Analysis of fungal pathogens in the environment of Branicki Palace in Białystok, Poland

Łukaszuk CR.[1], Zaremba KP.[2]

1. Department of Integrated Medical Care, Medical University of Białystok, Białystok, Poland
2. Home Care, Enfield, Greater London EN1, UK

ABSTRACT

Introduction: People spend about 90% of their time indoors. Most health problems associated with indoor air quality are caused by fungi. It is estimated fungi account for 70% of total indoor air microbial pollution.
Purpose: To analyze fungal pathogens isolated from indoor air of Branicki Palace in Białystok, Poland
Materials and methods: The research mycological material consisted of air collected from various rooms in Branicki Palace. Humidity and temperature of the tested rooms were also measured. The monitoring of airborne fungi pollution was done using a SAS SUPER 100 (pbi international) with international measure standards (EN 50081-1, EN 500 50082-1). Biological monitoring of wall surface contamination was performed using the Count-Tact applicator with Count-Tact plates.
Results: A total of 1140 CFU per m^3 of air were cultured in autumn and 580 CFU in winter. From the walls, a total of 124 CFU were cultured in autumn and 397 CFU in winter. CFU values in the investigated rooms ranged from 10 to 220 (mean 47 CFU) in autumn, and from 10 to 90 (mean 29 CFU) in winter. The most commonly isolated pathogens were: *Candida albicans, Aspergillus* sp., *non-Candida albicans,* and *Penicillium* sp.. The number of colonies isolated from the walls of all rooms in winter was greater than in autumn. The most commonly isolated pathogens were: *Aspergillus sp.* and *C. albicans* in autumn*; C. albicans and non-C. albicans* in winter.
Conclusions: In winter, the number of colonies isolated from walls in all rooms was significantly greater compared with autumn. *Candida albicans*, *Aspergillus* sp. and *Penicillium* sp. were the most commonly isolated fungal air pathogens, regardless of season. *C. albicans* and *Aspergillus* sp. were most commonly isolated from walls in autumn, while *C. albicans* and *non-C.albicans* in winter.
Key words: fungi, air pollution, Branicki Palace

***Corresponding author:**
Cecylia Łukaszuk
Department of Integrated Medical Care, Medical University of Białystok
15-096 Białystok, ul. M.C.Skłodowskiej 7a, Poland
Tel.:/Fax: +48857485528
e-mail: cecylia.lukaszuk@wp.pl

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INTRODUCTION

Branicki Palace is one of the most valuable aristocratic residences in Central and Eastern Europe, and the most important monument in Białystok. Its present form dates back to the time of Jan Klemens Branicki [1,2].

The Palace is located where, according to historical documents, in the 15th century stood a mansion belonging to the first owners of Białystok, the Raczkowicz family. In the 16th century, the Wiesiołowski family, the owners of the city at that time, built a two-story castle here. It is believed that Job Bretfus, court architect of King Sigismund Augustus, designed the castle. In the first decades of the 17th century, during another reconstruction, two ground-floor corner extensions were built on both sides of the main building. At the end of the 17th century, the castle was reconstructed by one of the most prominent Polish architects of the time, Tylman from Gameren, at the commission of Stefan Mikołaj Branicki, as Białystok was very seriously damaged during the Deluge [1,2].

The palace was rebuilt after 1841, when it served as an institute for noblewomen. The Palace survived in this somewhat different form until 1944. Until 1939, the facility functioned as a Regional Office. Then, during the German occupation, it was turned by the Nazis into a seat of the Białystok District administrative authorities [1,2]. Since 1950, the Branicki Palace has served as the seat of the Medical Academy (currently Medical University), which conducts systematic renovations and restorations to revive it to its 18th century glory [2]. The renovation works in Branicki Palace revealed the manner in which the mansion was built and extended over centuries (from the 16th to the 20th century) [3]. Part of Branicki Palace is occupied by university administrative offices, while the basement has been successively made available to the public.

With the progress of civilization, indoor air quality deteriorates. People spend about 90% of their time indoors; therefore, it is important to monitor the state of the air. Most health problems associated with indoor air quality are caused by fungi. It is estimated that fungi account for 70% of total indoor air microbial pollution [4].

Therefore, it is important to conduct mycological measurements, which are necessary to show the adverse impact of fungi present inside the Palace on the health of its occupants.

The aim of the study was to analyze fungal pathogens isolated from indoor air of Branicki Palace in Białystok, Poland.

MATERIALS AND METHODS

The research mycological material consisted of air collected from palace rooms, around entrances, and around main entrances to Branicki Palace. Simultaneously, humidity and temperature in the tested rooms were measured.

The monitoring of airborne fungi pollution was done using a SAS SUPER 100 (pbi international) with international measure standards (EN 50081-1, EN 500 50082-1). Sample has a flow rate of 100 liters air/min. At each site, a 100 liters sample was taken with the sampler placed at a height of 150 cm above floor level in the middle of the room, with all windows and doors closed.

Plates from SAS SUPER 100 were incubated. After incubation number of fungal colonies and number of fungi in air volume was counted. In according to producer, at the first part of investigation number of fungal colonies at plates (real number of colonies - RNC) was corrected on statistical probability multiple passage of particle through the same hole (number of colonies corrected). In according to formula, it was estimated CFU (colony-forming unit - number of colonies at 1000 L of air): X = (P x 1000) : V, where : V- volume of air sample , r – number of counted colonies at contact plate, P - corrected number of colonies (in according to producer instrument), X – number of colonies (CFU) at 1000 L (1 m^3) of air. Classification of isolated fungi was made with accordance to the current procedures.

Fungal identification was based on culture appearance and microculture characteristics [5].

We used TSI VelociCheck airflow flow velocity meter to measure relative air humidity, ambient temperature, surface temperature, and dew point.

Biological monitoring of surface contamination was performed using the Count-Tact applicator with Count-Tact plates (bioMerieux) containing medium compliant with the requirements of the Draft European Standard CEN/TC 243/WG2. The Count-Tact plates have a diameter of 55 mm and their surface is covered in slits. The convex meniscus of the agar enables direct collection of material to maintain hygiene of walls, floors, clothing, and equipment. The plates were placed in a special Count-Tact applicator (bioMerieux). The agar was applied directly onto the evaluated surface (force 500 g, time 10s). Following collection, samples were placed in an incubator at 37°C and incubated for 3 days.

The fungal colonies grown on the plates were counted and subjected to mycological identification. The obtained results are expressed as values of colony forming units per 1 cm^2 for each room, calculated using the following formula:

X= a : π r² ; **a** is the number of fungal colonies grown on the plates, **r** is the plate radius in cm.

Statistical analysis was performed using Statistica 10 PL software. Nonparametric methods were used during analysis: Mann-Whitney U test,

Wilcoxon matched pairs test, and Spearman's correlation coefficient. Results with $p<0.05$ were considered statistically significant.

RESULTS

Tables 1 and 2 show the values of measurements performed in autumn and winter inside Branicki Palace in Białystok.

The number of colonies (CFU/m^3) obtained in autumn ranged from 10 in Archives room 1 up to 220 in the basement beneath the columns. Winter CFU values ranged from 10 in Assembly Hall, Archives room 1, Psychology lecture hall, John II basement (in front of PRL basement) and 90 in the basement beneath the columns.

Air humidity in autumn ranged from 26.5% in the hallway leading to Assembly Hall up to 86.5% in John II basement. Indoor air humidity in winter ranged between 25.7% in Assembly Hall up to 67.6% in the basement beneath the columns.

Temperatures in autumn ranged from 16.6ºC in John II basement up to 22.2ºC in the hallway leading to Human Resources offices. Temperatures in winter ranged from 2.2ºC in the basement beneath the columns up to 22.1ºC in Human Resources offices.

In autumn, air circulation fluctuated between 0 m/s in the hallway leading to John II basement and the basement in front of PRL and 0.3 m/s in the hypocaust.

In winter, air circulation ranged between 0.01 m/s in Archives room 2 and 0.22 m/s in the hallway leading to Human Resources offices.

Table 1. Temperature, humidity, and air circulation measurement results and CFU in particular rooms in autumn

Room name	CFU [CFU/m^3]	Temperature [^{0}C]	Humidity [%]	Air circulation [m/s]
Assembly Hall	80	21.9	27.4	0.02
Hallway leading to Assembly Hall	80	22.1	26.5	0.06
Room with togas	60	21.4	33.7	0.04
Hallway leading to Human Resources offices	30	21.2	34.8	0.04
Human Resources offices	60	22	35.8	0.01
Hallway leading to office	30	22.1	37.3	0.05
Hallway leading to Archives room 2	30	20.5	48.5	0.02
Hall by entrance to the Palace	30	19.1	29.6	0.13
Office	30	20	43.7	0.06
Archives room 1	10	19	56.0	0.01
Archives room 2	30	18.9	40.6	0.08
Archives room 3	40	19.3	47.1	0.1
Psychology lecture hall	30	21.3	54.5	0.02
Total	**540**			
Hallway leading to John basement	40	19.1	78	0
Room I beneath University Promotion office	30	20.6	59.5	0.02
Hypocaust	90	21.3	64.9	0.3
John basement	40	22.1	57.2	0.01
John II basement (before PRL)	150	16.6	86.5	0.0
Basement beneath the columns	220	19.9	60.2	0.02
Total	**570**			
Outside the Palace	30	1.9	57.1	0.35
Total	**1140**			

Table 2. Temperature, humidity, and air circulation measurement results and CFU in particular rooms in winter

Room name	CFU [CFU/m^3]	Temperature [^{0}C]	Humidity [%]	Air circulation [m/s]
Assembly Hall	10	22	25.7	0.18
Hallway leading to Assembly Hall	30	22	29.4	0.02
Room with togas	40	19.6	33.9	0.03
Hallway leading to Human Resources offices	30	21.1	34	0.22
Human Resources offices	10	22.1	34.4	0.03
Hallway leading to office	30	19	36.1	0.02
Hallway leading to Archives room 2	20	19.1	39.7	0.03
Hall by entrance to the Palace	40	21.5	29.1	0.07
Office	80	20.3	36.2	0.06
Archives room 1	10	19.6	32	0.07
Archives room 2	20	18.4	34.1	0.01
Archives room 3	10	19.3	39.7	0.9
Psychology lecture hall	10	23	27.7	0.02
Total	**340**			
Hallway leading to John basement	20	20.8	33.9	0.1
Room I beneath University Promotion office	20	15.4	35.7	0.02
Hypocaust	60	2.4	63.8	0.02
John basement	30	17.4	35.1	0.03
John II basement (before PRL)	10	21.4	41.7	0.06
Basement beneath the columns	90	2.2	67.6	0.03
Total	**230**			
Outside the Palace	10	-2.9	59	1.03
Total	**580**			

The outdoor conditions at time of measurements were as follows: temperature in autumn and winter: 1.9ºC and 2.9º C, respectively; humidity in autumn and winter: 57.1% and 59%, respectively; air circulation in autumn and winter: 0.35 m/s and 1.03 m/s, respectively.

CFUs in different rooms in autumn and winter are shown in Figures 1 and 2, respectively.

The following fungal genera/species were cultured from the samples collected in Branicki Palace: *C. albicans (CA), non-Candida albicans (N-CA), Penicillium* sp. *(PS), Aspergillus* sp.*(AS), Acremonium sp. (ACS), Ulocladium (U), and Epicoccum sp. (ES). C. albicans* was the most common species, and was only absent in the office and Psychology lecture hall.

Fungal species occurring in single rooms included *Ulocladium* present in the hypocaust (3 colonies), *Epicoccum* sp. in the basement beneath the columns (1 colony), and *Acremonium sp.* in the hallway leading to the office (1 colony). Details are shown in Tables 3.

Figure 3 presents the number of fungal colonies grown in autumn from the walls of Branicki Palace.

A total of 124 fungal colonies, including 75 colonies from the office part of the Palace and the remaining 49 colonies from basement walls, were grown from the samples collected in autumn from the walls of the investigated rooms. The largest number of fungal colonies was obtained from hallway walls: leading to Assembly Hall and Human Resources offices (11 colonies each, mainly *Aspergillus* sp.), in the hall by the entrance to the Palace (8 colonies, mainly *Penicillium sp.* and *Aspergillus* sp.).

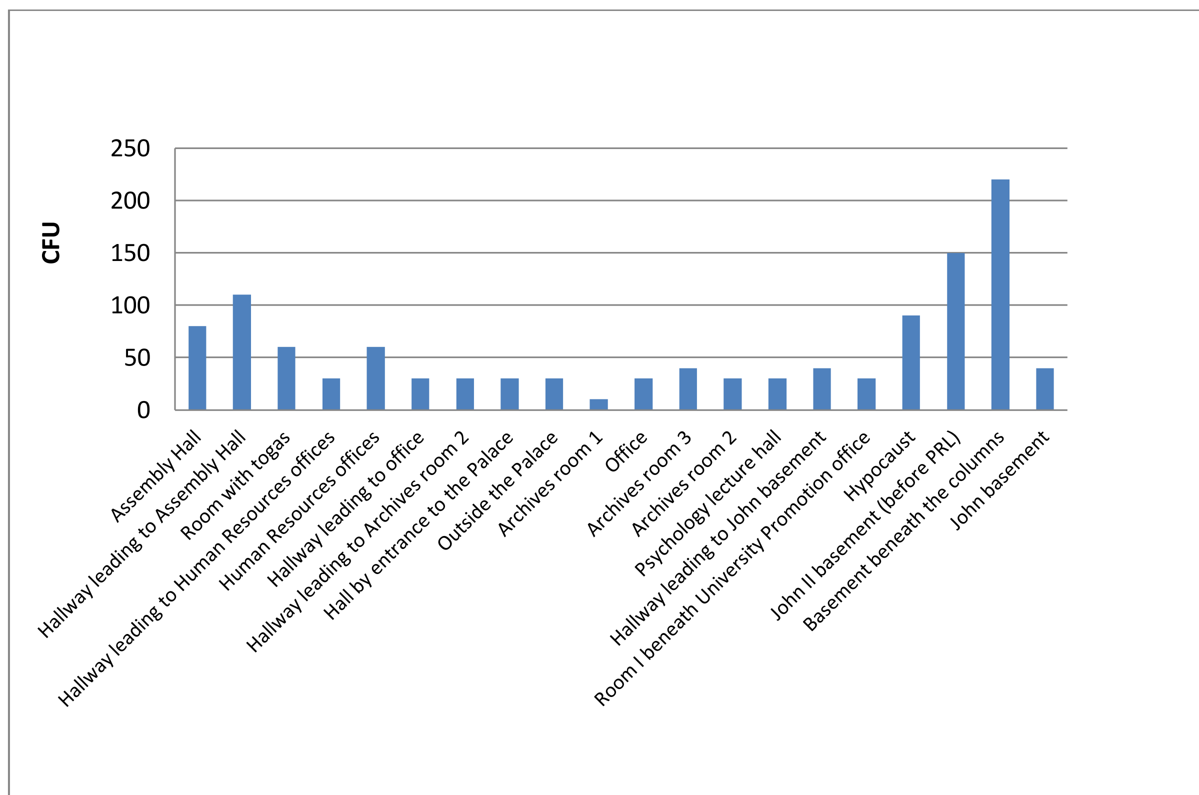

Figure 1. CFU values (CFU/m^3) depending on room, from cultures obtained in autumn

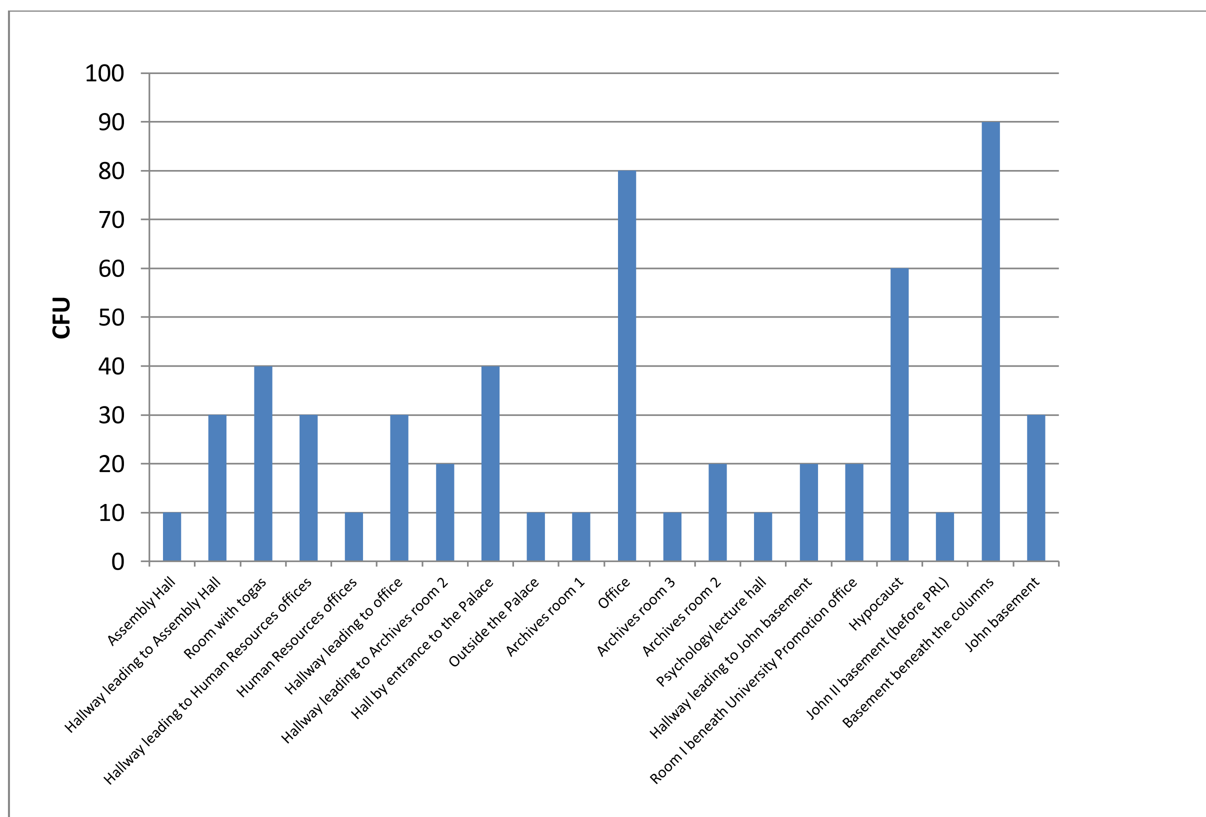

Figure 2. CFU values (CFU/m^3) depending on room, from cultures obtained in winter

Table 3. Fungi types grown in autumn and winter from samples collected in particular rooms

	CA	N-CA	PS	AS	ACS	U	ES	F
AUTUMN								
Assembly Hall	6	2	0	0	0	0	0	0
Hallway leading to Assembly Hall	6	1	0	1	0	0	0	0
Room with togas	2	1	0	3	0	0	0	0
Hallway leading to Human Resources offices	2	1	0	0	0	0	0	0
Human Resources offices	4	2	0	0	0	0	0	0
Hallway leading to office	2	0	0	0	1	0	0	0
Hallway leading to Archives room 2	2	0	1	0	0	0	0	0
Hall by entrance to the Palace	2	1	0	0	0	0	0	0
Outside the Palace	1	0	0	0	0	0	0	0
Archives room 1	1	0	0	0	0	0	0	0
Office	0	0	0	3	0	0	0	0
Archives room 3	2	0	2	0	0	0	0	0
Archives room 2	3	0	0	0	0	0	0	0
Psychology lecture hall	0	0	0	3	0	0	0	0
Hallway leading to John basement	1	1	1	1	0	0	0	0
Room I beneath University Promotion office	2	0	0	1	0	0	0	0
Hypocaust	2	1	2	1	0	3	0	0
John II basement (before PRL)	7	0	3	5	0	0	0	0
Basement beneath the columns	6	6	4	5	0	0	1	0
John basement	2	1	1	0	0	0	0	0
WINTER								
Assembly Hall	1	0	0	0	0	0	0	0
Hallway leading to Assembly Hall	2	0	1	0	0	0	0	0
Room with togas	1	3	0	0	0	0	0	0
Hallway leading to Human Resources offices	2	0	1	0	0	0	0	0
Human Resources offices	1	0	0	0	0	0	0	0
Hallway leading to office	2	1	0	0	0	0	0	0
Hallway leading to Archives room 2	2	0	0	0	0	0	0	0
Hall by entrance to the Palace	3	0	1	0	0	0	0	0
Outside the Palace	2	1	0	0	0	0	0	0
Archives room 1	1	0	0	0	0	0	0	0
Office	5	3	0	0	0	0	0	0
Archives room 3	1	0	0	0	0	0	0	0
Archives room 2	2	0	0	0	0	0	0	0
Psychology lecture hall	1	0	0	0	0	0	0	0
Hallway leading to John basement	1	0	0	0	0	0	0	1
Room I beneath University Promotion office	0	0	0	2	0	0	0	0
Hypocaust	0	0	5	1	0	0	0	0
John II basement (before PRL)	1	0	0	0	0	0	0	0
Basement beneath the columns	3	0	1	5	0	0	0	0
John basement	0	0	0	1	0	0	0	2

Candida albicans (CA), non-Candida albicans (N-CA), Penicillium sp. (PS), Aspergillus sp.(AS), Acremonium sp.(ACS), Ulocladium (U), Epicoccum sp. (ES)., Fusarium (F)

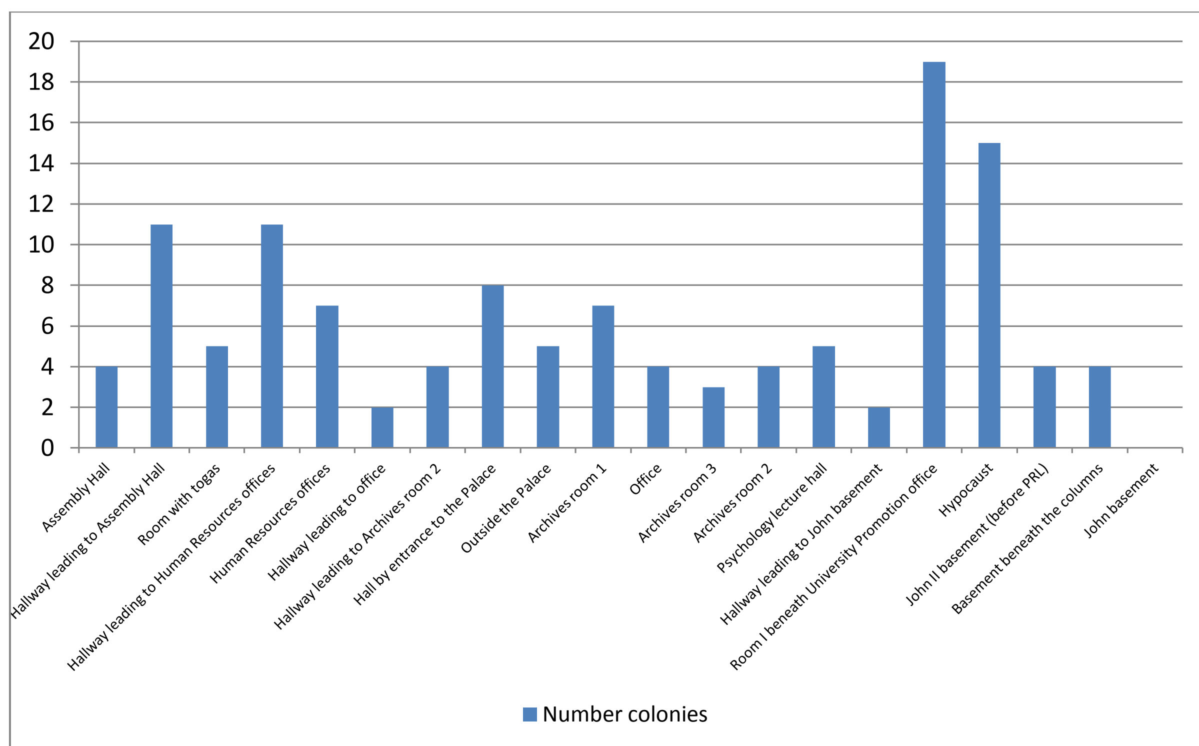

Figure 3. Number of fungal colonies grown in autumn from the walls of Branicki Palace

A total of 19 colonies were grown from the underground part in the hypocaust, and these mainly included: *Aspergillus niger* (9 colonies), 5 colonies of *C. albicans*, and 3 colonies of *Penicillium* sp.. A total of 15 colonies were grown from the walls of John II basement (5 *Penicillium* sp., 7 *C. albicans*, and 3 *Aspergillus* sp. colonies).

A total of 397 fungal colonies were grown from the samples collected in winter from the walls of the investigated rooms; 197 colonies were grown from samples collected in the office part of the Palace and the other 200 were grown from basement wall samples. Most colonies were obtained from the walls of Assembly Hall (51 colonies), the hallway leading to Archives room 2 (35 colonies), and the hall by the entrance to the Palace (30 colonies). In the basement part of the building, the largest number of fungal colonies were grown from samples taken from the walls in John II basement (52 colonies) and the hallway leading to John basement (50 colonies) (Fig. 4). *C. albicans* and *non-C. albicans*, *Penicillium* sp. and *Aspergillus* sp. were dominant.

Mean CFU values were $47/m^3$ in autumn and $29/m^3$ in winter. This indicates higher fungal colonization of the air in autumn. The impact of room type on CFUs and cultured fungi type is an important aspect. There were differences between the rooms in terms of climatic conditions in successive seasons. The Mann-Whitney U test was used for statistical analysis.

Mean humidity was 48.9% in autumn and 38.4% in winter. We found a significant (p= 0.0045) difference in air humidity between autumn and winter. Humidity was higher in autumn.

Mean air circulation was 0.04 m/s in autumn and 0.09 m/s in winter. We found no statistically significant differences in relation to indoor air circulation between autumn and winter. Details are not shown.

Mean temperature inside the Medical University was 19.5ºC in autumn and 17.2ºC in winter. We found no statistically significant differences in relation to room temperatures between autumn and winter. The temperature range was similar for all rooms, i.e. between 16.6ºC and 22.1ºC.

There were statistically significant differences between the numbers of colonies grown from the samples collected from walls in different rooms. In winter, the number of colonies in all rooms was significantly higher compared with autumn. The level of significance was p=0.0013 (Details are not shown). CFU values ranged between 10 and 220 in autumn and between 10 and 100 in winter. CFU values were higher in all rooms in autumn compared with winter. The level of significance was p=0.0013 (Wilcoxon matched pairs test).

We found a statistically significant (p=0.023) negative correlation (R= - 0.50) between winter air temperature and winter CFU values

(Spearman's correlation coefficient). The higher the temperature, the lower the CFU value in winter. This may be associated with a significant decrease in humidity in winter compared with autumn. Details are not shown.

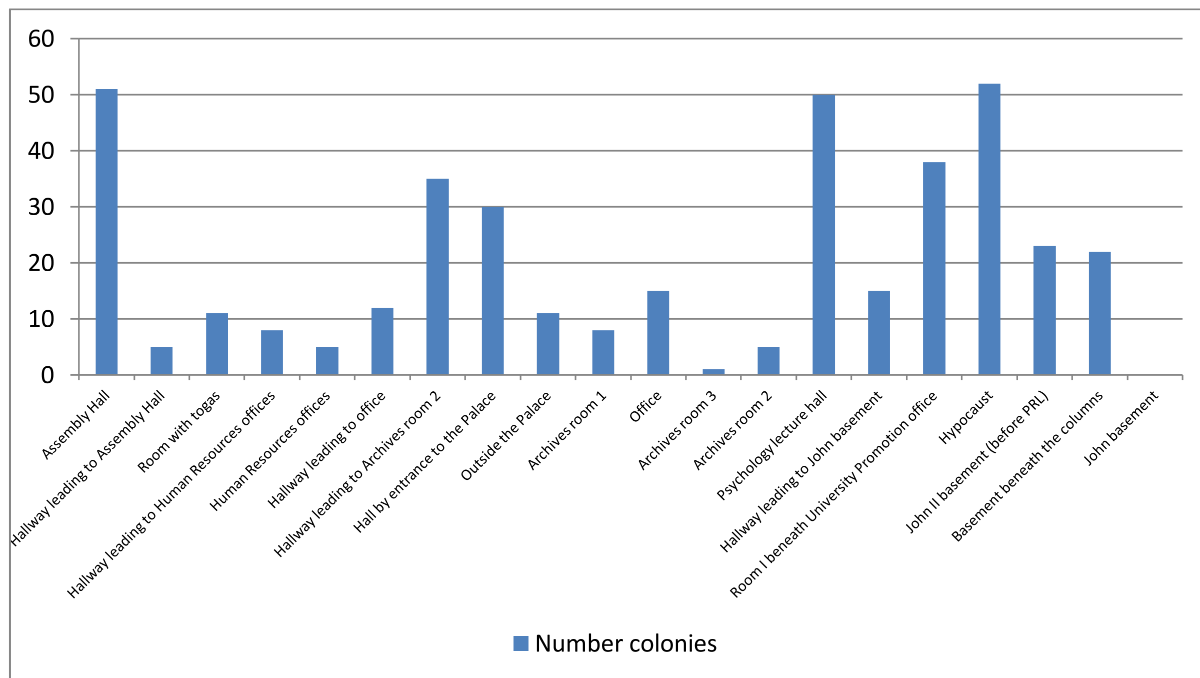

Figure 4. Number of fungal colonies grown in winter from the walls of Branicki Palace

DISCUSSION

It is estimated that fungi account for 70% of total indoor air microflora. Studies conducted in office buildings located in the USA and Brazil have found the presence of three species, i.e. *Penicillium* spp., *Aspergillus* spp., and *Cladosporium* spp. Fungi are a common cause of allergic rhinitis, asthma, conjunctivitis, and gastrointestinal inflammation [4].

Therefore, assessment of air contamination with harmful biological pathogens is an important undertaking.

Our study assessed the presence of fungal pathogens inside Branicki Palace, a historic building, which currently houses the Medical University in Białystok.

The research covered two seasons: autumn and winter. Air samples from rooms in Branicki Palace were collected using the SAS SUPER 100. Physical parameters, such as temperature, humidity and air circulation, which also affect the occurrence of fungi, were also assessed.

CFU values differed between the seasons. The number of colonies (CFU/m^3) obtained in autumn ranged from 10 in Archives room 1 up to 220 in the basement beneath the columns. Winter CFU values ranged from 10 in Assembly Hall, Archives room 1, Psychology lecture hall, John II basement (in front of PRL basement) and 90 in the basement beneath the columns. CFUs were higher in all rooms in autumn compared with winter.

According to the literature, the amount of fungi present in the air fluctuates depending on the season. It was shown that the amount increases in summer and autumn and decreases in spring and winter [6]. The ranges of fungal levels in outdoor air reported in the subject literature are 90-8230 CFU/m^3 in spring; 30-4500 CFU/m^3 in summer, and 40-4370 CFU/m^3 in autumn. Indoor concentrations of fungal aerosols considered within the normal range are 2-1440 CFU/m^3 in spring, 45-2050 CFU/m^3 in summer, 9-580 CFU/m^3 in autumn [7,8].

The results of our measurements were within the normal range observed in this season.

The rooms of Branicki Palace were classified into two groups:

- rooms for public use (offices) – Assembly Hall, hallway leading to Assembly Hall, Human Resources offices, hallway leading to Human Resources offices, office (a room occupied by the director and archives personnel) and hallway leading to office, Archives rooms (1,2,3), Psychology lecture hall, and main hall by entrance to the Palace, basement area – hypocaust (furnace of the floor heating system in the palace), John basement and hallway leading to this basement, two rooms beneath the University Promotion office, basement beneath the columns (temporarily occupied by Municipal Parks and Gardens

employees working to organize the gardens around Branicki Palace).

The bioclimate of office spaces comprises of a combination of physical, chemical and biological factors, which determine the air quality in the work environment in which no production processes or activities that could affect the well-being or the health of the personnel take place. In our studies, rooms conventionally referred to as offices or 'public use' had autumn CFU values ranging between 10 (Archives room 1) up to 110 (hallway leading to Assembly Hall). Winter CFU values ranged between 10 and 80, with a predominance of yeast-like fungi (*C. albicans* and *non-C.albicans)*.

Buczyńska et al. [9] found through their research, conducted in the offices of a company whose employees reported various respiratory symptoms, eye irritation, headaches and fatigue that the number of molds ranged from $0.33x10^2$ up to $3.34x10^2$ CFU/m^3. The highest fungus concentration was noted in a room used as archives. *Penicillium, Cladosporium, Aspergillus, Acremonium, Fusarium,* and *Botrytis*, were grown from the samples taken from these rooms [9].

Ogórek and Pląskowska [10] showed in their study, conducted in rooms for public use (in one university in Wrocław), that the mycological air contamination in the evaluated rooms varied in terms of both CFUs and fungi species composition. CFU values ranged from 37 up to 337 CFU/m^3, and were similar to those obtained in the present study. Research conducted by Mędrela-Kuder at al. [11] in the University of Physical Education in Krakow showed that the highest concentration of fungal spores was found in October and June, with *Aspergillus fumigates* being the dominant species. Assessment of health effects resulting from exposure to harmful and noxious factors present in office spaces is very difficult due to concomitant effects of multiple harmful factors, usually present at low concentrations, but for long periods of exposure [12].

Autumn CFU values for rooms conventionally referred to as the 'basement area' ranged between 30 in room 1 beneath the University Promotion office up to 220 in the basement beneath the columns. In winter, the obtained CFUs fluctuated between 10 in John II basement (before PRL) through 60 in hypocaust up to 90 in the basement beneath the columns. Fungi settle and grow in places where they have advantageous conditions, i.e. damp walls, poor ventilation or limited exposure to light, and these conditions prevail in the basements of Branicki Palace. *Aspergillus* sp. (9 colonies) and *Penicillium* sp. (6 colonies) were the main pathogens isolated from the sampled air.

Trojanowska et al. [13] assessed the occurrence of fungi in the crypts of St. Peter and St. Paul Church in Krakow. The aim of their study was to demonstrate the harmful effects of fungal spores on the health of people performing renovation works in the crypts. A total of 449 mold colonies were grown from the air samples (crypt 1). Penicillium was the most commonly isolated genus. In our studies, a total of 570 fungal colonies were isolated in autumn and 230 in winter from the basement area. *Aspergillus* sp. and *Penicillium* sp. were the most commonly grown fungi. The relationship between fungi exposure and asthma was first presented in 1726 by John Floyer, who described a patient developing asthma after repeated visits to a dungeon [14].

Fungi are organisms that are widespread throughout the globe. They are heterotrophic organisms, which only grow on substrates with adequate amounts of nutrients. In the external environment, their main habitat is the soil, where they feed on dead organic remains of plants and animals. A high relative humidity (more than 70%) and adequate temperatures promote the growth of fungi. Optimum growth temperatures range from 10ºC to 35ºC. Fungi development and release of spores depend on multiple factors, such as: local conditions, climatic factors (temperature, rainfall, wind, humidity), and time of day [14].

The temperature of the evaluated rooms ranged between 16.6ºC and 22.1ºC in autumn, and between 2.4º and 22.1ºC in winter. Indoor air humidity depends on external conditions, which are modified by building structure, ventilation, heating system or the direct activity of the room occupants [15]. Air humidity ranged from 26.5% to 86.5% in autumn and from 25.7% to 67.6% in winter. The obtained results confirm that fungal development is promoted in certain conditions.

We found also a simultaneous occurrence of the same fungi in the air as well as on the walls of the investigated rooms. *Aspergillus* and *Penicillium* were the most commonly isolated fungi and were more frequently isolated from the basement part of Branicki Palace, which had higher humidity levels compared with office spaces. Additionally, these rooms are dark and lack ventilation. The greatest number of *Aspergillus* colonies was grown from the hypocaust, which is a basement area of Branicki Palace, periodically occupied by gardening workers. Exposure to these pathogens may result in a number of serious health consequences.

Molds release mycotoxins, which are secondary metabolites produced under specific environmental conditions. Long-term activity of mycotoxins on the human body can, for example, induce cancer. Aflatoxins and ochratoxins produced by *Aspergillus* are the best-known mycotoxins [16, 17].

Indoor air quality has long been an important research problem. About 90% of our life is spent indoors, suggesting that conditions in such places should not be harmful to humans. However,

many people's health problems are due to the harmful effects of different physical, chemical and biological factors associated with closed areas.

CONCLUSIONS

1. We found differences in the amount and type of fungal pathogens cultured in autumn and winter. CFU values were higher in all rooms in autumn compared with winter.
2. In winter, the number of colonies isolated from walls in all rooms was significantly higher compared with autumn.
3. *Candida albicans*, *Aspergillus* sp. and *Penicillium* sp. were the most commonly isolated fungal air pathogens, regardless of season. *C. albicans* and *Aspergillus* sp. were most commonly isolated from walls in autumn, while *C. albicans* and *non-Candida albicans* in winter.

Conflicts of interest

The authors declare no conflict of interest in this paper.

Financial disclosure

None.

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