

The occurrence of anamorphic fungi in indoor space air at Cracow University of Physical Education

Występowanie grzybów anamorficzných w powietrzu pomieszczeń zamkniętych w Akademii Wychowania Fizycznego w Krakowie

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Material. Badaniami objęto pomieszczenia dydaktyczne i treningowe.

Wyniki. Warunki mikroklimatyczne panujące w pomieszczeniach sprzyjają rozwojowi grzybów. Najwięcej zarodników grzybów stwierdzono w powietrzu tunelu lekkoatletycznego (308 jtk/m³). Dominującymi rodzajami były: *Penicillium*, *Aspergillus*, *Cladosporium* i *Candida*. W obrębie rodzaju *Aspergillus* najczęściej wyisobniono gatunek *A. niger* (40,5%) w tunelu lekkoatletycznym i *A. fumigatus* w pomieszczeniu kontrolnym (30,0%) i dydaktycznym (24,0%). Wśród konidiów zaliczanych do rodzaju *Penicillium* w największym odsetku stwierdzono *P. lanosum* SC (28,8%) i PK (27,8%), *P. viridicatum* SC (22,4%), *P. chrysogenum* SG (20,8%) i PK (15,5%). Grzyby zaliczane do rodzaju *Cladosporium* reprezentuje gatunek *C. herbarium*.

Słowa kluczowe: zarodniki grzybów, powietrze wewnętrzne, Kraków

Material. The research encompassed teaching and training rooms.

Results. Microclimatic conditions of the rooms favored the development of fungi. The highest number of fungal spores was recorded in the air of the athletics tunnel (308 CFU m⁻³). The predominant genera were: *Penicillium*, *Aspergillus*, *Cladosporium* and *Candida*. Within the genus *Aspergillus*, the most frequently isolated species were *A. niger* in the athletics tunnel (40.5%) and *A. fumigatus* in both the auxiliary room (30.0%) and the teaching rooms (24.0%). Among the conidia of the genus *Penicillium* the highest percentage was reached by *P. lanosum* (LR-28.8% and G – 27.8%), *P. viridicatum* (LR – 22.4%), *P. chrysogenum* (G – 20.8% and AR – 15.5%). Fungi numbered among the genus *Cladosporium* were represented by the species *C. herbarum*.

Key words: fungal spores, indoor air, Cracow

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Introduction

Hawskworth et al (1995) estimate that the total number of fungi in the world amounts to 1.5 million of species, approximately 72 thousand (only 5%) of which have been classified [1]. The fungi kingdom encompasses an artificial "division" of mitosporic fungi (*Fungi Imperfecti*), which is an unclassifiable group comprising species which are similar as regards morphology and which arouse the greatest interest among doctors and biologists.

As stated in the classification from the latest edition of "Ainsworth & Bisby's Dictionary of the Fungi" (2001), the group of fungi which had been called *Fungi Imperfecti*, mitosporic fungi, or *Deuteromycetes* was termed "anamorphic fungi". According to the state of knowledge for the year 2001, 15,945 species within 2,547 genera number among anamorphic fungi [2].

Fungal spores are ubiquitous in nature and in human environment, existing in the air, water and soil, from the poles to the tropics. Being found seasonally in the air, they predominate from spring to autumn. Their occurrence reaches its peak in the summer months (July, August), which is related to the preponderance of the spores numbered among the genus *Cladosporium* and depends on current meteorological conditions. As regards indoor spaces, spores exist within them throughout the entire year. Spores present in rooms derive therefore from both outdoor and indoor sources. The species that predominate during the whole year in indoor spaces belong to the genera *Penicillium* and *Aspergillus* [3,4,5].

Refusal to comply with the rules of the proper handling of rooms that serve various purposes, restricting ventilation, or unfavourable microclimatic conditions encourage the development of fungi.

Aim

To analyse the occurrence of fungal spores in the air of the indoor spaces that provide place for teaching and training classes for university students. An attempt was made to establish the concentration of fungal spores on the basis of the BSL classification.

Material and Methods

The research encompassed teaching and training rooms located within the complex of buildings of Cracow University Academy of Physical Education. An auxiliary room (AR) serving the preparation of classes and characterised by less frequent presence of students and a lecture room (LR) were chosen as the teaching rooms. The training rooms included a gymnasium (G) and an indoor athletics tunnel (AT). Air samples were obtained through the sedimentation method, twice a week during the winters of 2005 and 2006 (from December to March). The winter season, when the ground is covered in snow, was chosen in order for spores found inside the rooms to be regarded as fully representative of the indoor source. The following agar bases were chosen for the growth of fungi: Saboraud agar with chloramphenicol, Czapek-Dox and Candida agar with chloramphenicol and gentamycin for selective detection of *C. albicans* produced by the MERCK company. Incubations having been conducted at temperatures of 27°C and 37°C for between 7 and 10 days, the grown fungal cultures were classified to species [6,7].

Results

The microclimatic conditions of the researched rooms were characterised by high relative humidity (from 80.9% to 87.7% on average) and high temperature (between 20.0°C and 22.0°C on average). The largest quantity of fungal spores was discovered in the air of the athletics tunnel (308.1 CFU m⁻³ on average) (Tab. I). Microclimatic conditions favour the development of fungi. The air of the teaching rooms was found to have the highest concentration of spores numbered among the genera *Penicillium* – 63.2%, *Aspergillus* – 24.0%, *Cladosporium* – 4.8% and *Candida* – 3.2%. In the auxiliary room, where students do not stay, the concentration of fungal spores was similar,

with genus *Penicillium* predominating (65.0%), then *Aspergillus* – 30.0% and *Candida* – 3.3% in turn. In the athletics tunnel the genus *Aspergillus* preponderated (45.5%), followed by *Penicillium* – 34.7% and *Cladosporium* – 7.9% (Fig. 1). As regards the air of the gymnasium, the genera *Penicillium* – 47.4%, *Aspergillus* – 29.2% and *Candida* – 9.1% were prevalent with the highest number of spores (Fig. 1).

32 fungi species were isolated from the air of the rooms. Within the genus *Aspergillus*, the most frequently isolated species were *A. niger van Tiegh.* in the athletics tunnel (40.5%) and *A. fumigatus Fresen.* both in the auxiliary room (30.0%) and the teaching rooms (24.0%). Among the conidia of the genus *Penicillium* the highest percentage was reached by *P. lanosum Westling* (LR-28.8% and G – 27.8%), *P. viridicatum Westling* (LR – 22.4%), *P. chrysogenum Thom* (G – 20.8% and AR – 15.5%). Fungi numbered among the genus *Cladosporium* were represented by the species *C. herbarum (Pers.) Link*, which amounted to 7.9% in AT and 5.8% in G (Tab. II).

The BSL classification was introduced on the basis of the biosafety of fungi. The fungi that were preponderant in the air of the teaching and auxiliary rooms were categorised as BSL-0 and BSL-2, whereas the training rooms were characterised by the dominance of the spores classified as BSL-1 and BSL-0 (Fig. 2).

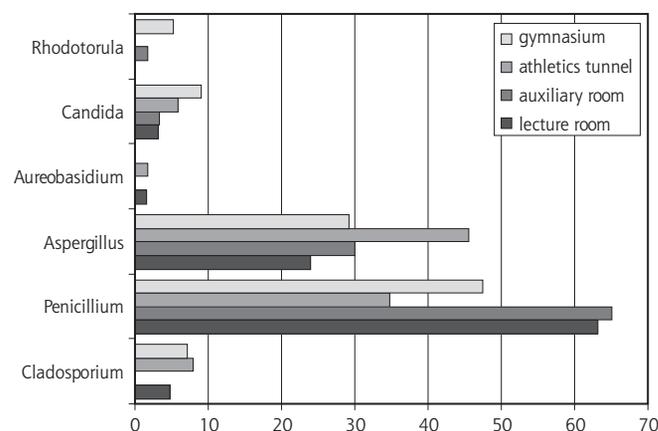


Fig. 1 A statement on fungal genera present in the air of the researched structures

Table I. Profile of microclimatic conditions and number of fungal spores in examined rooms

Re-searched rooms	Temperature °C				Relative humidity %				Number of fungi CFU m ⁻³			
	mean	min	max	SD	mean	min.	max.	SD	mean	min.	max.	SD
lecture room	21,6	19,8	24,1	1,2	80,9	63	97	10,0	204,2	67,3	1401,1	244,6
auxiliary room	22,0	19,5	23,5	1,4	81,3	69	92	12,3	160,5	63,7	573,3	135,2
athletics tunnel	21,3	17,1	28,5	2,2	83,8	56	98	12,3	308,1	63,7	2848,3	470,6
gymnasium	20,0	14,4	24,9	2,4	87,7	72	99	7,3	233,6	63,7	1082,9	219,6

SD – standard deviation

Table II. Percentages of the content of fungal species in the rooms' air

Species	Type of room			
	lecture room	auxiliary room	athletics tunnel	gymnasium
<i>Acremonium strictum</i> W. Gams	0,8	-	-	-
<i>Acremonium murorum</i> (Corda) W. Gams	-	-	-	2,0
<i>Aspergillus candidus</i> Link	-	-	-	3,9
<i>Aspergillus amstelodami</i> Thom & Church	-	-	-	0,6
<i>Aspergillus fumigatus</i> Fresen.	24,0	30,0	3,8	5,2
<i>Aspergillus flavipes</i> (Bainier & Sartory) Thom & Church	-	-	0,6	-
<i>Aspergillus niger</i> van Tiegh.	-	-	40,5	18,8
<i>Aspergillus ochraceus</i> G. Wilh.	-	-	-	0,7
<i>Aspergillus repens</i> (Corda) Sacc.	-	-	0,6	-
<i>Aureobasidium pullulans</i> (de Bary) G. Arnaud	1,6	-	1,8	-
<i>Candida albicans</i> (C.P.Robin) Berkhout	3,2	3,3	5,9	9,1
<i>Cladosporium cladosporioides</i> (Fresen.) G.A. de Vries	-	-	-	1,3
<i>Cladosporium herbarum</i> (Pers.) Link	4,8	-	7,9	5,8
<i>Mucor globosus</i> Fisch.	-	-	0,6	-
<i>Mucor spinosus</i> Tiegh.	-	-	1,2	-
<i>Penicillium chrysogenum</i> Thom	4,8	15,5	10,5	20,8
<i>Penicillium cyclopium</i> Westling	-	-	-	2,6
<i>Penicillium lanosum</i> Westling	28,8	27,8	10,5	7,8
<i>Penicillium glandicola</i> (Oudem.) Seifert & Samson	2,4	-	6,5	1,3
<i>Penicillium viridicatum</i> Westling	22,4	1,5	0,7	7,8
<i>Penicillium aculeatum</i> Raper & Fennell	2,4	9,3	1,3	3,2
<i>Penicillium commune</i> Thom	0,8	3,1	-	1,3
<i>Penicillium citrinum</i> Thom	-	6,2	0,7	-
<i>Penicillium expansum</i> Link	0,8	1,5	3,2	-
<i>Penicillium crustosum</i> Thom	0,8	-	1,3	1,9
<i>Penicillium funiculosum</i> Thom	-	-	-	0,7
<i>Phialophora bubakii</i> (Laxa) Schol-Schwarz	0,8	-	-	-
<i>Rhizopus nigricans</i> Ehrenb.	0,8	-	-	-
<i>Rhodotorula rubra</i> (Demme) Lodder	-	1,7	-	5,2
<i>Scopulariopsis brevicaulis</i> (Sacc.) Bainier	-	-	1,2	-
<i>Stemphylium botryosum</i> Wallr.	-	-	0,6	-
<i>Verticillium lactarii</i> Peck	0,8	-	0,6	-

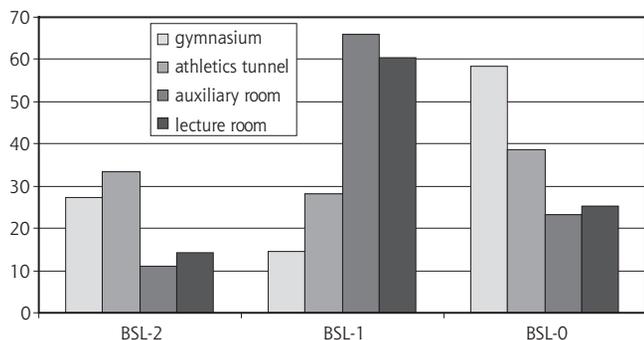


Fig. 2 The categorisation of fungi into the BSL classes

Discussion

The fungi found in indoor spaces do not show significant seasonal fluctuations. Fungal spores present in rooms derive from both outdoor and indoor sources. The indoor environment is characterised by the predominance of species numbered among the genera *Cladosporium* and *Aspergillus* as well as, in lower percentages, *Mucor*, *Cladosporium*, *Aureobasidium* [5]. This was confirmed by current research, in which the highest percentage of spores was found to belong to the genera *Penicillium* (*P. lanosum*, *P. viridicatum*, *P. chrysogenum*), *Aspergillus* (*A. niger*, *A. fumigatus*) and *Cladosporium* (*C. herbarum*).

As regards analysing the occurrence of spores in the air of rooms, it is usually living quarters that are selected as the subject of research, with teaching and training rooms being seldom chosen for the study of fungal spores concentration [8,9,10].

The study of teaching rooms at one of universities in Lodz showed significant concentration of fungal spores in the air: from 307 to 493 CFU m⁻³. Generally, 39 fungal species were isolated from the air of the researched rooms. The genera that predominated in the air of that environment proved to be *Penicillium* and *Aspergillus*, which amounted to 44% and 24% of all the fungi isolated. The majority of fungi were classified as BSL-1 and only three of the isolated species as BSL-2 [11].

Stobińska and Skrzycka (2002) conducted research into microbiological pollution, i.e. mainly bacteria and fungi present in the air of lecture rooms and laboratories. The authors found that it was bacteria that constituted the predominant microflora in the researched rooms, whereas fungi were primarily represented by the species *Penicillium* [12].

The preliminary study of teaching rooms at the Academy of Economics in Poznan showed the presence of spores belonging to the genera: *Penicillium*, *Aspergillus*, *Cladosporium* and *Alternaria*. The concentration of microorganisms tended to rise even six times in relation to the measurement taken in the morning, before classes [13].

Earlier continuous research (during one year) of the air of teaching and training rooms revealed the presence of the genera *Cladosporium*, *Penicillium* and *Aspergillus*, which was connected with the seasonal aerial occurrence of the genus *Cladosporium*, whose spores enter indoor spaces particularly in summer [4].

In the research undertaken in the gymnasiums of randomly chosen schools of different type in Krakow the recorded mean reached 640 CFU m⁻³. The authors proved the presence of 20 fungal genera, of which the

most frequently appearing spores were *Penicillium* and *Cladosporium* [14].

The research of the air of the gymnasium in Cracow was conducted depending on the time of use. After 3 years of being used, the air of the building contained from 171 to 494 CFU m⁻³. The preponderant fungi were those of the genera *Candida*, *Aspergillus*, *Penicillium*, *Scopulariopsis* [8]. The continuous study of the same gymnasium conducted between 1996 and 1999 showed 583.1 CFU m⁻³ on the average. Substantial relations between the quantity of fungi in the air of the gymnasium and the auxiliary room were discovered. The predominant spores belonged to the genera *Penicillium* and *Aspergillus*, with the following species among them: *A. fumigatus*, *A. niger*, *A. versicolor*, *P. viridicatum*, *P. expansum* and *Candida*. Fungi categorised as BSL-2 amounted to 15% in the air of the indoor sports structures [4].

It was in 1996, under the auspices of the European Confederation of Medical Mycology, that a classification based on the biosafety of the fungi that are potentially pathogenic for humans and animals (BSL – biosafety levels) was completed, terms from BSL-1 (saprotrophs, pathogens of plants) to BSL-3 (pathogens potentially capable of causing severe, deep mycotic infections in generally healthy people) (The register 1998) being introduced [15].

In the research presented, the majority of fungal species (33.3%) numbered among BSL-2 (*A. fumigatus*, *C. albicans*) were isolated from the air of the teaching and auxiliary rooms, while the air of the training rooms contained only 14.3%. Considering the fact that a significant concentration of fungal spores was discovered in the air of the rooms where students stayed, monitoring of the spore concentration is advisable.

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