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# Fungal aerosol in public utility buildings in the city of Kraków

## Aerozol grzybowy występujący w budynkach użyteczności publicznej na terenie miasta Krakowa

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[d] *analysis of results and drawing conclusions*
[e] *preparation of the manuscript*

## ABSTRACT

**Introduction**. The quality of indoor air is one of the most important factors affecting health and well-being of people, who inhale 10 $m^3$ of air every day and spend 80–95% of life indoors. The aim of this research was to evaluate the occurrence of airborne fungi, along with assessment of air pollution and microbiological hazard for humans, in 45 public utility buildings in Cracow.

**Material and methods.** The study was carried out in four groups of buildings, including teaching facilities of the University of Agriculture and Jagiellonian University, churches, shopping malls and hospitals with outpatient clinics. Four sites located in the open air were chosen as control. The air sampling was carried out with MAS-100 impactor. Fungi were enumerated on Malt Extract Agar and the results were expressed as colony forming units (CFU) per $m^3$ of air. The isolated fungi were identified by comparing macroscopic and microscopic observations with taxonomic monographs.

**Results**. Mean concentration of airborne fungi was highest in the teaching facilities (1970 CFU/$m^3$). These were also the sites where the largest range of fungal concentration was observed, i.e. from 0 to 23,300 CFU/$m^3$. The lowest mean concentration (99 CFU/$m^3$) including range was observed in hospitals (from 0 to 327 CFU/$m^3$). Species identification of the fungal isolates revealed the presence of allergenic fungi (*Alternaria* and *Cladosporium*) in the examined spaces. Moreover, some strains were also identified as potentially toxigenic species, such as *Penicillium expansum* or *Aspergillus niger.*

**Conclusions**. The concentration range of airborne fungi varied significantly between the tested spaces. Although the observed concentration of airborne fungi in the majority of buildings was quite low, detection of potentially toxigenic fungi indicates the need for monitoring of both concentration and composition of fungal aerosol in public utility buildings.

**Keywords**: bioaerosol, mold, public utility buildings

## STRESZCZENIE

**Wstęp**. Ludzie każdego dnia wdychają 10 $m^3$ powietrza oraz spędzają 80–95% czasu w pomieszczeniach, dlatego jakość powietrza we wnętrzach jest jednym z najważniejszych czynników wpływających na ludzkie samopoczucie i zdrowie. Celem badań była ocena stężenia grzybów pleśniowych w powietrzu oraz zagrożenia mikrobiologicznego związanego z kontaktem z zarodnikami grzybów pleśniowych w 45 budynkach użyteczności publicznej w Krakowie.

**Materiały i metody.** Badania przeprowadzono w czterech grupach budynków użyteczności publicznej – pomieszczeniach dydaktycznych Uniwersytetu Rolniczego w Krakowie oraz Uniwersytetu Jagiellońskiego, kościołach, galeriach handlowych oraz ośrodkach zdrowia. Grupę kontrolną stanowiły punkty pomiarowe zlokalizowane w środowisku zewnętrznym. Próbki powietrza pobierano przy użyciu impaktora MAS-100. Stężenie grzybów oznaczano na agarze słodowym a wyniki przedstawiono jako liczbę jednostek tworzących kolonie na podłożu hodowlanym (jtk), obecnych w 1 $m^3$ pobranego powietrza. Przynależność systematyczną wyizolowanych grzybów ozna-

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czano porównując obserwacje makroskopowe i mikroskopowe z kluczami diagnostycznymi.

**Wyniki**. Najwyższe średnie stężenie grzybów stwierdzono w powietrzu pomieszczeń dydaktycznych (1970 jtk/m$^3$). Tam też zaobserwowano największy zakres stężenia grzybów, tj. od 0 do 23 300 jtk/m$^3$. Najniższe średnie stężenie (99 jtk/m$^3$) oraz jego zakres stwierdzono w ośrodkach zdrowia (od 0 do 327 jtk/m$^3$). Identyfikacja gatunkowa wskazała na obecność w badanych budynkach grzybów potencjalnie alergennych, należących do rodzajów *Alternaria* i *Cladosporium*. Ponadto wśród badanych izolatów zidentyfikowano potencjalnie toksynotwórcze gatunki grzybów pleśniowych – *Penicillium expansum*, *Aspergillus niger*.

**Wnioski**. Stwierdzono szeroki zakres stężenia grzybów pleśniowych w powietrzu badanych budynków. Pomimo, że obserwowane stężenie grzybów w powietrzu większości budynków nie było wysokie, stwierdzenie obecności potencjalnie toksynotwórczych gatunków podkreśla konieczność monitorowania koncentracji i składu aerozolu mikrobiologicznego w budynkach użyteczności publicznej.

**Słowa kluczowe**: bioaerozol, grzyby pleśniowe, budynki użyteczności publicznej

## INTRODUCTION

The quality of indoor air is one of the most important factors affecting the health and well-being of people, who inhale 10 m$^3$ of air every day and spend 80–95% of their life indoors [1]. The air inhaled by people is abundantly populated with microorganisms, forming the so-called bioaerosol [2]. People, organic dusts, different types of materials collected in premises , as well as the air itself penetrating into the spaces from ventilation or air conditioning systems are the potential sources of air pollution [3].

Epidemiological studies showed that the exposure to high concentrations of airborne microorganisms, including mold fungi, often leads not only to allergy, asthma or allergic rhinitis but also to pneumonia or other infections [4, 5]. Biological factors, including fungal spores, can cause the sick building syndrome – it is a combination of symptoms that occur in people occupying one specific location [6]. Among bioaerosol-forming microorganisms, mould fungi are some of the most successful, as they are capable of surviving in diverse environments, due to their physiological versatility and genetic plasticity [6]. Their spores are produced in large numbers and are easily spread over a wide area. The presence of fungal spores and products of fungal metabolism can trigger various allergic reactions, including hypersensitivity pneumonitis, allergic rhinitis and some types of asthma [7]. Fungi usually enter the premises through ventilation or air conditioning systems, doors and windows, or as contaminants of building materials. Furthermore if there is increased moisture in a building, fungal growth and sporulation will most probably appear [8].

In the recent years there has been a growing number of allergy sufferers who also experience the presence of fungal spores in the air. Moreover, information obtained from the assessment of fungal aerosol can facilitate medical evaluations, determination of remedial measures or assessment of health hazards [8]. For this reason, it is essential to monitor the air quality in public utility buildings occupied with large numbers of people, frequently for longer periods of time. Therefore, the aim of this study was to assess the concentration of fungal aerosol in the selected groups of public utility buildings in Kraków. In addition, the isolated fungi were identified to evaluate the possible risk related to the exposure of people to potentially pathogenic and/or toxigenic fungal species.

## MATERIAL AND METHODS

The air sampling was conducted in 45 public utility buildings in the area of Kraków, divided into 4 groups: teaching facilities of the University of Agriculture (UR) and Jagiellonian University (UJ), encoded as U, churches (C), shopping malls (S) and healthcare facilities (hospitals and outpatient clinics, H). All sites which were selected for the study characterize large numbers of people present during the whole day. Shopping malls were the only sites where the air conditioning systems are being used, while in the remaining sites, there is a natural (gravitational) ventilation. Four sites located outdoors were used as control (T). The description of sampling sites is given in table I.

The air sampling was conducted in December 2012. The measurements were performed using a single-stage MAS-100 impactor (Merck, Switzerland). Air volume was 100 litres. The sampling was performed during the day, when the premises were occupied. During sampling, the impactor was placed

Table I. Location and characteristics of the study sites
Tabela I. Lokalizacja i charakterystyka punktów badawczych

| Code | Location | Code | Location | Code | Location | Code | Location | Code | Location |
|---|---|---|---|---|---|---|---|---|---|
| TEACHING FACILITIES | | CHURCHES | | SHOPPING MALLS | | HEALTH CARE FACILITIES | | CONTROL | |
| 1U | Laboratory classroom | 1C | Capuchin | 1S | Krakowska | 1H | Jagiellońskie Est. Clinic | 1T | Jordan's Park |
| 2U | Elevator | 2C | Św. Anna | 2S | Kazimierz | 2H | Złotego Wieku Est. Clinic | 2T | Planty meadow |
| 3U | Toilet | 3C | Franciszkanie | 3S | Plaza | 3H | Rydygiera Hospital | 3T | Polish Aviators Park |
| 4U | Vivarium | 4C | Dominikanie | 4S | M1 | 4H | Urocze Est. Clinic | 4T | Botanical Garden |
| 5U | Reading room | 5C | Św. Barbara | 5S | Carrefour | 5H | Szkolne Est. Clinic | | |
| 6U | Caffeteria | 6C | Arka Pana | 6S | Leroy Merlin | 6H | Żeromskiego Hospital | | |
| 7U | Gym | 7C | Matki Boskiej Częstochowskiej | 7S | Tesco | 7H | Borek Fałęcki Est. Clinic | | |
| 8U | Lecture hall 1 | 8C | Sanktuarium Łagiewniki | 8S | Biedronka | 8H | Neurology CMUJ | | |
| 9U | Lecture hall 2 | 9K | Niepokalanego Poczęcia Panny Marii | 9S | Solvay | 9H | Gynecology CMUJ | | |
| 10U | Corridor | 10C | Najśw. Serca Pana Jezusa | 10S | Zakopianka | 10H | Occupational Medicine | | |
| 11U | Locker room | 11C | Bazylika Mariacka | 11S | Bonarka | | | | |
| 12U | Dean's office | | | | | | | | |
| 13U | PCR laboratory | | | | | | | | |

at a height of 1.0–1.5 m above the floor (indoor sites) or the ground (outdoor, control sites) to simulate aspiration from the human breathing zone. The samples were collected on Petri dishes containing Malt Extract Agar (Oxoid, Great Britain). All measurements were conducted in 3 replicates. After sampling, the Petri dishes were incubated for 5–7 days at 25°C. Afterwards, the number of fungal colonies were counted and expressed as colony forming units per cubic meter of air (CFU/m$^3$). The actual colony count per each culture plate was corrected according to the positive hole correction table [9]. The isolated fungi were identified to the genus and/or species level by comparing the macroscopic and microscopic observations with diagnostic manuals [10, 11].

Statistica v. 10.0. software (StatSoft, US) was used to calculate the basic descriptive statistics and the Kruskal-Wallis test was applied in order to verify the significance of differences in the concentration of airborne fungi among different groups of public utility buildings.

## RESULTS

The results of the conducted analyses concerning the fungal aerosol concentration are summarized in table II. The concentration of airborne fungi in the majority of the examined premises was low and did not exceed $10^3$ CF/m$^3$, except for 4 sites, i.e. 4U (vivarium UR), 10U (corridor UR), 1C (Capuchins) and 4T (Botanical Garden). The mean concentration of fungal aerosol was highest in the teaching facilities of UR and UJ (1970 CFU/m$^3$). Those were the locations, where the largest range in the concentration of airborne fungi was also observed, i.e. from 0 to 23,300 CFU/m$^3$. Both the mean concentration of fungal aerosol and the range in the concentration of fungi were the lowest in

healthcare facilities. Statistical analysis of fungal concentrations indicated that there was no statistically significant difference among the examined groups of public utility buildings ($H = 1.37$, $p < 0.01$). The mean concentration of fungal colony forming units in healthcare facilities and shopping malls was even lower than the one recorded in the target sites (table II).

Table II. The concentration of fungal aerosol in the examined sites [CFU/m$^3$]
Tabela II. Stężenie aerozolu grzybowego w badanych miejscach [jtk/m$^3$]

| Code | Conc. | Code | Conc. | Code | Conc. | Code | Conc. | Code | Conc. |
|---|---|---|---|---|---|---|---|---|---|
| 1U | 73 | 1C | 1653 | 1S | 80 | 1H | 53 | 1T | 40 |
| 2U | 127 | 2C | 13 | 2S | 0 | 2H | 33 | 2T | 33 |
| 3U | 53 | 3C | 260 | 3S | 14 | 3H | 7 | 3T | 67 |
| 4U | 23300 | 4C | 687 | 4S | 427 | 4H | 14 | 4T | 1050 |
| 5U | 27 | 5C | 947 | 5S | 87 | 5H | 0 | | |
| 6U | 147 | 6C | 74 | 6S | 40 | 6H | 48 | | |
| 7U | 67 | 7C | 7 | 7S | 194 | 7H | 60 | | |
| 8U | 0 | 8C | 74 | 8S | 74 | 8H | 270 | | |
| 9U | 107 | 9C | 0 | 9S | 67 | 9H | 173 | | |
| 10U | 1473 | 10C | 7 | 10S | 173 | 10H | 327 | | |
| 11U | 87 | 11C | 160 | 11S | 87 | | | | |
| 12U | 47 | | | | | | | | |
| 13U | 100 | | | | | | | | |
| Mean | 1970 | Mean | 353 | Mean | 113 | Mean | 99 | Mean | 298 |
| Std. dev. | 6421 | Std. dev. | 533 | Std. dev. | 119 | Std. dev. | 117 | Std. dev. | 502 |

Species identification of isolated fungi revealed the presence of 26 fungal genera and/or species (table III). Among the examined spaces, the majority, i.e. 16 of all recorded taxa, were observed in the teaching facilities of UR and UJ, 14 different taxa were recorded in both shopping malls and healthcare facilities, 11 – in churches and 8 in the target sites located outdoors. The fungi that can be considered as allergenic, belonging to the genera *Alternaria* and *Cladosporium* were isolated in all of the studied spaces. For instance, the greatest isolation frequency of *A. alternata* was observed in churches, while the greatest percentage of these species was detected in the target sites. The greatest isolation frequency of *C. cladosporioides* was observed in the teaching facilities and the greatest percentage of this fungus was detected in hospitals. Moreover, also potentially genera and species were identified among the examined isolates (e.g. *Aspergillus niger* was observed in all premises and *Penicillium chrysogenum* was observed in teaching facilities, churches and shopping malls with the highest percentage in the university premises, table III).

## DISCUSSION

Epidemiological studies indicate that millions of people worldwide are exposed to different biological agents [12]. Unfortunately, there are no quantitative thresholds for admissible limits of microbiological contamination in Poland. This is why the Expert group for Biological Agents of the Interdepartmental Commission for Maximum Admissible Concentrations and Intensities for Agents Harmful to Health in the Working Environment at the Central Institute for Labour Protection – National Research Institute proposed the threshold values in occupational and non-occupational environments for different microbiological agents which can form bioaerosols, including fungi [12, 13]. The comparison of the results obtained in this study indicates that the concentration of fungal aerosol in all of the examined spaces, except for one site (i.e. 4U – vivarium), does not exceed the proposed maximum concentration acceptable for public service buildings. Detection of the highest concentration of fungal aerosol in the laboratory where different animals are kept for observation and research, is not

Table III. Frequency of occurrence and percentage [%] of each fungal genus and species in fungal aerosol of different groups of premises

Tabela III. Częstość izolacji i udział [%] poszczególnych rodzajów i gatunków grzybów w składzie aerozolu grzybowego badanych grup pomieszczeń

| Genus/species | Isolation frequency[1] | | | | | Percentage[2] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | U | C | S | H | T | U | C | S | H | T |
| *Aspergillus* spp. | 46.15 | 63.64 | 54.54 | 30 | 25 | 42.37 | 59.51 | 24.46 | 17.66 | 2.52 |
| *Penicillium spp.* | 30.77 | 36.36 | 36.36 | 30 | 50 | 31.62 | 21.66 | 29.20 | 6.50 | 35.80 |
| *Cladosporium cladosporioides* | 38.46 | 27.27 | 27.27 | 20 | 25 | 0.24 | 1.08 | 5.35 | 9.24 | 1.68 |
| *Alternaria alternata* | 15.38 | 18.18 | 9.09 | 30 | 50 | 0.06 | 4.46 | 1.85 | 4.87 | 6.30 |
| *Aspergillus niger* | 15.38 | 9.09 | 9.09 | 20 | – | 0.28 | 1.65 | 0.40 | 1.32 | – |
| *Penicillium chrysogenum* | 15.38 | 18.18 | 18.18 | – | – | 11.00 | 6.05 | 10.38 | – | – |
| *Alternaria longipes* | 15.38 | 9.09 | 9.09 | 10 | – | 0.09 | 0.39 | 1.61 | 7.21 | – |
| *Trichoderma viride* | 7.69 | – | 9.09 | 10 | 25 | 0.14 | – | 1.69 | 1.52 | 1.09 |
| *Botrytis cinerea* | – | 9.09 | – | 30 | – | – | 0.18 | – | 17.77 | – |
| *Rhizopus stolonifer* | 7.69 | – | – | – | 50 | 13.67 | – | – | – | 14.37 |
| *Penicillium citrinum* | 7.69 | 18.18 | 9.09 | – | – | 0.03 | 2.55 | 4.02 | – | – |
| *Fusarium* spp. | 15.38 | – | – | 10 | – | 0.09 | – | – | 1.22 | – |
| *Alternaria* spp. | – | – | 9.09 | 10 | – | – | – | 5.07 | 3.65 | – |
| *Aspergillus fumigatus* | 7.69 | 9.09 | – | – | – | 0.02 | 0.99 | – | – | – |
| *Aspergillus parasiticus* | 15.38 | – | – | – | – | 0.09 | – | – | – | – |
| *Aspergillus sydowii* | – | 9.09 | 9.09 | – | – | – | 0.99 | 4.51 | – | – |
| *Penicillium digitatum* | 7.69 | – | – | 10 | – | 0.13 | – | – | 8.73 | – |
| *Rhizopus nigricans* | – | – | 9.09 | 10 | – | – | – | 1.53 | 4.36 | – |
| *Absidia corymbifera* | – | – | – | 10 | – | – | – | – | 15.74 | – |
| *Cladosporium herbarum* | – | – | | – | 25 | – | – | – | – | 18.49 |
| *Mucor* spp. | 7.69 | – | – | – | – | 0.11 | – | – | – | – |
| *Penicillium expansum* | – | – | – | – | 25 | – | – | – | – | 19.75 |
| *Penicillium italicum* | – | – | 9.09 | – | – | – | – | 5.47 | – | – |
| *Trichoderma* spp. | 7.69 | – | – | – | – | 0.06 | – | – | – | – |
| *Verticillium albo-atrum* | – | – | – | 10 | – | – | – | – | 0.91 | – |
| *Verticillium* spp. | – | – | 9.09 | – | – | – | – | 0.72 | – | – |

[1] frequency of occurrence was determined as the number of sampling sites, in which a given genus or species was detected in relation to the total number of sampling sites (*częstotliwość występowania określono jako stosunek liczby miejsc poboru próbek w których stwierdzono występowanie danego rodzaju lub gatunku do wszystkich miejsc poboru*)

[2] percentage of microorganisms was determined as the relation of the number of colony forming units of a given genus or species to the number of CFU of all taxa identified in the course of the study (*udział procentowy mikroorganizmów określono jako stosunek liczby jednostek tworzących kolonie danego gatunku lub rodzaju do liczby jtk wszystkich taksonów stwierdzonych w badaniach*)

entirely unexpected, as it had been previously reported that animals themselves can be the source of bioaerosol [14], as well as stored grain and plant residues used for different purposes in animal breeding can be the abundant source of bacteria, fungi and their metabolites [15].

The concentration of fungal aerosol in healthcare facilities ranged from 0 to 327 CFU/m$^3$ (10H), with mean value of 99 CFU/m$^3$ (table II). The obtained values are lower than the ones recorded by Hsu et al. in hospitals in Taiwan [16], where the total concentration of fungal colony forming units ranged from 406 to 1414 CFU/m$^3$. Similar situation occurred in the case of shopping malls, where the fungal aerosol concentration ranged from 0 to the maximum of 427 CFU/m$^3$ (table II), compared with higher values recorded by Hsu et al. in hypermarkets [16], ranging from 123 to 1161 CFU/m$^3$. On the other hand, the values recorded for the teaching facilities were greater than the ones recorded by Kalwasińska

et al. in the library rooms of the Toruń University [2], which ranged from 0 to 420 CFU/m$^3$. Mean concentrations of fungi recorded by those authors in the air of different spaces of the university library were slightly different than the ones observed in our study. For instance, the mean value recorded in the corridor of the UR university (site 10U) reached 1473 CFU/m$^3$, while the one observed by Kalwasińska et al. in the main hall was 180 CFU/m$^3$ [2]. In our study the mean concentration of fungal CFUs in the air of the university cafeteria was 147, while that observed by Kalwasińska et al. was the greatest among the tested sites and reached 413 CFU/m$^3$ [2]. Finally, the second greatest concentration of fungal CFUs was found by Kalwasińska et al. in the toilet [2] while in our study the concentration of fungal aerosol in the toilet was among the lowest values, i.e. 53 CFU/m$^3$. In the presented study, the fungal aerosol concentration in the outdoor air (control sites) ranged from 33 to 1050 CFU/m$^3$ which, according to the limits set by the Polish Standard for the concentration of airborne fungi [17], allows the conclusion that the mentioned concentration falls within the limits of acceptable values.

In the case of the shopping malls, which were the only air-conditioned premises, the mean concentration of airborne fungi was the second lowest value recorded in the presented study (table II). Even though these premises are visited daily by large numbers of people, the fact that they are isolated from the outdoor environment could have affected the concentration of bioaerosol. Additionally, different studies confirm that the air-conditioned rooms are less contaminated than those where air-conditioning is not installed [2, 18], providing that the air-conditioning system is properly maintained.

In the presented study *Aspergillus* spp. and *Penicillium* spp. were the two most frequently detected fungal taxa. These are fungi that often occur in the natural environment, including soil, cereals, hay and other plant material, as well as in different types of food. Contact with those fungi may lead to various disorders, including respiratory, circulatory, immune and neurological diseases [19]. One of the fungal isolates was identified as *Aspergillus fumigatus* (isolated from two sites, i.e. 5U – reading room and 11K – St. Mary's Basilica). In accordance with the recommendations of the Ordinance of Minister of Health on hazardous biological agents in the workplace and on the health protection of workers occupationally exposed to these agents [20], this species is classified into Risk Group 2 of biological factors, i.e. those that can cause diseases in humans and may be dangerous to workers. Additionally, Alternaria and *Cladosporium* occurred very frequently and abundantly in the air of the examined public spaces. Fungi, which dominated in the presented study (*Aspergillus*, *Penicillium*, *Alternaria*, *Cladosporium*) are considered as the most allergenic airborne fungi [21]. The most frequently occurring fungal genera, detected in this study, are also reported as the most common by other authors. For instance, in the study by Menteşe et al. [22], conducted in different indoor spaces, including public facilities, *Penicillium* spp., *Aspergillus* spp. and *Cladosporium* spp. were the most frequently observed fungal genera. Also, Pastuszka et al. in their study conducted in healthy and mould contaminated homes in Upper Silesia detected the presence of different species of *Cladosporium*, *Alternaria* and *Penicillium* [23].

## CONCLUSIONS

Based on the results obtained it may be concluded that the levels of airborne fungi may vary considerably, depending both on the group of public utility buildings and the particular use of individual sites. Nevertheless, except for one sampling site, the concentration of airborne fungi in the examined public premises fell within the acceptable limit. Additionally, it can be concluded that good ventilation and air conditioning of the public spaces may decrease the concentration of fungal aerosol; thus it is advisable to apply those solutions, particularly in the spaces where the greatest concentrations of airborne fungi were detected. The presence of potentially allergenic and toxin induced fungi emphasizes the need for continuous monitoring of air quality, particularly in public utility buildings, which are visited by large numbers of people each day.

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