. devoid of the protective function of a biofilm under conditions of a lack of food substrate and low humidity does not exceed 10%. The main route of transmission of pathogenic *Candida* isolates between ontospheres of children seems to be the direct route or air-droplet route, because staying outside the host's body longer does not eradicate the fungus but leads to misadaptation of thermophilicity, followed by the loss of capability to colonize a macroorganism [29].

The periodically and cyclically conducted mycological assessment of indoor air in different types of buildings is important for a number of reasons. It may be treated as one of the elements of biomonitoring. The knowledge of the composition of building mycobiota enables determining its technical condition and the need for potential repair. The species composition of fungi indicates also the functionality of rooms and health of their users because 1/3 of the species composition of fungi occurring in buildings is usually linked with a human body and requires or tolerates a temperature of 37ºC for growth. It seems that most of the incidental isolates obtained in the reported experiment were transferred by the users from the external environment or from their place of residence. Among these, there is a tangibly thermophilic species, predominant in the studied school buildings – *A. fumigatus*. It was classified to the second biosafety class, for it belongs to species with a relatively high ability to survive in vertebrate tissues, while in individuals with immunity disorders it may induce severe opportunistic infections. It is especially dangerous to asthmatic and allergic patients, who may often be found among schoolchildren. According to the Structural Database of Allergenic Proteins [30], 26 allergens of *A. fumigatus* have been identified, that may occur in mycelium and in spores. The air aerosol of the rooms where *A. fumigatus* occurrs may also be contaminated by its mycotoxins (fumiclavin, fumitremorgens) produced by some strains [31]. Hence, the presence of this species in the school environment may pose a real threat to children and school workers. Especially, according to Piontek [32], a highly toxic fungus growing on the wall ranging from 1 to 10 cm^2 can affect the health of users of the premises.

The main parameter for the development of fungi is humidity, the humidity ratio, and more specifically the potential of the substrate to the ambient temperature in the building. The classic reference to the initial mycological assessment of facilities are Niukša graphs [33], which determine mycosafety parameters. In the examined areas oscillated temperature and humidity border the safe area [33]. In the spring the higher values of the parameters resulted in an increase in the diversity of fungi, but their quantity was safe for users of the premises.

Fungi have various requirements in terms of humidity. For instance, *Aspergillus* may grow at water activity of 0.8, although *A. glaucus* adapted its metabolism to humidity of 0.6. In contrast, *A. versicolor* is an indicatory species of damp rooms [34] and its presence indicates poor draining of water from a building. It also seems to be a thermophilic species, for in the reported experiment it was isolated only from the samples incubated at a temperature of 37ºC. In turn, other species of the "versicolor" group were often noted in the samples incubated at 25ºC, which is, however, inconsistent with literature data [35]. The determination of the discussed fungi species poses many difficulties. It is likely that the *A. versicolor* names reported in literature refer to a group and not to a species, which may be deduced based on the lack of extension by its name.

A similar and high number of thermophilic species of the genus *Aspergillus*, to that of the school rooms, was observed in a significantly warmer climate – in dormitories in Egypt [36], whereas those species were hardly ever [37] or never [38] isolated in Polish flats.

In spite of the fact that in the spring season the number of fungi in the external environment is the lowest throughout the year [36, 39], the high number of species noted in the examined rooms once the heating had been switched off was rather a result of the indoor air and school surrounding [8]. Considering the previous studies, it seems that it is the autumn taxonomic spectrum of fungi that may be found typical of the examined school environment, and species incapable of growth at a temperature of 37ºC and occurring only in one type of room – to be typical of this spectrum. Usually, these are fungi of the genus *Penicillium*: in a classroom – *P. egyptiacum* (current name: *Eupenicillium crustaceum*), in a hallway – *P. jensenii*, in toilets – *P. baarnense* (current name: *Eupenicillium baarnense*), *P. frequentans*, *P. kapuscinskii* (current name: *Penicillium canescens*), *P. psittacinum* (current name: *Penicillium commune*), as well as *Paecilomyces variotii* and *P. viridis*. In the case of toilets, typical turned out to be *Acremonium spinosum* and *Chrysosporium zonatum*. Likewise for a school dressing room, apart from fungi of the genus *Penicillium* (*P. corylophilum*), typical appeared to be *Rhizomucor pusillus* and *Aspergillus funiculosus*, and for the shower room – *Cladophialophora boppi* and *Paecilomyces marquandi*. No characteristic species were determined for the sports locker room. It is possible that further research will allow for selection of groups of fungi typical of the different facilities of school.

So far, investigations of the indoor bio-aerosol of rooms in this part of Europe have not demonstrated such a wide species spectrum of fungi [40], as is the case in this study. It seems that it may be attributed, to a great extent, to the adopted research method. Though the parallel incubations at various temperatures resulted in the appearance of sterile (non-sporulating) isolates that could not be identified, they primarily enabled completion of the taxonomic spectrum of mycobiota of school buildings. It may not be excluded that the sterile isolates (ca. 10%) also constitute a potential reservoir, posing threat to the users of buildings or affecting widely understood processes of building biodegradation.

Conclusions

Aspergillus fumigatus may be acknowledged as a permanent component of mycobiota of school/public buildings in this part of Europe, and its number in room facilities should be systematically monitored.

The room facilities with the widest species spectrum of fungi in the school environment are toilets, which have been found to pose the greatest risk of mycotic infections of various etiology to schoolchildren and staff.

In spite of the fact to microfungi isolated from school rooms occur in an external environment which constitutes their primary reservoir for building contamination, their ability to survive on constructing materials and in the bioaerosol of rooms is high. The prevalence and species abundance of fungi in schools should be continuously monitored, with special attention paid to thermophilic isolates owing to their high environmental plasticity and potential pathogenic properties.

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