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Micromycetes as a Hidden Health Risk in Ventilation Systems of Panel Buildings: A Case Study

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Abstract

Indoor air quality is an important determinant of human health and is shaped by the interactions among physical, chemical, and biological factors. Airborne fungi represent a significant component of indoor microbiota and are associated with allergic reactions, respiratory diseases, and opportunistic infections, particularly in vulnerable populations. Ventilation systems are essential for air exchange but may also function as reservoirs and dispersal pathways for fungal propagules, especially in older residential buildings with natural ventilation where dust and moisture enable fungal persistence. Despite the widespread presence of prefabricated panel buildings in Central Europe, data on fungal communities within their ventilation systems remain limited. This study addresses this gap by characterizing fungal communities in the ventilation shafts of multi-unit panel buildings using a combination of culture-based and DNA-based approaches. Micromycetes were detected in all sampled systems, with no significant differences among cities. *Cladosporium* dominated fungal assemblages, with a mean relative abundance of 15.7% and maxima up to 63%. Cultivation yielded 45 viable isolates, mainly *Aspergillus*, *Penicillium*, and *Cladosporium*, confirming ventilation systems as overlooked reservoirs relevant to indoor air quality and health.

1 Introduction

In recent years, increasing attention has been given to the presence of air microbiota in indoor environments, particularly regarding its impact on human health (Barberán et al. 2015; Kumar et al. 2022). The most active population in their productive age spends more than 90% of their time indoors (Schweizer et al. 2007). Ventilation systems and air-conditioning units can serve as reservoirs and transmission vectors for pathogenic microorganisms, potentially leading to severe health outcomes such as Legionnaires' disease (Wüthrich et al. 2019) or various fungal or viral infections (Li et al. 2007; Liu et al. 2021b; Kumar et al. 2021; Shiraishi et al. 2023).

Around 47% of Europe's population lives in flats (Eurostat 2024). In the Czech Republic, just under a third of the population (31%) lives in prefabricated panel buildings (ČSÚ 2014). Construction of these buildings began in the 1950s, with the oldest housing estates built in the 1960s (Lipták and Pindel 2016). In older panel-buildings, ventilation systems were originally designed to extract stale air, particularly from kitchens and bathrooms. However, many of these systems have not received adequate maintenance or cleaning since construction (Kolářová 2007).

Over time, this neglect leads to the accumulation of dust and grease layers that are not only highly flammable but also serve as reservoirs for diverse microbial consortia, including fungal propagules. These established communities may re-enter living spaces due to various physical and environmental factors: differences in air pressure/temperature between the interior and exterior of residential units; changes in weather; temperature gradients; the configuration and geometry of the ventilation ductwork; and the placement and design of exhaust or air outlet points within apartments. Such conditions facilitate the mobilization and dispersion of microbial particulates indoors, which can, in turn, exacerbate

respiratory disorders, including asthma, allergic rhinitis, hypersensitivity pneumonitis, and other related pathologies.

The rising incidence of infections caused by micromycetes, particularly among immunocompromised individuals, has become a major public health concern. These infections can present in diverse clinical forms, including aspergillomas, chronic pulmonary aspergillosis, allergic bronchopulmonary aspergillosis, invasive aspergillosis, pneumonitis, and other severe diseases (Denning 1998; Mousavi et al. 2016). Conidia of these fungi - such as *Aspergillus*, *Penicillium*, *Fusarium*, *Paecilomyces*, and *Scopulariopsis* genera are highly hydrophobic and easily dispersed through air and dust, making inhalation almost unavoidable (Kwon-Chung and Sugui 2013). This airborne spread can trigger allergic reactions in susceptible individuals and lead to opportunistic infections. Indoor environments with elevated humidity are particularly problematic, as representatives of genera such as *Aspergillus* and *Penicillium* readily colonize damp building materials, forming visible mold on walls and other surfaces.

In the Czech Republic, limits for the occurrence of microorganisms in indoor environments are defined or recommended by two documents: Decree No. 6/2003 Coll., which applies exclusively to residential rooms, and the European guideline EUR 14988, which distinguishes between two categories of facilities: residential rooms and households (Rubinová 2015). A similar situation applies across Europe, where ventilation requirements are generally based on harmonized standards such as EN 15665 and EN 16798-1, implemented through national regulations. These standards define performance criteria and recommended ventilation rates for residential buildings rather than prescribing specific systems. In contrast, microbiological limits for indoor air are not uniformly regulated at the EU level; most countries rely on guidelines or indicative values, while strict limits apply only to controlled environments such as cleanrooms.

Despite acknowledged health risks and established general regulations, comprehensive studies specifically on fungal contamination in the aging ventilation infrastructure of prefabricated panel buildings remain lacking. Previous research on indoor air quality has predominantly centered on modern heating, ventilation, and air-conditioning (HVAC) systems (Prussin II and Marr 2015) or general household dust (Barberán et al. 2015; Gangneux et al. 2020). These studies have demonstrated that mechanical ventilation systems can harbor diverse fungal communities, whose abundance fluctuates with seasonal variations and maintenance protocols (Zemitis et al. 2026), or that hybrid exhaust systems typical of mid-20th-century panel buildings can present a distinctly different microenvironment. The long-term accumulation of organic matter and grease in these unmaintained vertical shafts creates unique ecological niches for microbial growth that are not comparable to modern HVAC filters. Currently, there is a significant lack of data characterizing the precise mycobiome within these structures.

In this study, we collected dust samples from ventilation systems in several older panel buildings across the Czech Republic. To assess fungal diversity, we employed both culture-based methods and DNA-based analyses. Cultivation enabled the isolation of viable fungal strains under controlled laboratory conditions, while molecular techniques provided a comprehensive characterization of the entire fungal

community, including non-culturable taxa. We then compared the composition and structure of these fungal communities across different locations and seasonal periods.

2 Materials and methods

2.1 Description of sampling sites

Several panel buildings were selected for this study based on specific inclusion criteria: ease of access, suitable conditions for operational measurements, residents' willingness to cooperate, and the absence of prior cleaning or renovation of the ventilation system. The selected buildings were all prefabricated panel structures, representative of typical residential construction from the socialist era (1960–1985). These were apartment buildings with natural ventilation, relying on convection driven by temperature differences- a principle commonly applied in residential buildings worldwide. Such buildings account for approximately 50% of the residential housing stock in the Czech Republic. Sampling was conducted in eight cities across the Czech Republic: Prague (3 buildings), Brno (4), Liberec (2), Zlín (1), Hořovice (1), Otrokovice (1), Brumov-Bylnice (2), and Český KrumLov (1) (Fig. 1).

Sampling was deliberately focused on the internal surfaces and accumulated dust within the ventilation ductwork, as the primary aim of the study was to characterize the fungal reservoir within the system itself rather than the resulting airborne exposure in occupied spaces. Surface swabs and bulk dust collection were therefore the most appropriate sampling methods for this purpose.

2.2 Sample handling

To evaluate microbial contamination across different functional areas of residential ventilation systems, the collected samples were classified into four groups (K, WC, K + WC, and D). This classification was based on the specific sampling location (room type) and the physical nature of the collected material (surface swab/accumulated dust). The rationale for this division was to distinguish between room-specific microbial profiles (kitchen vs. bathroom), mixed-source environments, and the overall accumulated microbiome within the ductwork. The groups are defined in Table 1.

Table 1
Sample classification

Sample	Description
K (Kitchen)	Surface swab samples collected from the exhaust vents located in kitchens.
WC (Toilet/Bathroom)	Surface swab samples collected from the exhaust vents located in toilets or bathrooms.
K + WC (Mixed duct)	Surface swab samples collected from a shared ventilation duct serving both the kitchen and the toilet/bathroom. This specific structural setup was present and sampled exclusively at the Technická street site (Brno, Czech Republic)
D (Dust)	Composite dust samples representing the mixed, accumulated material from the entire duct system at a given sampling site.

2.3 Sampling procedures

Surface swab samples (groups K, WC, and K + WC) were collected from an area of approximately 10 x 10 cm at the kitchen, bathroom/toilet and toilet exhaust vents. To assess the efficacy of the maintenance process, sampling was performed both before and after cleaning the ventilation system. After each sampling, the swab tip was detached and placed into a 2 mL microtube filled with DNA Shield solution (Zymo Research, Irvine, California, USA), sealed, and stored in a freezer at -20°C until DNA extraction.

Additionally, dust (group D) was collected from the filter in the air extraction unit during the ventilation system's cleaning procedure to provide a more comprehensive overview of the mycobiome across the entire ventilation system (Fig. S1E). Dust samples were collected after all exhaust vents at the sampling site had been cleaned. Two complementary dust sampling methods were used: first, a surface swab of the collected dust was taken and processed identically to the vent swabs (placed in a 2 mL microtube with DNA Shield at -20°C). Second, in accordance with the downstream extraction kit manufacturer's protocol, approximately 250 mg of a composite bulk dust sample was collected from the equipment filters into sealable plastic bags and subsequently stored at -20°C until further analysis.

2.4 Cultivation

Cotton swabs obtained from the Brno, Czech Republic, sampling were also used to cultivate micromycetes. Four types of media were used for the cultivation of yeasts and filamentous fungi: Nutrient Agar (Oxoid, United Kingdom), Actinomycete Isolation Agar (HiMedia, India), Endo Agar (HiMedia, India), and Wort Agar (Merck, Germany). The media were prepared according to the manufacturers' instructions (Supplementary file).

The swab or dust sample in physiological saline solution was vortexed, and the swab was discarded. A serial dilution was prepared, and 0.1 mL of each dilution was plated onto agar plates. Nutrient agar was incubated at 37°C and 22°C, Actinomycete Isolation Agar at 22°C, Endo Agar at 37°C, and Wort Agar at 30°C. Plates were incubated in a thermostat for 24 to 48 hours before individual colonies were selected for micromycete isolation.

The cultivation experiment was conducted as a pilot study using samples from Brno, Czech Republic, to assess the viability and diversity of fungal propagules in the ventilation systems. Based on the results obtained, confirming the presence of viable and potentially harmful fungal taxa, DNA-based screening and high-throughput sequencing were subsequently applied to the full sample set from all cities.

2.5 DNA isolation

Colonies of micromycetes were picked and purified. DNA was isolated using the DNeasy UltraClean Microbial Kit (Qiagen, USA). Amplification of the ITS region was performed using primers ITS1 and ITS4 described by White et al. (White et al. 1990). The PCR protocol included the following steps: initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 1 minute, annealing at 54°C for 1 minute, and extension at 72°C for 1 minute. A final extension at 72°C for 10 minutes was

performed after the last cycle. The purified PCR product was subsequently sequenced and identified using the BLAST algorithm.

DNA from swab samples for Illumina sequencing was extracted using the Quick-DNA Fecal/Soil Microbe Kits (Zymo Research, Irvine, California, USA) according to the manufacturer's protocol. After extraction, DNA concentration and purity were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA) to ensure suitability for downstream applications. The purified DNA samples were then stored at -20°C to preserve integrity until further processing and sequencing.

After DNA isolation, part of the ITS region was amplified. Modified versions of the ITS1 (TCCGTAGGTGAACCTGCGG) and ITS2 (GCTGCGTTCTTCATCGATGC) primers [17] were used in the PCR, enabling dual-index barcoding. Primers were modified for the use of metabarcoding according to Pichler et al. (Pichler et al. 2018) and the Earth Microbiome Project protocol (Thompson et al. 2017) and was performed in a total reaction volume of 25 µL, containing 10 µL of Platinum II HotStart PCR Master Mix (Thermo Fisher Scientific), primers at a final concentration of 200 nM, and 2 µL of extracted DNA. Following amplification, PCR products were purified using AMPure XP Beads (Beckman Coulter, Brea, CA), quantified with a Qubit 4 fluorometer (Thermo Fisher Scientific, Waltham, MA), and assessed for quality using a Fragment Analyzer (Agilent Technologies, Santa Clara, CA). The prepared libraries were subsequently sequenced on an Illumina MiniSeq platform (Illumina, San Diego, CA) employing MidOutput reagent kits with paired-end reads of 2 × 150 bp.

2.6 Sequence Analysis

The data were obtained from two sequential sequencing runs. The raw sequencing data were processed using the DADA2 package (v1.16) (Callahan et al. 2016) in accordance with the ITS DADA2 workflow, which included identification and removal of primer sequences from the raw data by cutadapt, followed by quality filtering (removing ambiguous bases, and applying an expected error threshold of 2), dereplication, denoising, read merging, and chimera removal. Taxonomy was assigned using the RDP classifier against the UNITE ITS database (Abarenkov et al. 2024), resulting in a high-resolution amplicon sequence variant (ASV) table. For subsequent analyses, all samples were rarefied to 9700 reads. The raw fastq files are available under BioProject PRJNA1403450.

2.7 Data evaluation

Using experimental data, the basic statistical parameters (M-mean, SD-standard deviation, and M ± SD) were calculated. Statistical analyses were planned prior to data collection. The Kruskal-Wallis test, followed by Dunn's post hoc test with Bonferroni correction, was selected a priori to compare alpha diversity indices (Chao1, Shannon, Simpson) across sample types (K, WC, K + WC, D) and sampling cities, given the non-normal distribution of microbiome data. Temporal variation in fungal community composition throughout the year was not formally tested because the limited number of repeated sampling time points precluded robust statistical inference. The plots were built using the software package OriginPro 2023 (OriginLab Corporation, Northampton, MA) and R (version 4.4.3) within the RStudio environment (version 2024.12.1).

The taxonomic data were analyzed using the microeco package (version 1.16.0) (Liu et al. 2021a). Functional prediction of metabolic pathways was processed through microeco package function trans_func, using FungalTraits database (version 1.2) (Põlme et al. 2020).

3 Results

High-throughput sequencing of the ITS region revealed a diverse fungal community within the ventilation systems of the sampled panel buildings. Across all sites, the sequencing data identified numerous common indoor taxa. Culture-based analyses complemented these findings by isolating viable strains under controlled laboratory conditions. Although cultivation recovered fewer taxa than sequencing, it provided essential insights into the viability and potential growth of fungi in ventilation systems.

3.1 Alpha and beta diversity

Alpha and beta diversity metrics were calculated to assess fungal community structure across cities, building types, and ventilation system categories. The Chao1 index reflects the species richness of a given community. Statistical differences between sample types were assessed using the Kruskal-Wallis test followed by Dunn's post-hoc test with Bonferroni correction. Chao1 index values ranged from 11 to 509, with no statistically significant differences found among individual cities or sample types (Kruskal-Wallis test, $p > 0.05$) (Fig. 2).

However, indices reflecting species evenness in the distribution and abundance of detected species (Shannon and Simpson) revealed distinct patterns by sampled environment rather than geographic location. While no significant differences were observed among cities, sample types showed considerable variation. Mixed dust samples exhibited higher evenness indices, as expected, since dust collects from the entire duct system and represents a broader, more evenly distributed community. Conversely, swabs from the common duct (combining the kitchen and WC) showed significantly lower evenness than those from individual ducts (Kruskal-Wallis test, $p < 0.05$).

Beta diversity was analysed using the Bray-Curtis dissimilarity index, which quantifies differences in microbial community composition based on relative abundance. The resulting distance matrix was visualized using non-metric multidimensional scaling (NMDS) (Fig. S2). No significant clustering of samples was observed by the city in which they were collected. Instead, grouping was primarily influenced by sample type, with dust samples clustering together, and by specific building conditions, which will be further discussed below. A similar trend was observed using the Jaccard index, which focuses on the presence or absence of microbial communities, visualized through Principal Coordinates Analysis (PCoA) (Fig. S2).

3.2 Relative abundance

3.2.1 Core taxonomic composition of the ventilation mycobiome

Overall, the mycobiome of the studied environments proved to be rich and highly consistent across different cities. The most abundant fungal order across all samples was *Cladosporiales* (relative abundance ranging from 0.9% to 63%), with *Cladosporium* being the universally dominant genus detected in every single sample. Its mean relative abundance was 15.7 ± 12.0%, and, with few exceptions, the dominant species was *C. herbarum*. Other frequently represented orders included *Chaetothyriales*, *Pleosporales*, *Mycosphaerellales*, and *Eurotiales* (Fig. 3).

Beyond *Cladosporium*, the microbial profiles consistently featured a core group of genera (Fig. S3). *Alternaria* (primarily *A. infectoria* and *A. tenuissima*) was present in 87% of samples (mean 3.4 ± 4.4%, max 25%), and *Neoascochyta* in 83% of samples. The genera *Penicillium* (mainly *P. corylophilum*, *P. palitans*, and *P. chrysogenum*) and *Neophaeococcomyces* were both detected in 78% of samples. Although *Naganishia*, *Spiralomyces*, and *Rachicladosporium* were found in only 60% of samples, they occasionally reached high maximum relative abundances ranging from 21% to 27%.

Notably, the genus *Aspergillus* was highly prevalent (mean 3.5 ± 5.3%, max 32.6%) and exhibited high species diversity within the ventilation systems, with up to 20 species identified (Fig. S4). The most prevalent were *A. penicilloides*, *A. ruber*, and *A. sydowii*, though potentially harmful species such as *A. flavus* and *A. fumigatus* were also detected, highlighting potential health implications.

3.2.2 Influence of specific building conditions and duct types

While the general mycobiome was consistent, specific building histories and duct functions significantly altered microbial profiles in certain locations. The most prominent outlier was a building on Technická street (Brno, Czech Republic), which had been unused for an extended period. Samples from this location (K + WC ducts) clustered entirely separately in the beta diversity analysis (Fig. S2) and were responsible for the low alpha diversity evenness. Taxonomically, this unused environment was characterized by dominance of the order *Chaetothyriales* (up to 67% in three of four samples), almost exclusively represented by the genus *Exophiala*. Additional prevalent genera at this location included *Devriesia* (~ 10%) and *Paraphoma* (~ 10%).

Furthermore, specific duct types within individual buildings showed distinct variations, as observed in two buildings on Jozefy Faimonové street (Brno, Czech Republic). In the first building, the WC duct swab and dust sample were heavily dominated by the order *Dothideales* (genus *Aureobasidium*, up to 68%), whereas the kitchen duct swab from the exact same building was dominated by the order *Pleosporales* (genus *Didymella*, 49%). In the second building, WC duct swabs were uniquely dominated by the genus *Bjerkandera* (order *Polyporales*, 52%). These findings indicate that while geographical location has minimal impact, specific microenvironments (kitchens vs. bathrooms) and building usage history can drastically shift the fungal community composition.

3.3 Fungal Traits

Using the Fungal Traits database (Põlme et al. 2020), functional traits were assigned based on the identified taxa, resulting in a trait profile of micromycetes shown in Fig. 4. This profile was largely consistent across samples, with the most notable differences observed in samples from Brno, Czech Republic, specifically from Technická street and Jozefy Faimonové street.

Most identified fungi were litter saprotrophs, utilizing substrates such as fruit, leaves, seeds, wood, and feces or other animal-derived materials. The detected species require a certain level of moisture for growth, which is provided by the ventilation system (see measurements in Fig. 4).

Most dominant species formed mycelial structures, although yeast-like fungi and dimorphic fungi, which can switch between morphological forms depending on environmental conditions, were also present.

Importantly, the predicted pathogenicity suggests that the detected species are primarily opportunistic pathogens, capable of causing disease in immunocompromised or otherwise vulnerable individuals.

3.4 Cultivation

While molecular profiling (NGS) provides a comprehensive overview of the fungal communities present in the ventilation systems, it cannot differentiate between DNA originating from living cells and environmental DNA from dead or degraded biomass. Therefore, culture-based analysis was employed to determine the viability and colonization potential of the fungal propagules accumulated in the dust.

Colonies of microorganisms that had grown on the plates inoculated during sampling in Brno, Czech Republic were collected and purified to obtain pure cultures. DNA was isolated, and the isolates were identified. A total of 45 isolates were obtained, the majority of which belonged to the genus *Aspergillus* (17 isolates) (Fig. 5). Other abundant genera included *Penicillium* (7 isolates) and *Cladosporium* (6 isolates). The sequences of the isolated strains are deposited in GenBank database (Accession numbers: PX674411-PX674454).

The diversity of isolated species within each genus was broad. From the genus *Aspergillus*, the following species were identified: *A. flavus, A. oryzae, A. fumigatus, A. niger*, and *A. ochraceus*. Within the genus *Penicillium*, the isolates included *P. camemberti, P. corylophilum, P. crustosum, P. chrysogenum*, and *Penicillium sp.* The genus *Cladosporium* was represented by *C. cladosporioides, C. halotolerans*, and *Cladosporium sp.*

The isolation of micromycetes from swabs confirmed their viability and highlighted their potential health risks in residential buildings, particularly regarding allergy development and immunocompromised individuals.

4 Discussion

Ventilation systems act as critical modulators of indoor air quality (IAQ). When dust and organic matter accumulate in ductwork, they create favorable microenvironments that provide nutrient-rich substrates

for fungal colonization. It has been discovered that airflow within these duct networks is not always strictly unidirectional. The occurrence of reverse airflow driven by weather conditions was confirmed (as evidenced by time-series measurements during extreme meteorological events/storms) and was also shown to depend on the local air-exhaust capabilities within the panel-building apartments (i.e., particularly in the absence or inactivity of rooftop ventilation units). This means that fungal spores, hyphal fragments, secondary metabolites (such as mycotoxins or volatile organic compounds) and contaminated dust accumulating even in exhaust ducts can be actively pushed back into the inhabited indoor spaces. This mechanism effectively transforms an unmaintained ventilation system from an air-purifying mechanism into a reservoir and vector for continuous bioaerosol exposure, directly deteriorating IAQ.

In this study, DNA was isolated from eight cities across the Czech Republic, from a total of 15 buildings, using both swab samples and dust collected from entire ventilation systems. The samples revealed high diversity of micromycetes in both swabs and dust. Dust exhibited greater diversity and evenness in the representation of individual taxa, likely because its content originated from across the entire system. Nevertheless, when examining microbial composition in individual samples - whether by city or sample type - the microbial profile was similar across all samples, except for the abandoned Technická street and Josefy Faimonové street.

4.1 The context of the results in comparison to other studies

Although most detected species were saprophytic or plant pathogens, representatives with potential to cause disease in immunocompromised individuals were also present. Their viability was confirmed through cultivation, where the most dominant genera were *Aspergillus*, *Penicillium* and *Cladosporium*. These genera were also the most prevalent in the NGS sequencing data. The results correspond with findings by Fukutomi & Taniguchi (2015), who reported these three genera as the most common airborne molds in indoor environments. The study by Golofit-Szymczak et al. (2013) also investigated micromycetes and yeasts. Frequently cultivated genera from dust included *Acremonium* sp., *Alternaria* sp., *Aspergillus* sp., *Penicillium* sp., and *Rhodotorula mucilaginosa*. Almost all these genera, except *Acremonium* sp., were successfully cultured during this work. Similarly, other studies of indoor air in different parts of the world led to the isolation of these genera and species, as in our study, showcasing the fungal population in indoor environments (Horner et al. 2004; Bensch et al. 2018).

In recent worldwide study, *Cladosporium* was the most common isolate from indoor air, with *C. halotolerans* being the most frequently isolated species (Bensch et al. 2018). *C. cladosporoides* was also the most dominant isolate, being the most prevalent in indoor and outdoor air, in the study in Atlanta, USA (Horner et al. 2004). Other the most prevalent genera in the study were *Alternaria*, *Aspergillus*, *Penicillium* and *Epicoccum*, which again correspond with our results. However, in our study, the most abundant species was *C. herbarum*, followed by *C. cladosporioides* and *C. halotolerans*. *C. herbarum* was

also the dominant *Cladosporium* species in the study of the fungal microbiome of damp buildings (Andersen et al. 2011).

Alternaria alternata was not identified in the data from Illumina sequencing, however, it was isolated and identified during cultivation experiments. This may be due to the challenging process of isolating DNA from fungi and insufficient lysis of the fungal cell wall. The genus Alternaria is commonly isolated or detected in indoor air (Horner et al. 2004).

Although the genus *Penicillium* was not detected in all samples, its prevalence exceeded 10% in some samples. The most dominant species in the samples, *P. corylophilum*, is often detected in damp indoor environments and has been associated with sick building syndrome (Bok et al. 2009). It was also the dominant species isolated in high-humidity crawl spaces in houses in Sweden (Bok et al. 2009). Both *Penicillium* species were also detected in water-damaged or damp building materials [28] or in air and indoor wall samples from Polish schools built using prefabricated concrete (Ejdys and Biedunkiewicz 2011).

In the context of indoor air, *Aspergillus* was present in high occurrence in hospital intensive care units, which pose a risk for the development of COVID-19- and influenza-associated pulmonary aspergillosis [31], or primary health care centers, where the CFU surpassed the legislative limits, giving emphasis on ventilation system maintenance (Viegas et al. 2019). *Aspergillus* was also prevalent in the indoor air of asthmatic patients, especially A. flavus and A. fumigatus, posing a health risk and aggravating patients' conditions (Hedayati et al. 2010).

4.2 The posing danger of the detected fungal representatives

The most dominant genus present in all samples was *Cladosporium*. Members of the genus *Cladosporium* are saprobic molds, some of which have adapted to plant parasitism, while others significantly contribute to the contamination of both plant- and animal-based food products. Several species of *Cladosporium* can cause superficial, subcutaneous, systemic, and central nervous system infections (Raman Sharma 2010). Due to their small size (3–35 μm), *Cladosporium* spores can be easily inhaled and reach the lower respiratory tract. Exposure may lead to inhalant allergies, including allergic rhinitis, sinusitis, conjunctivitis, mold asthma, and allergic alveolitis (Knutsen et al. 2012). *C. herbarum* plays an important role in allergy development, as it contains about 60 antigens (Kurup et al. 2002).

The genus *Alternaria* is among the most prevalent in our samples. Representatives of the genus *Alternaria* are widespread molds primarily associated with soil ecosystems, where they contribute to the decomposition of organic matter. *Alternaria* species, in conjunction with other indoor fungal contaminants, have been implicated in the etiology of sick building syndrome (Abel-Fernández et al. 2023). Species of *Alternaria* may lead to allergic sinusitis and rhinitis, bronchial asthma, hypersensitivity pneumonitis, skin infections, and bronchopulmonary mycosis (Hernandez-Ramirez et al. 2021). In our study, the most prevalent species were *A. infectoria* and *A. tenuissima*. *A. tenuissima* is an opportunistic

plant pathogen, whereas *A. infectoria* causes cutaneous infections, posing a risk to immunocompromised individuals (Lopes et al. 2013).

Penicillium is a genus of fungi with major importance in the environment and in pharmaceutical production. They are found in soil, air, and on decaying fruits, vegetables, and meat. Diseases caused by infection with any *Penicillium* species are collectively referred to as penicilliosis. *Penicillium* species such as *P. citrinum*, *P. chrysogenum*, *P. digitatum*, *P. expansum*, and *P. marneffei* have been reported to be associated with various human infections, including keratitis, endophthalmitis, otomycosis, pneumonia, endocarditis, and urinary tract infections. Invasive infections have been described as being caused by *P. chrysogenum* (Lyratzopoulos et al. 2002).

The genus *Aspergillus* represents a cosmopolitan group of fungi that plays a vital role in the saprophytic decomposition of organic matter. These fungi are commonly found in a wide array of environments, including soil, decaying plant material, indoor air, and household dust. Their ecological adaptability and low water demand allow *Aspergillus* species to thrive in both natural ecosystems and human-made environments.

The most clinically relevant *Aspergillus* species are *A. fumigatus*, *A. flavus*, *A. terreus* and *A. niger* (Arastehfar et al. 2021). *Aspergillus* can cause infections from non-invasive to invasive in both immunocompromised and immunocompetent individuals (Kwon-Chung and Sugui 2013). Non-invasive infections primarily include allergic sinusitis, allergic bronchopulmonary aspergillosis (ABPA), fungal balls in the sinus or lung (aspergilloma), chronic pulmonary aspergillosis, and superficial infections such as otitis externa and onychomycosis (Denning et al. 2018). For invasive infections, invasive aspergillosis is perhaps the most devastating of *Aspergillus*-related diseases, which is associated with high case fatality, exhibiting mortality rates that may exceed 90% in certain severely compromised patient populations (Dagenais and Keller 2009).

Aspergillus fumigatus is one of the most common pathogens, causing 90% of all Aspergillus infections (Denning 1998). The conidia can more effectively adhere to the epithelium of the respiratory tract, due to the negative charge of the conidia (Wasylnka and Moore 2000; Wasylnka et al. 2001; Kwon-Chung and Sugui 2013). The fungus's ability to produce a range of lytic enzymes and toxins that cause necrotic effects on cells also contributes to its pathogenic importance (Adam et al. 2018; Strømland et al. 2021). *Aspergillus flavus* is another clinically important species of the genus *Aspergillus*, the second most common cause of aspergillosis in certain geographic regions, especially in tropical and subtropical climates (Klich 2007; Hedayati et al. 2007).

Exophiala jeanselmei complex and *E. spinier* complex are well-documented human pathogens. Clinical manifestations include mycetoma (especially for *E. jeanselmei* complex), localized cutaneous infections, subcutaneous cysts, endocarditis and cerebral and disseminated infections. Phaeohyphomycosis caused by *Exophiala* species has been reported in both normal and immunosuppressed patients. *Exophiala dermatitidis* has been isolated from plant debris and soil and is a recognised causative agent of mycetoma and phaeohyphomycosis in humans (Zeng et al. 2007; Kirchhoff et al. 2019). *E. spinifera*

has a worldwide distribution and is a recognized causative agent of mycetoma and phaeohyphomycosis in humans. Zeng et al. (2007) presented an overview of the medically important *Exophiala* species.

4.3 Study limitations

Several limitations of this study should be acknowledged. Culture-based analysis was conducted solely on samples from Brno, Czech Republic, as a pilot study to confirm the viability of fungal propagules and to guide subsequent DNA-based screening and sequencing conducted across all sampling sites. Although cultivation results were consistent with the sequencing data, the geographic representativeness of culture-based findings remains limited, and future studies should include cultivation from a broader range of locations. All sampling was performed within a limited time window and does not capture temporal variation in fungal community composition throughout the year. Seasonal fluctuations in temperature, humidity, and outdoor fungal spore loads are known to influence indoor mycobiomes, and longitudinal sampling would be needed to fully characterize these dynamics in passive ventilation systems of residential buildings.

5 Conclusion

Ventilation systems in residential buildings, particularly those employing passive functionality, represent a potential health hazard for occupants. Prolonged neglect and insufficient maintenance of these systems result in the accumulation of microbial and fungal organisms within the infrastructure. Organic and particulate matter entering the system, such as kitchen oil residues, animal hair and fur, and pollen and plant debris from outdoor environments, serve as substrates that support microbial proliferation and active growth. Fungal spores may be transported into living spaces by pressure fluctuations, thereby degrading indoor air quality and contributing to the onset of allergic reactions or asthma. Considering the widespread implementation of passive ventilation systems in households across Central and Eastern Europe, this issue poses a significant risk to millions of homes.

Species identified in our study include allergens, asthma-inducing organisms, and fungi that can cause severe pulmonary diseases in immunocompromised individuals. Genera regarded as critical include *Cladosporium*, *Alternaria*, *Aspergillus*, and *Penicillium*. The cultivation confirmed the viability of the fungal spores. These taxa were frequently detected within the ventilation system microbiome, with the first two genera being nearly ubiquitous. Notably, the taxonomic profile exhibited remarkable similarity across different urban environments, indicating minimal influence of local ecological conditions.

Declarations

Conflicts of Interest

The authors declare no conflicts of interest.

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Author Contribution

Conceptualization: M.V., T.V., N.H. Methodology: M.V., N.H., D.N., J.L., P.L., M.N. Investigation: M.V., S.V., B.D., N.H., D.N. (DNA isolation, sequencing), J.L. (DNA isolation, sequencing), D.P. (sampling, ventilation system cleaning), L.P. (sampling, ventilation system cleaning), P.L. (physical measurements, airflow), M.N. (physical measurements, airflow). Formal analysis: S.V., B.D., N.H. Funding acquisition: T.V. Project administration: T.V. Supervision: M.V., T.V. Writing – original draft: M.V., S.V. Writing – review & editing: M.V., T.V., N.H. All authors reviewed the manuscript.

Data Availability

The data that support the findings of this study are openly available in the National Center for Biotechnology Information (NCBI) at https://www.ncbi.nlm.nih.gov/, reference number PRJNA1403450.

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Figures

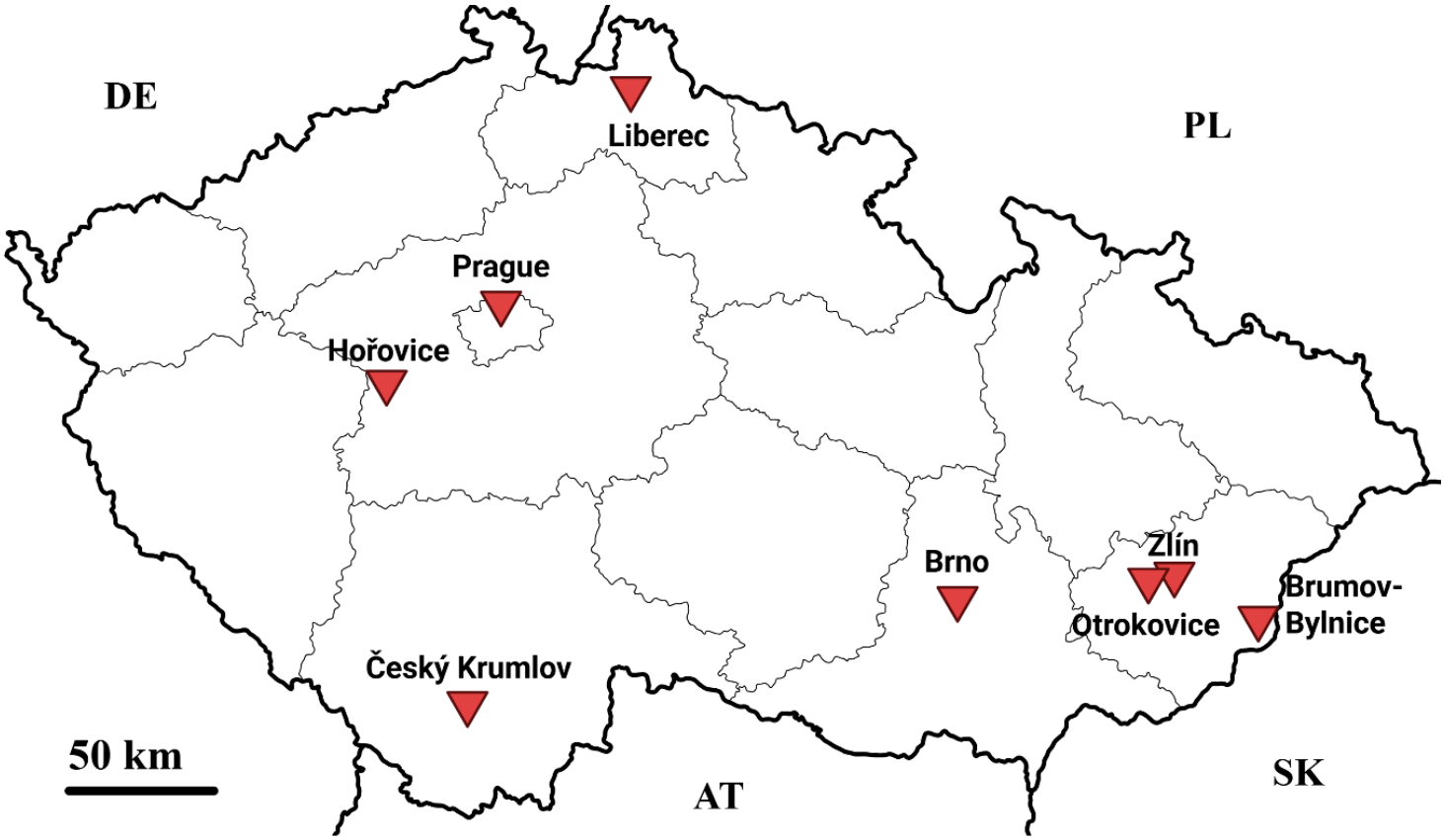


Figure 1

The map of the Czech Republic with the location of sampled cities.

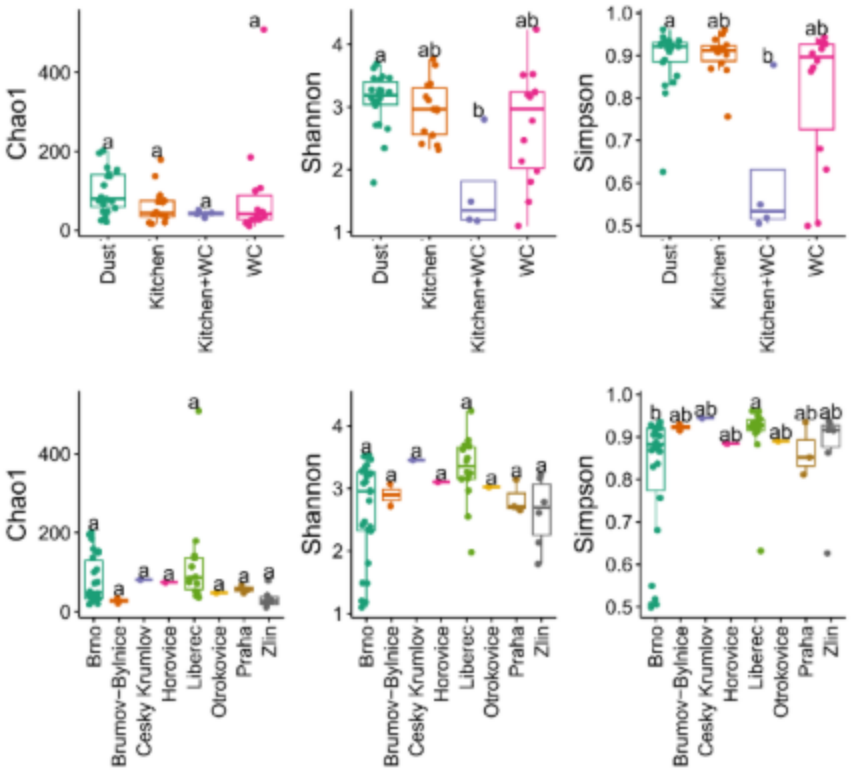


Figure 2

Alpha diversity matrices (Chao1, Shannon and Simpson) of sample groups. Above each box, letters indicate statistically significant differences between groups based on Kruskal-Wallis test followed by Dunn's post-hoc test (KW, $p < 0.05$). Groups sharing the same letter are not significantly different from each other.

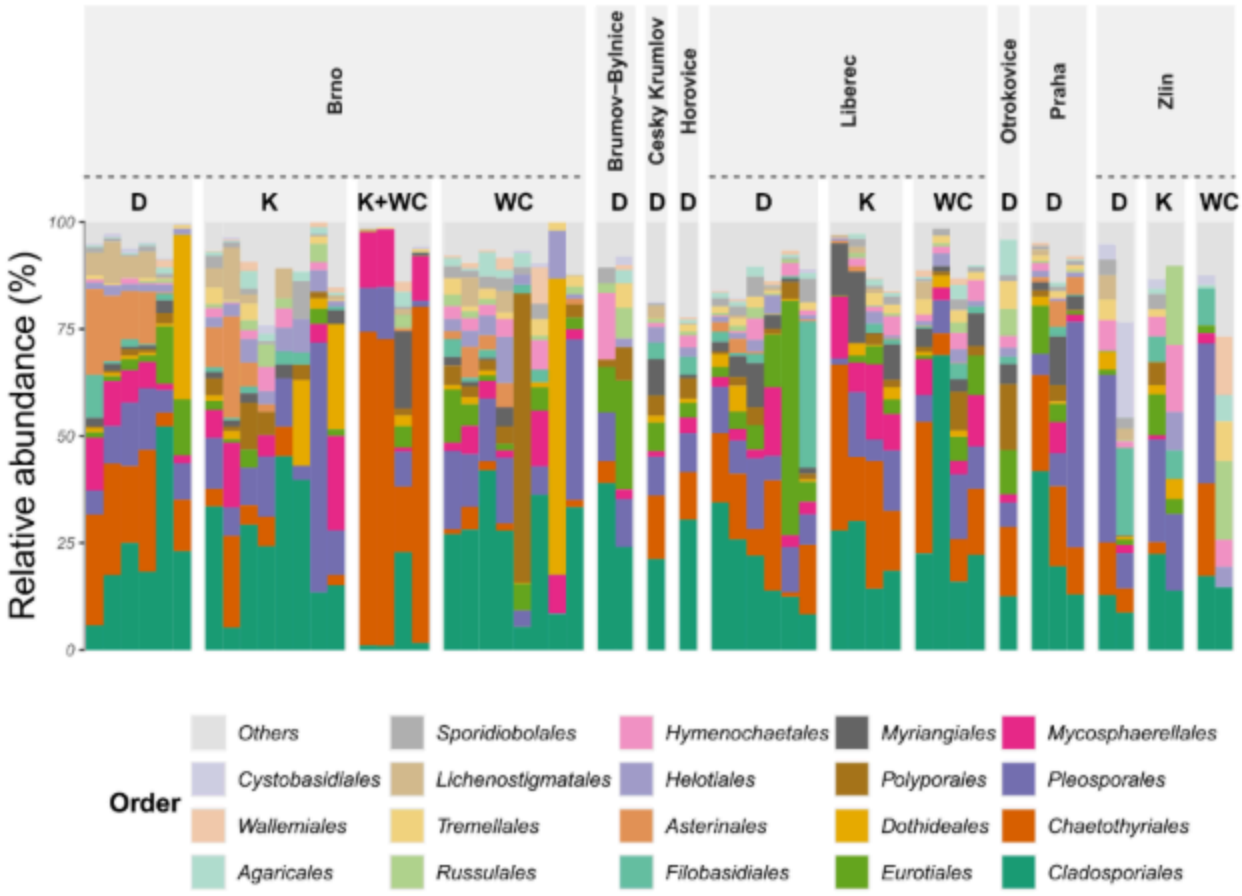


Figure 3

Bar plot displaying the fungal composition at the order taxonomic level. The samples from the cities are divided based on matrix: D - Dust, mixed sample from the duct; K - Kitchen duct swab; WC - WC duct swab; K+WC - swab from the common duct for the kitchen and WC (only Technická street, Brno, Czech Republic).

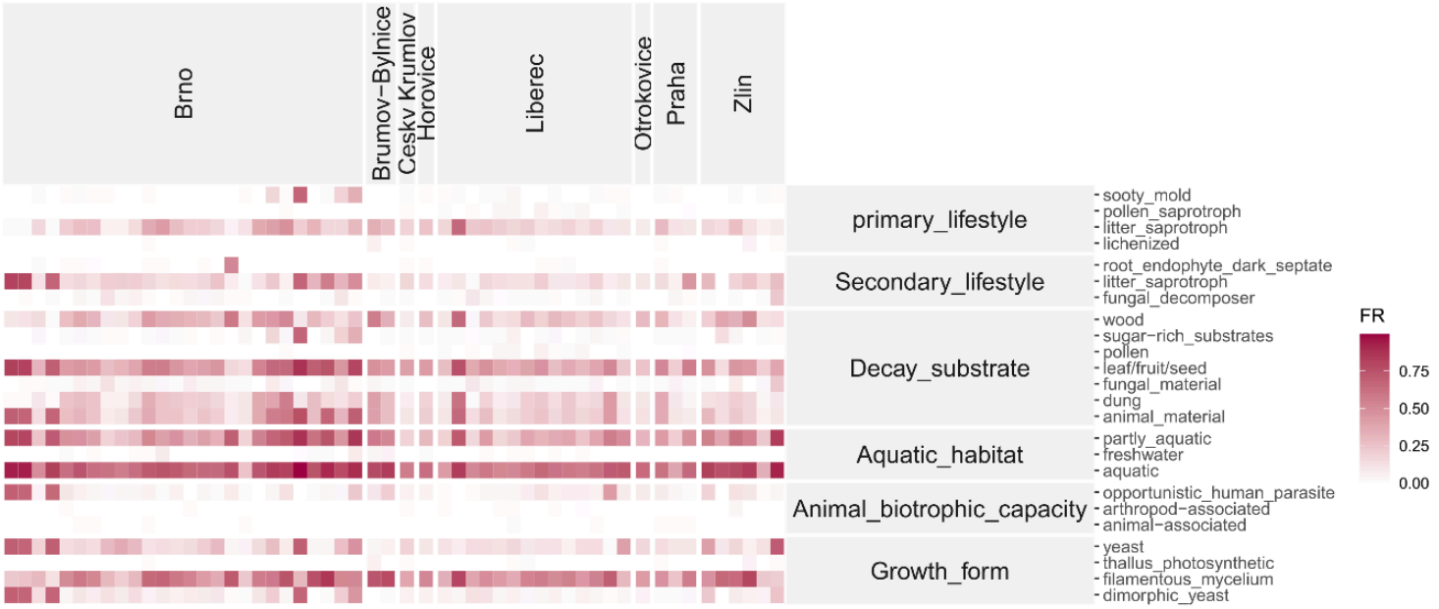
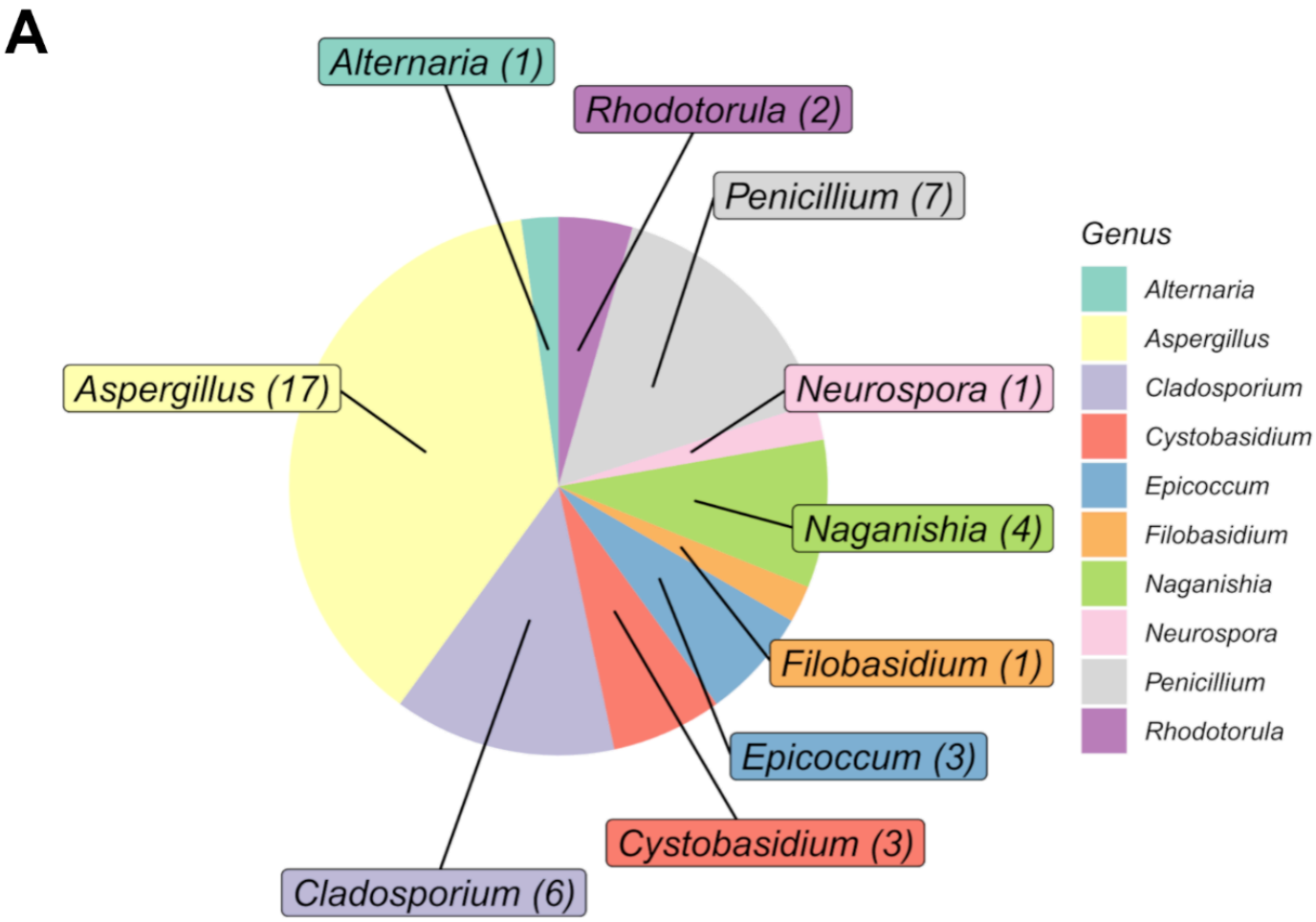


Figure 4

The fungal characteristics as it was predicted by assignment to Fungal Traits database, separated based on the sampled city.



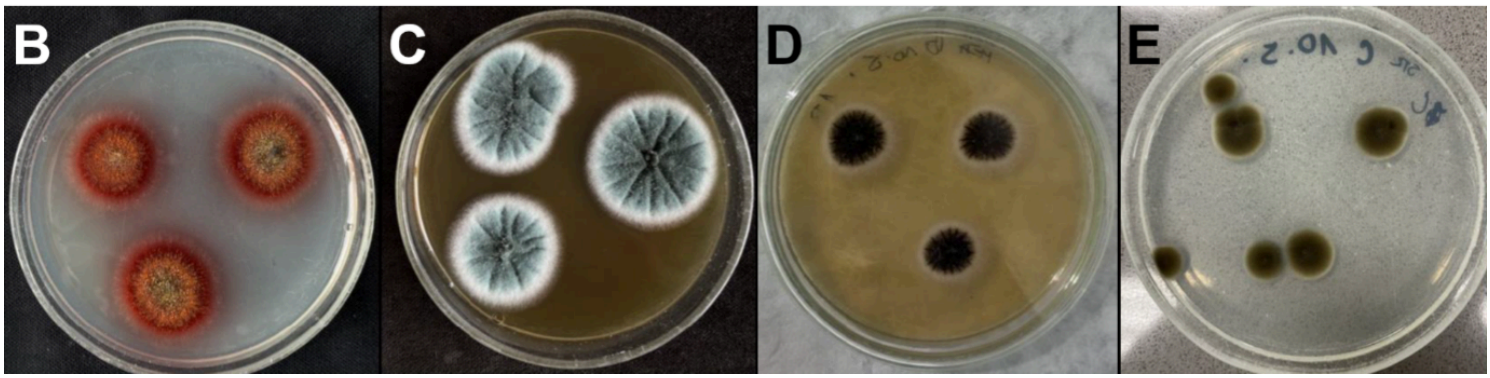


Figure 5

A: Number of isolates obtained from sampling in Brno, Czech Republic, displayed at the genus taxonomic level. B-E: Photos of the cultivated isolates from the swabs. B: *Epicoccum nigrum*, C: *Aspergillus fumigatus*, D: *Aspergillus niger*, E: *Cladosporium halotolerans*

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