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Indoor Air Quality Assessment in Selected Warsaw Flats: Quantitative and Qualitative Analysis of Airborne Fungi

Ocena jakości powietrza wewnętrznego w wybranych mieszkaniach w Warszawie – analiza ilościowa i jakościowa grzybów obecnych w bioaerozolu

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Abstract: This study investigates the sanitary condition of indoor air in thirty flats across thirteen districts of Warsaw, Poland, by analysing the concentration and diversity of airborne microscopic fungi. A total of 270 air samples were collected from bathrooms, kitchens, and bedrooms using the MAS-100 Eco® impact sampler and cultured on YGC medium. Fungal colony forming units (CFU m⁻³) were quantified and identified to the genus level. The results revealed that fungal counts exceeded acceptable thresholds in 70% of bathrooms and kitchens, and in 97% of bedrooms, with concentrations ranging from 1 × 10² to over 2.5 × 10³ CFU m⁻³ of the air. Thirteen fungal genera were identified, with *Cladosporium* (100% of flats), *Penicillium* (90%), and *Alternaria* (60%), *Aureobasidium* (57%), and yeast-like fungi (53%) being the most prevalent. Statistical analysis showed significant correlations between fungal abundance and flat characteristics and residents' habits including location, year of construction, building material, floor area, presence of pets, smoking habits, cleaning frequency, number of windows, ventilation frequency, and bathroom fixtures. The findings highlight the importance of regular monitoring of indoor fungal presence to mitigate health risks and improve living conditions.

Keywords: indoor air quality, bioaerosol, residential environments, fungal load, Warsaw

Streszczenie: Przeprowadzono ocenę mikrobiologicznej jakości powietrza wewnętrznego w trzydziestu mieszkaniach zlokalizowanych w trzynastu dzielnicach Warszawy, na podstawie analizy stężenia oraz różnorodności grzybów przenoszonych drogą powietrzną. Łącznie pobrano 270 próbek bioaerozolu z łazienek, kuchni i sypialni, wykorzystując próbnik powietrza MAS-100 Eco® oraz podłoże YGC. Przeprowadzono ilościową ocenę grzybów obecnych w bioaerozolu (CFU·m·³) oraz ich identyfikację taksonomiczną na poziomie rodzaju. Badania wykazały, że grzybów przekraczało dopuszczalne normy w 70% łazienek i kuchni oraz w 97% sypialni, osiągając od 1 × 10² do ponad 2,5 × 10³ jednostek tworzących kolonie m·³ powietrza. Zidentyfikowano trzynaście rodzajów grzybów, z dominacją *Cladosporium* (100% mieszkań), *Penicillium* (90%), *Alternaria* (60%), *Aureobasidium* (57%) oraz grzybów drożdżopodobnych (53%). Analiza statystyczna wykazała istotne zależności pomiędzy liczebnością grzybów a cechami mieszkań oraz nawykami mieszkańców, takimi jak: lokalizacja, rok budowy, materiał konstrukcyjny, powierzchnia użytkowa, obecność zwierząt domowych, palenie tytoniu, częstotliwość sprzątania, liczba okien, częstotliwość wentylacji oraz wyposażenie łazienek. Uzyskane wyniki podkreślają znaczenie regularnego monitorowania obecności grzybów w powietrzu wewnętrznym w celu ograniczenia ryzyka zdrowotnego i poprawy warunków życia.

Słowa kluczowe: jakość powietrza wewnętrznego, bioaerozol, pomieszczenia mieszkalne, skażenie grzybami, Warszawa

Introduction

Indoor air quality (IAQ) has become an increasingly important public health concern, especially in urban environments where people spend the majority of their time indoors. According to the World Health Organization (WHO 2014), people typically spend up to 90% of their daily time indoors – in homes, workplaces, and public buildings. Nevertheless, indoor air is often more polluted than outdoor air, posing significant health risks due to the presence of various biological and chemical contaminants (Khan and Karuppayil 2012; Tran et al. 2020; Asril et al. 2023; Upadhyay 2023; Al-Shaarani and Pecoraro 2024).

Among the biological pollutants, airborne microscopic fungi, commonly referred to as moulds, are particularly concerning. These microorganisms can proliferate in indoor environments under favourable conditions such as high humidity, poor ventilation, and the presence of organic materials (e.g., wood, textiles, or dust), which provide nutrients for fungal growth (Upadhyay 2023). Fungal spores become airborne and form part of the bioaerosol, which can be inhaled or come into contact with skin, eyes, or mucous membranes, potentially leading to allergic reactions, respiratory infections, and other health issues (Khan and Karuppayil 2012).

Health implications of exposure to indoor fungi are well documented (Kumar et al. 2021). Certain genera, such as Aspergillus, Penicillium, Cladosporium, Alternaria, and Fusarium, are known to produce mycotoxins - secondary metabolites that can cause immunosuppression, neurotoxicity, and even carcinogenic effects (Kumar et al. 2021; Upadhayay 2023; Al-Shaarani and Pecoraro 2024; Hurraß et al. 2024). Moreover, the phenomenon of Sick Building Syndrome (SBS) and Building-Related Illness (BRI) or Dampness and Mould Hypersensitivity Syndrome (DMHS) has been linked to microbial contamination in indoor air, particularly in poorly ventilated or inadequately maintained buildings (Valtonen 2017; Kramer et al. 2021; Goudarzi and Reshadatian 2024).

Despite the growing awareness of these risks, Poland currently lacks comprehensive legal regulations governing acceptable levels of microbial contamination in indoor air. The only national standard (PN-89/Z-04111/03) (PKN 1989), which provided guidelines for assessing fungal concentrations in atmospheric air, was withdrawn in 2015 and has not been replaced (Chmiel et al. 2015). This regulatory gap underscores the need for continued research and monitoring of indoor air quality, particularly in private residential settings, which are often overlooked in favour of public buildings such as offices, schools, sports facilities, libraries, and hospitals (Małecka-Adamowicz et al. 2019; Takaoka, Motoko and Norbäck 2020; Cyprowski et al. 2023).

The aim of this study was to evaluate the sanitary condition of indoor air in selected Warsaw flats by using airborne culturable fungi as bioindicators. By analysing fungal abundance and diversity in different rooms (bathrooms, kitchens, and bedrooms) and correlating these findings with flat characteristics and lifestyle factors, the study aimed to identify the main drivers of fungal occurrence and provide practical insights to support the improvement of indoor air quality in residential settings.

1. Materials and Methods

1.1. Study area and sampling design

Air quality was assessed in 30 residential flats located across 13 districts of Warsaw, Poland. The flats were selected on a voluntary basis, with efforts made to ensure that their distribution across Warsaw's districts was as balanced as possible. The primary inclusion criterion was that each flat had a separate and functional kitchen, bedroom, and bathroom, which enabled standardized air sampling in three distinct room types. Three replicate air samples were collected from each room, resulting in a total of 270

samples. Sampling was conducted over a two-week period from 17 to 31 May 2023.

Each flat owner completed a detailed questionnaire comprising 23 questions (both open and closed). The questionnaire concerned the building characteristics (e.g. year of construction, building material), number of residents and their habits (e.g. cleaning frequency, ventilation practices, having pets, smoking indoors, etc.), and flat characteristics (e.g. total floor area, bathroom fittings, type of flooring, type of heating etc.) (Tab. S₁).

1.2. Air sampling procedure

Air samples were collected using the MAS-100 Eco® air sampler (MERCK, Germany) according to standards such as ISO 14698-1/2 (ISO 2003). The sampler operates by drawing air through a perforated lid (400 holes) and directing it onto a 90 mm Petri dish containing a selective agar medium. The flow rate was set at 100 dm³/min.

Sampling volumes were adjusted based on room type. In bathrooms – 3 samples of 20 dm³ each (due to higher expected fungal contamination), in kitchens and bedrooms – 1 sample of 20 dm³ and 2 samples of 50 dm³.

1.3. Culture and identification of fungi

Selective glucose-peptone medium YGC (Yeast Extract Glucose Chloramphenicol Agar) with yeast extract and chloramphenicol (which restricts bacterial growth) was used to isolate fungi from collected samples. After sampling, Petri dishes were incubated at 25°C for 5 days. Fungal colonies were then counted and corrected using Feller's statistical correction table, which accounts for the probability of multiple spores entering a single hole and forming a single colony (Feller 1950).

The corrected colony count was used to calculate the concentration of

colony-forming units per cubic meter of air (CFU m⁻³) using the formula:

CFU
$$m^{-3} = a \times 1000/V$$

where:

a – corrected colony count from Feller's table;

V – volume of sampled air (in litres).

Fungal colonies were identified to the genus level based on morphological characteristics under a light microscope, using identification keys by Gilman (1959), Barnett (1960), Marcinkowska (2012), and Koval et al. (2016).

1.4. Statistical analysis

To assess the influence of environmental and household variables on fungal abundance, one-way ANOVA was performed using Statistica software (Statistica ver.14, 2023). To determine intra-group differences, posthoc comparisons were performed using the Least Significant Difference (LSD) test at a significance level of p < 0.05. Fungal counts were log-transformed prior to analysis to meet the assumptions of normality.

To evaluate the generic diversity of airborne fungi, the Shannon diversity index (H') and Evenness index (J') were calculated for each room type in each flat, based on the relative abundance of fungal genera. These calculations were performed using PAST software (version 4) (Hammer et al. 2020). A one-way ANOVA was also conducted to compare Shannon index values across room types. In addition to means and standard deviations, medians were reported to account for potential skewness in diversity distributions.

2. Results

2.1. General characteristics of the surveyed flats and characteristics of residents' habits

The study was conducted in 30 residential flats located within urban areas of 13 administrative districts of Warsaw, Poland. Over 60% of the surveyed flats were constructed during the second half of the 20th

¹ Tables marked with "S" are accessible in a separate "Supporting Data" file entitled Appendix 1 at the end of this article.

century, while the remaining units were built between 2011 and 2021. The predominant construction materials were brick and hollow blocks. Flooring materials were primarily wooden parquet and ceramic tiles. The flats varied in size, with floor areas ranging from 28 m 2 to 80 m 2 . The number of windows per flat ranged from 2 to 10. Each flat was inhabited by one to four residents (Tab. S₁).

All residents adhered to the habit of taking off their shoes upon entering the house. There were no pets in 70% of the flats surveyed. Only four residents declared cigarette smoking indoors.

Cleaning frequency was relatively evenly distributed: 47% of households reported cleaning once a week, while 43% cleaned twice a week. Ventilation practices varied; although 90% of residents reported airing their homes daily, only 37% did so continuously throughout the day. The remaining households ventilated their homes for less than eight hours per day. Subjective assessments of indoor humidity levels varied considerably between rooms (Tab. S₁).

2.2. The airborne fungal concentrations in the air of the surveyed flats

The analysis of fungal concentrations in the air in the 30 flats showed a considerable variation both between flats and between the rooms studied (kitchen, bathroom and bedroom) within a specific flat.

Fungal concentration in the kitchen exceeded the permissible exposure limit [according to Krzysztofik (1992) after Górny (2004) above 300 CFU m⁻³ of air] in 23 flats, in the bathroom similarly, in 22 flats out of 30 surveyed.

Fungal counts in the bedroom ranged widely, from 109 CFU m⁻³ in flat 19 to over 2450 CFU m⁻³ in flat 12 (Fig.1). The acceptable threshold for fungal density in the bedroom according to Krzysztofik (1992) of 100 CFU m⁻³ was exceeded in all flats (Fig. 1). Flats 8, 9 and 12 showed the highest average fungal concentrations, with values exceeding 1700 CFU m⁻³ in some rooms. In particular,

flat 12 showed extreme values in the bedroom (2450 CFU m⁻³) and kitchen (1770 CFU m⁻³). These concentrations exceeded the recommended thresholds by nearly 25-fold in the bedroom and almost 6-fold in the kitchen, respectively. Flats 14, 15, 16, 19 and 30 showed consistently low fungal concentrations, in most cases below 300 CFU m⁻³ in the rooms surveyed (Fig. 1).

Room-specific trends were also observed. In flats 3, 8, 9, 10, 12, 14, 20, 22, 25, 26, and 30, the bedroom had higher fungal levels than the kitchen or bathroom. In several flats (flats 1, 2, 12, 14, 17, 19, 27, 28, and 30), higher fungal concentrations were found in kitchens than in bathrooms (Fig. 1).

2.3. Frequency of fungal genera in the indoor environment

Ten mould genera were identified among the fungi isolated from indoor bioaerosols: Cladosporium, Penicillium, Aspergillus, Alternaria, Trichoderma, Fusarium, Aureobasidium, Mucor, Botrytis and Chaetomium. In addition, yeasts and yeast-like fungi were detected, including representatives of the genera Saccharomyces, Geotrichum and Candida.

Cladosporium was the most frequently detected genus, present in 100% of the flats and in almost all samples taken from the rooms surveyed (with the exception of one bedroom), confirming its dominance in indoor air (Fig. 2).

Penicillium also showed a high frequency of occurrence, being detected in 90% of flats overall. It was particularly prevalent in kitchens (76.7%) but was also common in bathrooms and bedrooms (70% each). Alternaria was identified in 60% of flats, with the highest frequency observed in bedrooms (43.3%). Genus Aureobasidium and yeast were detected in around 55% of flats. Their occurrence was more frequent in the bathrooms, especially for yeast (in 53% of the samples) (Fig. 2). Aspergillus, although less frequent overall, was detected in 33.3% of flats it showed a relatively high frequency in bedrooms (23.3%). Other genera such as Botrytis,



Figure 1. Concentration of airborne fungi (CFU m⁻³) in the kitchen, bathroom, and bedroom across 30 flats, visualized using a heat map (values ranging from 0 to 300 CFU m⁻³ are represented by a colour gradient from light green through green to orange, while values exceeding 300 CFU m⁻³ are shown in shades from orange to red, indicating increasing fungal load)

Chaetomium, Fusarium, Mucor and Trichoderma were detected sporadically, with a frequency of less than 20% in the rooms surveyed (Fig. 2).

2.4. Relative Abundance and Diversity of Fungal Genera in Indoor Across Flats and Room Types

An inter-flat and intra-flat comparison reveals that while some genera are ubiquitous, others exhibit room-specific preferences. *Cladosporium* was the dominant

genus in nearly all flats and room types, often accounting for more than 60% and up to 100% of the total fungal community (Fig. 3).

In some flats, specific fungal profiles were observed. Flats 4, 11, and 12 showed a unique pattern with *Penicillium* dominance across all three rooms. Flat 8, compared to other flats, exhibited a notable presence of *Fusarium* in the kitchen and *Alternaria* in the bedroom. In flats 2 and 5, particularly in

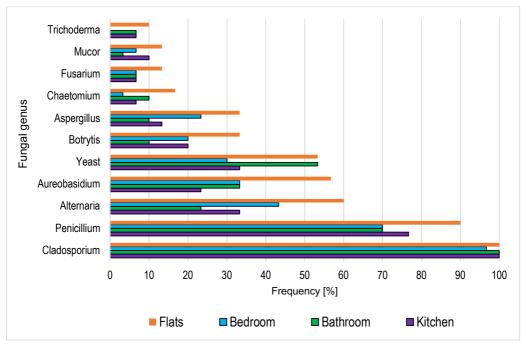


Figure 2. Relative frequency (%) of selected airborne fungal genera detected in the kitchen, bathroom, and bedroom, and across all 30 flats

the bathrooms, yeasts were present in high abundance. Less common genera, such as *Aureobasidium* (in flat 3, bathroom, and flat 20, bedroom) and *Botrytis* (in flat 27, bathroom), occurred sporadically but reached locally high relative abundances (Fig. 3).

Although statistical analysis did not confirm significant differences in fungal genus diversity between room types (F(2,87) = 1.1275; p = 0.3285), Shannon index-based analysis indicated that bathrooms had slightly higher diversity compared to bedrooms and kitchens (Fig. 4).

In the bathrooms, the number of fungal genera ranged from 1 to 6, with more flats containing three or more fungal genera, whose abundance was more evenly distributed, as reflected in the higher Pielou evenness indices (J'). Consequently, Shannon index values ranged from 0.18 to 1.55 (median = 0.77) (Tab. 1). In bedrooms, the number of genera ranged from 2 to 6, with Shannon index values between 0.06 and 1.38 (median = 0.69). Kitchens showed a genus count ranging from 1 to 7,

with Shannon index values from 0.09 to 1.51 (median = 0.58) (Tab. 1).

2.5. Influence of flat characteristics and habits of residents on indoor fungal concentration

The results of the one-way analysis of variance (ANOVA) revealed significant associations between airborne fungal concentration and several flat-related variables, including location, year of construction, building material, floor area, presence of pets, smoking habits, cleaning frequency, number of windows, ventilation frequency, and bathroom fixtures (Table 2).

Flat location had a significant impact on fungal abundance (Tab. 2). In districts located on the eastern (right) bank of the Vistula River (Praga Północ, Praga Południe, Targówek, Białołęka, and Wawer) the average number of CFU m⁻³ of air was 7.7×10^2 , which was significantly higher than in central districts on the left bank (Śródmieście, Ochota, Mokotów) with 5.6×10^2 (LSD post hoc test p = 0.0071), and in non-central districts (Żoliborz, Bielany,

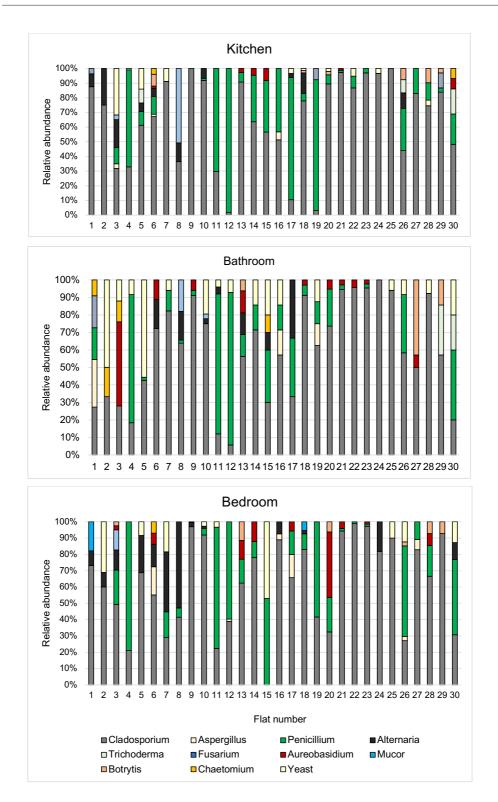


Figure 3. Variability of relative fungal abundance (%) by room type (kitchen, bathroom and bedroom) in flats

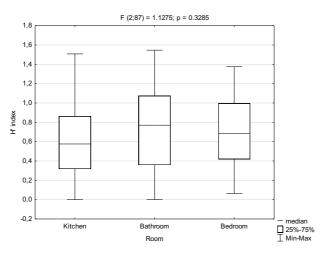


Figure 4. Shannon's fungal diversity index (H') in three room types (kitchen, bathroom, bedroom) based on samples from 30 flats

Table 1. Fungal genus richness (S), Shannon diversity index (H'), and Pielou's evenness index (J') across different room types (kitchen, bathroom, bedroom) and flats

Flat number	Kitchen	Bathroom	Bedroom (S) H'/J'				
Flat number	(S) H'/J'	(S) H'/J'					
1	(3) 0.45/0.41	(5) 1.55/0.96	(2) 0.75/0.69				
2	(2) 0.56/0.81	(3) 1.01/0.92	(3) 0.89/0.81				
3	(6) 1.51/0.84	(4) 1.22/0.88	(6) 1.38/0.77				
4	(3) 0.68/0.62	(3) 0.75/0.68	(2) 0.51/0.74				
5	(5) 1.18/0.74	(3) 0.76/0.70	(3) 0.80/0.73				
6	(7) 1.15/0.59	(3) 0.78/0.71	(5) 1.27/0.79				
7	(2) 0.30/0.43	(3) 0.58/0.53	(4) 1.33/0.96				
8	(3) 0.98/0.89	(4) 0.96/0.69	(3) 0.87/0.79				
9	(1) 0/0	(3) 0.36/0.33	(3) 0.15/0.14				
10	(3) 0.33/0.29	(4) 0.73/0.53	(4) 0.37/0.26				
11	(2) 0.61/0.88	(4) 0.69/0.50	(3) 0.67/0.61				
12	(2) 0.09/0.12	(3) 0.47/0.43	(3) 0.74/0.67				
13	(3) 0.36/0.33	(5) 1.28/0.79	(4) 1.07/0.78				
14	(3) 0.79/0.72	(3) 0.80/0.73	(3) 0.68/0.62				
15	(3) 0.90/0.82	(5) 1.51/0.94	(2) 0.69/1.00				
16	(3) 0.86/0.79	(4) 1.15/0.83	(3) 0.42/0.38				
17	(5) 0.62/0.38	(3) 1.10/1.00	(4) 1.00/0.72				
18	(5) 0.75/0.46	(3) 0.36/0.32	(4) 0.62/0.45				
19	(3) 0.41/0.37	(4) 1.07/0.78	(2) 0.68/0.98				
20	(4) 0.44/0.32	(3) 0.71/0.65	(4) 1.23/0.89				
21	(3) 0.14/0.13	(3) 0.25/0.23	(3) 0.25/0.23				
22	(3) 0.48/0.44	(2) 0.18/0.26	(2) 0.06/0.09				
23	(2) 0.14/0.20	(3) 0.22/0.20	(3) 0.15/0.13				
24	(2) 0.15/0.21	(1) 0/0	(2) 0.47/0.68				
25	(1) 0/0	(2) 0.22/0.32	(2) 0.33/0.47				
26	(5) 1.37/0.85	(3) 0.89/0.81	(5) 1.12/0.70				
27	(2) 0.46/0.66	(3) 0.90/0.82	(3) 0.57/0.52				
28	(4) 0.83/0.60	(2) 0.27/0.39	(4) 0.97/0.70				
29	(4) 0.59/0.43	(3) 0.96/0.87	(2) 0.26/0.38				
30	(5) 1.35/0.84	(4) 1.33/0.96	(4) 1.22/0.88				

Variable	df	F-value	p-value
Location	2	4.060	0.0183
Year build	2	9.893	0.0001
Building material	3	10.165	0.0000
Floor area	2	18.744	0.0000
No. of residents	1	0.028	0.867
Pets	2	14.088	0.0000
Smoking	1	8.237	0.0044
Cleaning frequency	2	11.64	0.00001
Hoover type	2	2.514	0.083
Window number	2	5.174	0.0063
Ventilation frequency	2	8.990	0.0002
Floor type	3	1.283	0.281
Sanitary fittings (only for bathrooms)	2	7.229	0.0013
Perceived humidity separately for:			
kitchen	2	1.905	0.1710
bathroom	2	1.167	0.3160
bedroom	2	0.730	0.3954

Table 2. Variation in fungal abundance (CFU m^{-3}) explained by flat characteristics and residents' behaviour based on one-way ANOVA (statistically significant variables at $p \le 0.05$ are indicated in bold)

Bemowo, Wola, and Ursus) with 6.8×10^2 CFU m⁻³ (LSD p = 0.0364).

Fungal concentrations were significantly higher in flats built between 2011 and 2021 (mean 9.0×10^2 CFU m⁻³) compared to those built between 1986-2010 (5.5×10^2 CFU m⁻³; LSD p = 0.00005) and 1953-1985 (5.8×10^2 CFU m⁻³; LSD p = 0.0004).

Flats constructed with hollow bricks had significantly higher fungal concentrations (mean 8.7×10^2 CFU m⁻³) than those built with traditional bricks (mean 5.1×10^2 CFU m⁻³; LSD p = 0.00001), concrete blocks (mean 5.2×10^2 CFU m⁻³; LSD p = 0.00002), or prefabricated panels (mean 5.5×10^2 CFU m⁻³; LSD p = 0.0078).

A clear relationship was observed between flat size and fungal concentration. Small flats (28-40 m²) had significantly higher concentrations (9×10^2 CFU m⁻³) than mediumsized flats (41-65 m²; 7.2×10^2 CFU m⁻³; LSD p = 0.0000) and large flats (>66 m²; 4.2×10^2 CFU m⁻³; LSD p = 0.00005).

Fungal abundance was significantly lower in flats with cats $(3.3 \times 10^2 \text{ CFU m}^{-3})$ compared to those with dogs $(7.7 \times 10^2 \text{ CFU m}^{-3})$;

LSD p = 0.0000). Flats without pets had similar concentrations to those with dogs $(6.9 \times 10^{2} \text{ CFU m}^{-3})$.

Smoking was associated with significantly higher fungal concentrations. In smoking households, the mean was 10.0×10^2 CFU m⁻³, compared to 6.3×10^2 CFU m⁻³ in non-smoking flats (LSD p = 0.0044).

Cleaning frequency also influenced fungal levels. Flats cleaned once every two weeks had significantly lower concentrations $(2.7\times10^2 \text{ CFU m}^{-3})$ than those cleaned once a week $(7.4\times10^2 \text{ CFU m}^{-3}; \text{ LSD p} = 0.000002)$ or twice a week $(6.4\times10^2 \text{ CFU m}^{-3}; \text{ LSD p} = 0.000002)$.

The number of windows was significantly associated with fungal abundance. Flats with 3-4 windows had higher concentrations $(7.7\times10^2~\text{CFU m}^{-3})$ than those with 2 windows $(6.3\times10^2~\text{CFU m}^{-3}; \text{LSD p} = 0.0343)$ or more than 5 windows $(5.7\times10^2~\text{CFU m}^{-3}; \text{LSD p} = 0.0022)$.

Ventilation frequency significantly influenced fungal concentrations. Flats ventilated whole the day had higher fungal levels

(mean 7.3×10² CFU m⁻³) than those ventilated less frequently (mean 6.3×10² CFU m⁻³).

Finally, bathroom fixtures significantly influenced fungal levels. Bathrooms with open shower cabins had higher concentrations $(9.3\times10^2~CFU~m^{-3})$ than those with bathtubs $(5.2\times10^2~CFU~m^{-3};LSD~p=0.0034)$ or closed shower cabins $(5.2\times10^2~CFU~m^{-3};LSD~p=0.0011)$.

3. Discussion

The results of this study show that indoor air in residential flats contains significant levels of airborne fungi, often exceeding the thresholds previously considered acceptable in national guidelines. Although the Polish Standard PN-89/Z-04111/03, which specified limits for fungal concentrations in atmospheric air, was withdrawn in 2015 and has not been replaced, it remains a commonly used reference in scientific literature and technical assessments (Górny 2004). According to this standard, fungal concentrations above 100 CFU m-3 in bedrooms and 300 CFU m⁻³ in kitchens and bathrooms are considered indicative of poor air quality (Krzysztofik (1992) after Górny (2004)). In the present study, 97% of bedrooms and approximately 70% of kitchens and bathrooms exceeded these thresholds. indicating widespread fungal occurrence. This level of exposure may pose health risks, particularly for sensitive individuals, including those with asthma, allergies, or immunosuppression (Kumar et al. 2021; Upadhayay 2023; Al-Shaarani and Pecoraro 2024; Hurraß et al. 2024).

At the European level, there are currently no harmonized legal regulations specifying acceptable concentrations of airborne fungi in indoor environments. Existing EU Directive 2008/50/EC (Directive 2008/50/EC) on air quality focus predominantly on chemical pollutants (PM 2.5, NO₂, and volatile organic compounds VOCs), while biological contaminants, including mould spores, remain outside the scope of quantitative regulatory limits. In the absence of binding national and EU standards, the assessment

of indoor air contamination with fungi is based solely on expert recommendations, such as those provided by the World Health Organization (WHO 2009) regarding indoor air quality in the context of dampness and mould exposure. These guidelines emphasize the importance of preventive measures, including effective moisture control and adequate ventilation, rather than the establishment of fixed numerical thresholds for fungal concentrations.

The most frequently isolated fungal genera - Cladosporium, Penicillium, Alternaria, Aureobasidium, and yeast-like fungi - are consistent with those commonly reported in indoor environments across Europe and beyond (Anees-Hill et al. 2022; Al-Shaarani and Pecoraro 2024; Šunić et al. 2025). This consistency highlights the widespread nature of these fungi in indoor air and underlines their potential importance as bioindicators of indoor air quality. Although species-level identification can provide more precise information regarding health risks, the genus-level identification applied in this study was sufficient for assessing the sanitary condition of indoor air and recognizing general patterns of fungal occurrence in residential environments (Anees-Hill et al. 2022; Šunić et al. 2025). The mere detection of these genera is widely acknowledged as an indicator of poor air quality and potential health concern, regardless of species-level differences. While health risks may vary within a given genus, the presence of these fungi at elevated concentrations already indicates the need for monitoring and preventive action (WHO 2009). As demonstrated by our study, even relatively new residential buildings face the issue of high concentrations of fungal spores in indoor air. Notably, newer buildings (built between 2011 and 2021), despite being constructed from one of the more moisture-resistant materials, showed a higher fungal load, probably due to tighter insulation and inadequate ventilation, which can trap moisture and organic particles indoors and are the main

causes of fungal growth (Carpino et al 2023; Loukou et al. 2024).

Among the many characteristics of flats, location significantly influenced the abundance of airborne fungi. Flats located in the eastern districts of Warsaw showed higher concentrations of fungi in the air, which may be related to local microclimatic conditions shaped, among other factors, by the speed and dominant directions of winds, which is confirmed by the research of other authors (Rodríguez-Rajo et al. 2005; Sadyś et al. 2015). In Warsaw, the dominant wind directions are westerly and north-westerly (Weather Spark 2023), potentially facilitating the transport of fungal spores from big forest areas to the north-west of the city, such as the Kampinos National Park, as well as smaller forest complexes located directly across the Vistula, including the Młociński Park and the Bielański Forest. In our study, ventilation practices also had a significant effect on indoor fungal levels, which, combined with the fact that locally fungal densities in outdoor air may be higher, could explain the higher concentration of fungi in the indoor environment through the influx of spores from outside via open windows (Lee et al. 2006; Ye et al. 2021).

While external sources of fungal spores and factors beyond the control of the occupants can contribute to indoor air pollution and reduced air quality, internal factors, particularly those related to household practices and the habits of the occupants, appear to have a greater impact on the concentration and diversity of airborne fungi. The higher fungal concentrations observed in flats that were cleaned and ventilated more frequently throughout the whole day can be explained by the combined effect of resuspension and airflow dynamics, whereby dust deposited on various surfaces, and within it, fungal spores, pollen or bacteria are again lifted into the air by airflow (Veillette et al. 2013; Qian et al. 2014). Frequent cleaning - especially using dry methods such as sweeping or vacuuming without HEPA filters – could disrupt the deposition

of fungal spores on floors, carpets and furniture, reintroducing them into the air. This process has been shown to increase levels of bioaerosols in the air (Kanaani et al. 2008; Knibbs et al. 2012). When such cleaning is combined with continuous ventilation, especially natural ventilation through open windows, this could further facilitate the movement and distribution of spores in the indoor environment. Air flow, rather than removing contaminants, could keep spores in suspension longer or even draw in additional spores from the external environment as mentioned above, especially in areas with a high fungal load. Thus, both frequent cleaning and prolonged ventilation could inadvertently contribute to elevated fungal levels indoors.

In the context of the resuspension phenomenon, the lower airborne fungal concentrations observed in flats with cats than with dogs can be explained by differences in the animals' behaviour and their influence on indoor particle dynamics. Cats tend to move more gently and with less force than dogs, generating less air movement that would otherwise cause resuspension of settled fungal spores from surfaces such as floors and carpets. Dogs especially larger or more active dogs could generate stronger air currents increasing the possibility of resuspension of spores and their distribution throughout the house. In addition, cats are more likely to stay indoors than dogs, reducing the introduction of fungal spores from outside on fur or paws (Fujimura et al. 2010; Hickman et al. 2022; Šunić et al. 2025). Hygiene practices of cat owners could also play a role - more frequent wet cleaning could reduce microbial accumulation without causing widespread resuspension.

Bathroom facilities significantly influenced fungal abundance (Lian and de Hoog 2010; Adams et al. 2013). Open shower cabins were associated with higher levels of fungi compared to baths or closed cabins, probably due to greater dispersion of moisture and increased surface area for fungal colonisation. Open showers allow water vapour and

droplets to spread more freely in the bathroom, increasing ambient humidity and promoting condensation on walls, ceilings and other surfaces. Constantly sustained humidity creates ideal conditions for the growth of fungi, particularly genera such as Cladosporium, Penicillium, and Aspergillus (Ozoaduche and Idemudia 2021). Closed shower cabins and baths, on the other hand, tend to retain moisture more effectively, limiting its spread and allowing surrounding surfaces to dry more quickly, while open showers allow moisture to penetrate porous materials, such as grout or wooden elements, which could serve as long-term reservoirs for fungal colonisation (Loukou et al. 2024).

Despite the lack of legally binding standards in Poland regarding fungal contamination inside buildings, the results of this study clearly indicate an urgent need to update regulations and establish air quality standards for indoor environments. The absence of such regulations significantly hinders effective public health interventions, especially in light of the growing number of reports on the adverse health effects of indoor fungal exposure (Valtonen 2017; Kramer et al. 2021; Goudarzi and Reshadatian 2024).

Conclusions

The conducted study clearly indicates the widespread presence of fungal spores in residential flats across Warsaw. High concentrations of fungal spores such as Cladosporium, Penicillium, and Alternaria, exceeding proposed indoor air quality standards, may pose a significant health risk to residents, particularly individuals with respiratory conditions, allergies, or weakened immune systems. The findings show that poor indoor air quality affects both older and newer buildings; however, in newer constructions, fungal concentrations were on average twice as high, likely due to limited ventilation and moisture retention in well-insulated interiors.

The analyses revealed significant correlations between fungal concentrations and

both flat characteristics and occupant habits. Factors such as location, year of construction, building material, floor area, presence of pets, smoking indoors, cleaning frequency, number of windows, ventilation practices, and bathroom fixtures had a noticeable impact on the fungal load in indoor air. Particularly noteworthy is the observation that frequent cleaning and intensive ventilation (practices generally considered health-promoting) may under certain conditions lead to increased bioaerosol concentrations due to the phenomenon of resuspension.

The lack of legally binding standards in Poland regarding acceptable levels of fungal concentration in indoor air hinders effective preventive and intervention measures. Therefore, until appropriate standards are implemented, it is essential to continue scientific research in this area and intensify educational and informational efforts. Raising public awareness about the risks associated with indoor fungal presence can help reduce health hazards and improve the quality of life for residents.

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Appendix 1: Supporting Data for Indoor Air Quality Assessment in Selected Warsaw Flats: Quantitative and Qualitative Analysis of Airborne Fungi

Table S₁. Selected flat characteristics relevant to indoor fungal occurrence and air quality (the data was collected through a detailed questionnaire with 23 specific questions, completed by residents during air sampling)

	2 2																									
	Perceived humidity ⁵	M/M/M	M/M/M	M/M/M	M/H/M	M/M/D	M/M/D	M/M/D	M/M/M	D/W/W	Q/H/W	D/H/D	M/H/M	a/a/a	M/M/M	M/H/M	M/M/D	M/M/D	M/M/D	M/M/M	M/M/M	M/H/M	M/M/M	M/M/M	M/M/M	M/H/D
	Sanitary fittings ⁴	ESC	ESC	OSC	OSC	OSC	ESC	Bath	OSC	ESC	OSC OSC	OSC	OSC	OSC OSC	Bath	ESC	Bath	ESC	OSC OSC	OSC	Bath	Bath	OSC	Bath	ESC	Bath
	Flooring type 3	۵	P,T	_	٩,	P,T	_	_	_	7	Ь	_	P, T, PCV	Ь	P,T	P, T, PCV	1′L	P, T, PCV	7	P,T	Ь	_	P, T, PCV	P,T	P,T	PCV
	Ventilation Freq.	/8h	>20h	/8>	>20h	not often	not often	>20h	<8h	/8>	>20h	>20h	/8h	>20h	/8>	<8h	/8h	/8h	>20h	<8h	<8h	>20h	/8>	>20h	not often	/8h
	Cleaning Freq. ²	2/7	1/7	2/7	1/7	1/7	2/7	2/7	2/7	1/7	2/7	1/7	1/7	2/7	1/7	1/7	2/7	1/7	2/7	1/14	1/7	2/7	1/7	1/7	2/7	2/7
	Smoking	No	No	No	No	No	No	No	No	No	No	Yes	Yes	No	No	No	Yes	Yes	No	No	No	No	No	No	No	No
	Pets	Dog	None	None	None	None	None	None	Dog	None	None	None	None	Dog	None	None	Cat	None	None	None	Dog	None	Cat	None	Cat	None
,c	No. of residents	4	4	2	2	2	4	2	2	4	2	2	4	2	4	4	2	4	2	2	2	4	2	2	2	2
I	Floor area [m²]	50	65	44	28	44	37	61	53	62	70	40	56	62	48	69	38	59	58	34	80	20	94	49	57	87
- a	Building material ¹	Brick	LPS	읲	Concrete	ピ	LPS	읲	ピ	HB	윞	읲	ピ	Brick	Concrete	위	LPS	Brick	윞	Brick	Brick	LPS	Brick	LPS	Concrete	Concrete
	Year built	1960	1970	2016	1963	2015	1968	2020	2020	2000	2020	2005	2021	1960	1963	2011	1971	1954	1975	1956	1953	1975	1955	1968	2013	1980
	Location	Śródmieście	Targówek	Bemowo	Śródmieście	Żoliborz	Praga Płn.	Praga Płd.	Białołęka	Białołęka	Wawer	Praga Płn.	Bernowo	Wola	Wola	Wola	Praga Płd.	Śródmieście	Tarówek	Żoliborz	Ochota	Bemowo	Ochota	Ochota	Białołęka	Bielany
	Flat No.		2	2	4	5	9	7	∞	6	10	1	12	13	14	15	16	17	18	19	20	21	22	23	24	25

Perceived humidity ⁵	D/D/D	M/H/D	M/H/M	M/M/D		
Sanitary fittings ⁴	ESC	ESC	ESC	ESC		
Flooring type 3	P,T	7	Laminate	1'1		
Ventilation Freq.	/8h	48>	>20h	<8h		
Cleaning Freq. 2	1/7	2/7	2/7	1/7		
Smoking	No	No	No	No		
Pets	Cat	None	Dog	None		
No. of residents	2	2	4	4		
Floor area [m²]	99	52	45	77		
Building material ¹	Brick	Brick	HB	兕		
Year built	1993	2006	2004	1994		
Location	Mokotów	Mokotów	Ursus	Bielany		
Flat No.	27	28	29	30		

(*- LPS – large-panel system, HB – hollow blocks; * – 1/7 – once a week, 2/7 – twice a week, 1/14 – once every fortnight; 3 – P – parquet, T – tiles, L – laminate, PCV – Polyvinyl Chloride (flooring material); * – ESC – enclosed shower cabin, OSC – open shower cabin; * – D-dry, M – moderate, H – humid in order kitchen/bathroom/bedroom)