

Profile and distribution characteristics of culturable airborne fungi in residential homes with children in Beijing, China

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Abstract

A survey was conducted to evaluate the characteristics of airborne fungi in 31 residential homes in Beijing that housed children. Indoor fungal concentrations in the homes ranged from 62 to 3498 colony forming units (CFU)/m³ (mean 837 CFU/m³). A total of 225 fungal isolates belonging to 24 genera and 65 species were identified in the air. The most common fungi were *Penicillium*, *Cladosporium*, *Aspergillus*, and *Alternaria* based on concentration proportion and frequency. Approximately 40% of the total number of isolated fungal species belonged to *Penicillium*, which represented the maximum proportion of the total fungal concentration at ~32%, followed by *Cladosporium*, *Aspergillus*, non-sporing fungi, *Monilia*, and *Alternaria*. The most frequently occurring fungal species were *Cladosporium cladosporioides* (8.44%), *Penicillium chrysogenum* (5.33%) and *Penicillium funiculosum* (4.89%). Fungal concentrations in residential homes with a male child were significantly higher than those with a female child (** $P < 0.01$). The highest fungal concentration was detected in summer, followed by spring and autumn, and the lowest was in winter (** $P < 0.01$). These results provide a database of airborne fungi exposure in Beijing homes, and suggest that the gender of children in residences has a significant influence on both the concentration and composition of airborne fungi.

Keywords

Penicillium, Residential home, Bioaerosol, Gender, Indoor air quality

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Introduction

The growing health problems experienced by home dwellers exposed to various indoor air contaminants are a worldwide social concern, because people today spend most of their time confined indoors.¹ Airborne fungi such as moulds and yeasts are ubiquitous in indoor and outdoor environments, and nearly 10% of the world's human population has fungal allergies.^{2,3} Fungi and their by-products are significant causes of adverse effects on human health such as respiratory disorders, hypersensitivity pneumonitis, and toxic reactions.^{4,5} More than 80 genera of fungi have been associated with symptoms of respiratory tract allergies, and over 100 species have been implicated in serious human

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and animal infections.⁶ Fungal organisms such as *Alternaria*, *Aspergillus*, *Penicillium*, and *Cladosporium* spp. are responsible for an array of respiratory conditions, from allergic rhinitis to asthma.⁷ *Aspergillus fumigatus* may be responsible for 90% of all invasive aspergillosis, and *Aspergillus niger* can also cause a range of invasive pulmonary diseases.⁸

The adverse health effects of the fungi have been studied globally and many studies have assessed the presence of airborne fungi in indoor environments.^{9–19} The impact of different fungal species in indoor environments is a cause of concern among the scientific community. However, the level of exposure to fungal contamination and its implications on human health needs to be identified. There is a need to provide baseline information about the abundance, composition, and distribution of fungal communities in the atmosphere and their possible impacts on human health that will be helpful for the decision makers. The collection of detailed information about airborne fungi around the globe is needed to provide more firsthand data. In this regard, the US Environmental Protection Agency conducted a five-year Building Assessment Survey and Evaluation to characterize the determinants of indoor air quality, including the dispersal of airborne pathogens such as bacteria and fungi.²⁰ The German Federal Environmental Office also initiated a research project to determine background concentrations of indoor mould, to standardize measurement procedures.²¹ However, little is known about the characteristics of airborne fungi in the indoor environments of China.

Typically, people spend more than 90% of their time in indoor environments such as homes, schools, offices, shopping malls, etc. These areas pose an ongoing risk of exposure to airborne fungi. Due to their age and greater susceptibility to environmental contaminants, children may be more likely than adults to suffer from the potential health hazards of airborne fungi²²; the rate of infections is age dependent and children under three years old are at greatest risk.²³ Activities of children are concentrated mainly in residential homes, given their low action abilities. There is therefore a particularly acute need for studies of airborne fungi in residential environments where children reside. Our objective was to evaluate the abundance and composition of culturable airborne fungi in Beijing residential households that include children. The results of this study provide not only a database about the exposure of children to airborne fungi in residential homes, but also a framework of reference for future research on airborne fungi in other environments.

Materials and methods

Selection of sampling sites

Beijing is China's political and cultural centre, with a population of ~19.6 million permanent residents at the end of 2010 (main data bulletin of the Sixth National Census, 2010). The city is situated in a warm temperate zone with a territorially monsoon climate.

Field sampling of indoor bioaerosols was conducted in 31 residential homes (16 with a male child versus 15 with a female child) selected by the following criteria: presence of only one child (male or female) aged 1–10 years, no visible mould, no smell of mould, and no previous or present moisture damage evident based on homeowner's information. Households were selected from different areas of Beijing City: three apartments were located in the east, six in the south, four in the west, 14 in the north, and four in the centre. The average age of children was 5.2 y, and the means within the five areas were E=4 y, S=5 y, W=6 y, N=5 y, and C=6 y. The largest apartment sampled had an area of 125 m², and the smallest was 40 m². The air temperature in the study sites during sampling time ranged from 9.2°C to 28.6°C, due to heating systems in winter. The relative humidity in the air at the study sites ranged from 32% to 61%.

Description of the air sampler

An FA-1 sampler (an imitation of the Andersen sampler, made by the Applied Technical Institute of Liaoyang, China) was used to collect culturable airborne fungi inside the selected homes. The sampler was sterilized in a hot oven at 180°C for 2 h, and the heating happened 24 h before each sampling time. Samplers washed with 5% bleach and 70% ethanol solution before each measurement in the sampling site. The sampler contained six stages: each stage had a plate with 400 holes of uniform diameter through which air was drawn at 1.7 m³/h and allowed to contact Petri dishes containing agar. Airborne particles were thus separated into six size fractions with diameters of 7.0 µm (stage 1), 4.7–7.0 µm (stage 2), 3.3–4.7 µm (stage 3), 2.1–3.3 µm (stage 4), 1.1–2.1 µm (stage 5), and 0.65–1.1 µm (stage 6).

Sampling methods

Indoor air sampling was performed at the centre of each home's living room to collect a representative sample from 31 selected apartments, between December 2009 and November 2010. Sampling device was operated at a sampling flow rate of 1.7 m³/h and

was stationed on a platform at a height of 1.5–2 m from the apartment wall. The indoor samplers were oriented vertically relative to the ground. Residents remained at home during air sampling and performed their normal activities, and heating, ventilating and Air-conditioning (HVAC) was allowed to run normally in summer (air conditioning) and winter (heating). Air samples were collected in each indoor site for 3 min in triplicate, three times per day, and this continued for three consecutive days of each of the four seasons in a year. Sampling was carried out during spring, summer, autumn, and winter, respectively. At each sampling time, the FA-1 sampler was loaded with 9.0 cm Petri dishes containing Sabouraud agar, with chloramphenicol to inhibit bacterial growth. Exposed culture dishes were incubated for 72 h at 25°C.²⁴

Counting fungal samples

After incubation, the colony forming units (CFU) on each plate were counted, and the concentration of the samples was expressed as CFU per cubic metre of air (CFU/m³). Due to the unavoidable superposition of colonies when microbial particles impact the same location on the plate through the same sieve pore, calculation of the number of colonies was adjusted using equation (1), and this was expressed as CFU/m³ via calculation of equation (2)²⁵

$$P_r = N \left(\frac{1}{N} + \frac{1}{N-1} + \frac{1}{N-2} + \cdots + \frac{1}{N-r+1} \right) \quad (1)$$

$$C(\text{CFU}/\text{m}^3) = \frac{(P_1 + P_2 + P_3 + P_4 + P_5 + P_6) \times 1000}{t(\text{min}) \times F(L/\text{min})} \quad (2)$$

where P_r is the revised colony number in every stage, N is the number of sieve pores in every stage of the sampler, r is the number of colonies sampled at that time point, C is the airborne fungal concentration, P_1 through P_6 are the revised number of colonies in every stage in the sampler, t is the duration of sampling time, and F is the rate of air flow in the sampler during sampling.

Fungal identification

After incubation and counting, the fungal colonies growing on each dish were identified microscopically to the genus level based on the morphology of observed hyphae, conidia, and sporangia. Fungal colonies subcultured onto malt extract agar that had not developed sporulating structures after 14 days were described as 'non-sporulating isolates'. The fungal isolates were then identified further using the molecular method described below. Each pure isolate was homogenized in liquid

culture medium, and DNA was extracted using the cetyl trimethyl ammonium bromide method.²⁶ The internal transcribed spacer (ITS) region of the fungal rRNA genes was amplified using the following universal primer set: ITS₁ (5'-TCCGTAGGTGAACCTGCGG-3') and ITS₄ (5'-TCCTCCGCTTATTGATATGC-3').^{27,28} The reaction mixture (50 µl) consisted of 0.3 µl Taq polymerase, 2 µl dNTP, 5 µl 10 × polymerase chain reaction (PCR) buffer, 2 µl of each primer, and 1.0 µl (ca. 10 ng DNA) of template. The amplification programme was as follows: initial denaturation at 94°C for 5 min, 30 cycles of 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 30 s, followed by a final extension at 72°C for 10 min. The PCR products were detected using electrophoresis on a 1% agarose gel. The sequences were obtained by the Beijing Genomics Institute, China and were analysed with the BLAST programme of the National Center for Biotechnology Information, USA (<http://www.ncbi.nlm.nih.gov/Blast.cgi>). Sequences showing the highest similarity to those of the clones were extracted from GenBank.

Statistical analysis

Fungal concentrations in the atmosphere vary significantly at different sampling sites among residential homes. Therefore, three measures of the central tendency of fungal concentration were calculated (mean, median, and geometric mean) using SPSS Version 19.0, to obtain the broadest picture of the results. The proportions of fungal samples represented by different genera were expressed as percentages, calculated using Microsoft Office Excel 2010. ANOVA was used to compare fungal concentrations among different sampling sites and times.

Results

Identity of fungal groups

Description of fungal taxa. A total of 225 fungal isolates belonging to 24 genera and 65 species of culturable airborne fungi were identified from 31 residential homes in Beijing (Table 1). Approximately 40% (26 species) of the total number of species belonged to the genus *Penicillium*. A further eight species of *Aspergillus* (12.3%), six species of *Cladosporium* (9.2%), two species of *Alternaria* (3.1%), and five species of *Phoma* (7.7%) were identified in the fungal samples. The most frequently occurring fungal species in residential homes were *Cladosporium cladosporioides* (8.4%), *Penicillium chrysogenum* (5.3%), *Penicillium funiculosum* (4.9%), *Cladosporium sphaerospermum* (4.0%), *Aspergillus versicolor* (3.6%), and *Alternaria tenuissima*

Table 1. The types, number of isolates, and percentages of airborne fungal genera identified in family homes in Beijing.

Fungal groups	Number of isolates	Percentage (%)
<i>Alternaria</i>	12	5.33
<i>Alternaria alternata</i>	3	1.33
<i>Alternaria tenuissima</i>	8	3.56
<i>Alternaria</i> sp.	1	0.44
<i>Aspergillus</i>	21	9.33
<i>Aspergillus flavus</i>	2	0.89
<i>Aspergillus fumigatus</i>	3	1.33
<i>Aspergillus nidulans</i>	1	0.44
<i>Aspergillus ochraceus</i>	1	0.44
<i>Aspergillus sydowii</i>	4	1.78
<i>Aspergillus unguis</i>	1	0.44
<i>Aspergillus versicolor</i>	8	3.56
<i>Aspergillus westerdijkiae</i>	1	0.44
<i>Beauveria</i>	2	0.89
<i>Beauveria bassiana</i>	2	0.89
<i>Chaetomium</i>	1	0.44
<i>Chaetomium globosum</i>	1	0.44
<i>Cladosporium</i>	40	17.78
<i>Cladosporium cladosporioide</i>	19	8.44
<i>Cladosporium cucumerinum</i>	3	1.33
<i>Cladosporium</i> sp. F42	1	0.44
<i>Cladosporium</i> sp. H1	2	0.89
<i>Cladosporium sphaerospermum</i>	9	4.00
<i>Cladosporium tenuissimum</i>	6	2.67
<i>Cochliobolus</i>	2	0.89
<i>Cochliobolus lunatus</i>	1	0.44
<i>Cochliobolus</i> sp.	1	0.44
<i>Epicoccum</i>	1	0.44
<i>Epicoccum nigrum</i>	1	0.44
<i>Eurotium</i>	3	1.33
<i>Eurotium herbariorum</i>	1	0.44
<i>Eurotium repens</i>	1	0.44
<i>Eurotium rubrum</i>	1	0.44
<i>Fusarium</i>	2	0.89
<i>Fusarium</i> sp.	2	0.89
<i>Lecanicillium</i>	5	2.22
<i>Lecanicillium psalliotae</i>	5	2.22
<i>Leptosphaerulina</i>	1	0.44
<i>Leptosphaerulina chartarum</i>	1	0.44
<i>Neosartorya</i>	1	0.44
<i>Neosartorya hiratsukae</i>	1	0.44
<i>Penicillium</i>	81	36.0
<i>Penicillium adametzii</i>	1	0.44
<i>Penicillium adametzioides</i>	1	0.44
<i>Penicillium brevicompactum</i>	3	1.33

(continued)

Table 1. Continued

Fungal groups	Number of isolates	Percentage (%)
<i>Penicillium chrysogenum</i>	12	5.33
<i>Penicillium cinnamopurpureum</i>	1	0.44
<i>Penicillium citrinum</i>	2	0.89
<i>Penicillium commune</i>	3	1.33
<i>Penicillium crustosum</i>	2	0.89
<i>Penicillium digitatum</i>	7	3.11
<i>Penicillium expansum</i>	2	0.89
<i>Penicillium funiculosum</i>	11	4.89
<i>Penicillium glabrum</i>	2	0.89
<i>Penicillium italicum</i>	1	0.44
<i>Penicillium marneffei</i>	1	0.44
<i>Penicillium olsonii</i>	1	0.44
<i>Penicillium oxalicum</i>	2	0.89
<i>Penicillium pinophilum</i>	5	2.22
<i>Penicillium polonicum</i>	4	1.78
<i>Penicillium purpurogenum</i>	3	1.33
<i>Penicillium radicum</i>	1	0.44
<i>Penicillium skrjabinii</i>	1	0.44
<i>Penicillium spinulosum</i>	1	0.44
<i>Penicillium toxicarium</i>	1	0.44
<i>Penicillium verruculosum</i>	2	0.89
<i>Penicillium vinaceum</i>	1	0.44
<i>Penicillium viridicatum</i>	3	1.33
<i>Penicillium</i> sp.	7	3.11
<i>Peyronellaea</i>	3	1.33
<i>Peyronellaea glomerata</i>	3	1.33
<i>Phoma</i>	8	3.56
<i>Phoma eupyrena</i>	1	0.44
<i>Phoma exigua</i>	1	0.44
<i>Phoma glomerata</i>	1	0.44
<i>Phoma herbarum</i>	1	0.44
<i>Phoma medicaginis</i>	4	1.78
<i>Pleosporales</i>	2	0.89
<i>Pleosporales</i> sp.	2	0.89
<i>Schizophyllum</i>	1	0.44
<i>Schizophyllum commune</i>	1	0.44
<i>Scolecobasidium</i>	1	0.44
<i>Scolecobasidium humicola</i>	1	0.44
<i>Talaromyces</i>	3	1.33
<i>Talaromyces flavus</i>	3	1.33
<i>Tilletiopsis</i>	2	0.89
<i>Tilletiopsis albescens</i>	2	0.89
<i>Trametes</i>	1	0.44
<i>Trametes ochracea</i>	1	0.44

(continued)

Table 1. Continued

Fungal groups	Number of isolates	Percentage (%)
<i>Tritirachium</i>	1	0.44
<i>Tritirachium</i> sp.	1	0.44
<i>Ulocladium</i>	2	0.89
<i>Ulocladium multiforme</i>	1	0.44
<i>Ulocladium obovoideum</i>	1	0.44
<i>Zygomycete</i>	1	0.44
<i>Zygomycete</i> sp.	1	0.44
No identification	28	12.44
No identification	28	12.44

(3.6%), followed by *Penicillium digitatum* (3.1%), *Cladosporium tenuissimum* (2.7%), *Penicillium pinophilum* (2.2%). Numerous other fungal species were detected less frequently (Table 1).

Table 2 demonstrates the concentration proportion and frequency of culturable airborne fungi in residential homes. The frequencies of *Penicillium*, *Cladosporium*, *Aspergillus*, and *Alternaria* were 95.7%, 89.4%, 62.6%, and 45.6%, respectively. The frequencies of the other fungal genera, except for the unidentified group, varied from 0.6% to 36.8%. The fungal concentration proportion of *Penicillium* was 32.2%, followed by *Cladosporium* (22.7%), *Aspergillus* (7.7%), non-sporing fungi (3.1%), *Monilia* (2.0%),

Table 2. Frequency and concentration proportion of culturable fungi in family homes with a male and female child in Beijing.

Fungal groups	Male child		Female child		Total	
	Frequency (%)	Concentration percentage (%)	Frequency (%)	Concentration percentage (%)	Frequency (%)	Concentration percentage (%)
<i>Alternaria</i>	44.90	1.88	46.15	2.27	45.6	1.91
<i>Aspergillus</i>	58.50	7.42	65.38	8.44	62.6	7.74
<i>Cladosporium</i>	92.52	22.68	87.36	24.32	89.4	22.67
<i>Lecanicillium</i>	14.29	1.34	19.78	1.99	16.7	1.55
<i>Monilia</i>	36.73	2.27	36.26	1.64	36.8	2.02
No identification	93.20	24.23	96.70	25.66	94.2	24.27
No sporing	24.49	2.76	17.58	2.60	21.0	3.14
<i>Penicillium</i>	93.88	32.96	97.25	28.67	95.7	32.23
<i>Phoma</i>	19.05	0.68	20.88	0.64	20.1	0.70
<i>Saccharomyce</i>	8.84	0.53	6.04	0.34	7.6	0.37
<i>Talaromyces</i>	3.40	0.06	4.40	0.18	4.0	0.13
<i>Ulocladium</i>	0.68	0.02	0.55	0.01	0.6	0.02
<i>Bipolaris</i>	4.76	0.14	4.40	0.21	4.6	0.16
<i>Fusarium</i>	4.76	0.08	3.30	0.09	4.3	0.08
<i>Beauveria</i>	4.08	0.66	5.49	0.25	4.9	0.60
<i>Eurotium</i>	8.16	0.54	6.04	0.15	7.3	0.23
<i>Mucor</i>	1.36	0.04	0.55	0.01	0.9	0.03
<i>Cochliobolus</i>	2.72	0.04	4.95	0.09	4.0	0.08
<i>Epicoccum</i>	4.08	0.10	1.65	0.02	2.7	0.05
<i>Trametes</i>	0.00	0.00	2.75	0.11	1.5	0.08
<i>Pleosporales</i>	0.00	0.04	0.55	0.01	0.6	0.03
<i>Chaetomium</i>	1.36	0.01	2.20	0.08	1.8	0.04
<i>Neosartorya</i>	1.36	0.05	2.75	0.22	2.1	0.10
<i>Zygomycete</i>	2.04	0.07	0.55	0.02	1.2	0.03
<i>Tilletiopsis</i>	6.12	0.27	2.75	0.15	4.3	0.23
<i>Peyronellaea</i>	0.00	0.00	1.10	0.05	0.6	0.02
<i>Schizophyllum</i>	6.80	1.02	10.99	1.74	9.4	1.17
<i>Tritirachium</i>	1.36	0.13	0.55	0.02	0.9	0.08

Table 3. Seasonal variation pattern of culturable airborne fungal frequency and concentration proportion in family homes with children in Beijing.

Fungal groups	Spring		Summer		Autumn		Winter	
	Frequency (%)	Concentration percentage (%)	Frequency (%)	Concentration percentage (%)	Frequency (%)	Concentration percentage (%)	Frequency (%)	Concentration percentage (%)
<i>Alternaria</i>	65.43	2.62	77.78	4.24	46.97	1.94	14.29	0.69
<i>Aspergillus</i>	46.91	4.16	92.06	18.43	65.15	6.11	55.46	6.10
<i>Cladosporium</i>	91.36	32.13	90.48	25.48	98.48	24.70	83.19	16.15
<i>Lecanicillium</i>	14.81	1.07	26.98	1.66	24.24	3.93	10.92	0.91
<i>Monilia</i>	25.93	0.85	82.54	3.88	28.79	1.22	23.53	2.00
No identification	100.00	26.19	96.83	30.31	96.97	26.91	89.92	20.38
No sporing	6.17	0.62	0.00	0.00	0.00	0.00	52.94	6.97
<i>Penicillium</i>	97.53	29.96	92.06	12.77	98.48	24.04	94.96	44.08
<i>Phoma</i>	27.16	0.90	31.75	0.60	22.73	0.71	7.56	0.50
<i>Saccharomyce</i>	3.70	0.10	1.59	0.01	1.52	0.08	15.97	1.05
<i>Talaromyces</i>	6.17	0.26	4.76	0.13	6.06	0.16	0.84	0.02
<i>Ulocladium</i>	0.00	0.00	0.00	0.00	1.52	0.04	0.84	0.02
<i>Bipolaris</i>	0.00	0.00	3.17	0.04	3.03	0.07	9.24	0.43
<i>Fusarium</i>	7.41	0.16	4.76	0.09	4.55	0.10	0.84	0.03
<i>Beauveria</i>	1.23	0.06	9.52	0.23	9.09	1.64	2.52	0.12
<i>Eurotium</i>	4.94	0.46	20.63	0.79	3.03	0.15	3.36	0.08
<i>Mucor</i>	0.00	0.00	1.59	0.05	0.00	0.00	1.68	0.05
<i>Cochliobolus</i>	1.23	0.02	9.52	0.10	6.06	0.18	1.68	0.03
<i>Epicoccum</i>	2.47	0.05	4.76	0.04	3.03	0.05	1.68	0.08
<i>Trametes</i>	0.00	0.00	0.00	0.00	4.55	0.18	1.68	0.06
<i>Pleosporales</i>	0.00	0.00	0.00	0.00	0.00	0.00	1.68	0.07
<i>Chaetomium</i>	1.23	0.01	3.17	0.03	3.03	0.13	0.84	0.04
<i>Neosartorya</i>	0.00	0.00	1.59	0.04	7.58	0.58	0.84	0.06
<i>Zygomycete</i>	0.00	0.00	3.17	0.13	1.52	0.03	0.84	0.03
<i>Tilletiopsis</i>	6.17	0.38	11.11	0.54	0.00	0.00	1.68	0.02
<i>Peyronellaea</i>	0.00	0.00	1.59	0.04	0.00	0.00	0.84	0.06
<i>Schizophyllum</i>	0.00	0.00	6.35	0.08	39.39	7.00	0.00	0.00
<i>Tritirachium</i>	0.00	0.00	3.17	0.31	1.52	0.05	1.68	0.00

and *Alternaria* (1.9%). Their concentration proportion of these taxa varied from 1.9% to 32.2% and represented ~93% of the total fungal colonies.

Effect of child gender on fungi found in homes

A total of 23 and 26 genera of airborne fungi were identified in residences that housed a male and a female child, respectively (Table 2). The frequencies of *Penicillium*, *Cladosporium*, *Aspergillus*, *Alternaria*, and *Monilia* in residential homes with a male child were 93.9%, 92.5%, 58.5%, 44.9%, and 36.7%, respectively, whereas those in residential homes with a female child were 97.3%, 87.4%, 65.4%, 46.2%, and 36.3%, respectively. The concentration proportions of *Penicillium*, *Cladosporium*, *Aspergillus*, *Monilia*, and *Alternaria* in residential homes with a male child were

33.0%, 22.7%, 7.4%, 2.3%, and 1.9%, respectively, whereas those in residential homes with a female child were 28.7%, 24.3%, 8.4%, 1.6%, and 2.3%, respectively. The concentration proportion of *Penicillium* was significantly higher in residential homes that had a male child (* $P < 0.05$), whereas that of *Cladosporium* was significantly higher in homes with a female child (* $P < 0.05$).

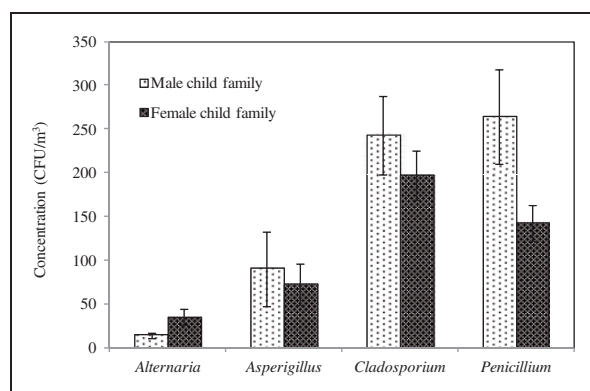
Fungi identified in different seasons

Table 3 demonstrates the concentration proportion and frequency of culturable airborne fungi in residential homes across different seasons. In spring, summer and autumn, *Cladosporium* was shown to be the dominant fungal species, with the maximum fungal concentration proportion and accounted for 32.1%, 25.5%, 24.7%, respectively. *Penicillium* was the

Table 4. Concentration data on total airborne fungi in family homes with children in Beijing (CFU/m³).

Family homes	Mean	Median	Geomean	Minimal level	Maximal level
Male child family	906a	668	607	63	3498
Female child family	764b	572	542	62	2644
General	837	633	561	62	3498

The significance level is 0.01.

**Figure 1.** The most common airborne fungi in family homes with a male and female child in Beijing.

second most concentrated group isolated from the samples, followed by *Aspergillus* and *Alternaria*. Their concentration proportion varied from 1.9% to 30.0%. In winter, *Penicillium* contributed 44.1% of the maximum fungal concentration, followed by *Cladosporium*, *Aspergillus*, and *Monilia*. The highest and lowest concentration proportion of *Penicillium* were observed in winter and summer ($*P < 0.05$), respectively, whereas those of *Cladosporium* were found in spring and winter, respectively ($*P < 0.05$).

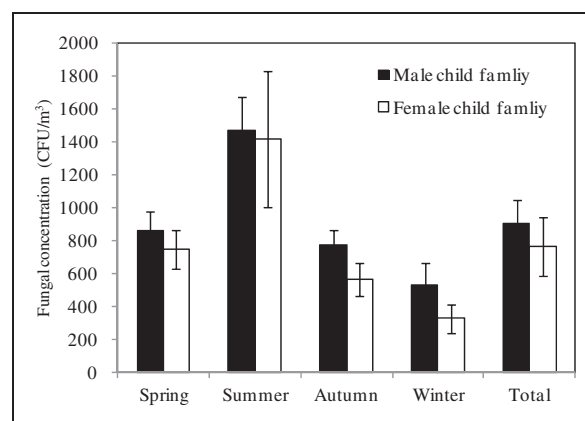
Patterns of fungal concentrations

Overall concentration

The concentration of culturable fungi in homes ranged from 62 to 3498 CFU/m³, with a mean of 837 and a median of 633 CFU/m³ (Table 4).

Effect of child gender on fungal concentration

Significantly higher fungal concentrations were found in homes with a male child ($**P < 0.01$). The mean concentrations were 906 CFU/m³ (range 63–3498) in homes with a male child and 764 CFU/m³ (range 62–2644) in those with a female child. The concentrations of *Penicillium*, *Cladosporium* and *Aspergillus* in households with male children were much higher than those

**Figure 2.** Seasonal variation pattern of total culturable fungal concentration in family homes with children in Beijing.

female children ($**P < 0.01$), whereas the reverse was true for concentrations of *Alternaria* ($**P < 0.01$) (Figure 1).

Fungal concentrations in different seasons

The total fungal concentrations found in homes differed significantly among seasons of the year. Concentrations peaked in summer (June–August), followed by spring (March–May) and autumn (from September to November), and they were lowest in winter (December–February) ($**P < 0.01$) (Figure 2).

The same seasonal patterns of variation were observed in both *Alternaria* and *Aspergillus* concentrations in homes over the course of the year. The highest concentration of *Alternaria* was recorded in summer, in homes with a male (41 CFU/m³) or female child (118 CFU/m³), whereas the lowest *Alternaria* concentration occurred in winter, in homes with a male (slightly >2 CFU/m³) or a female child (<1 CFU/m³). Similarly, the highest *Aspergillus* concentrations in summer were approximately 458 and 236 CFU/m³ in homes with a male or female child, respectively, and the lowest *Aspergillus* concentration in winter was slightly >8 and <11 CFU/m³ in homes with a male and female child, respectively. *Cladosporium* concentrations were highest in summer (378 CFU/m³), followed by autumn and winter, and lowest in spring (177 CFU/m³)

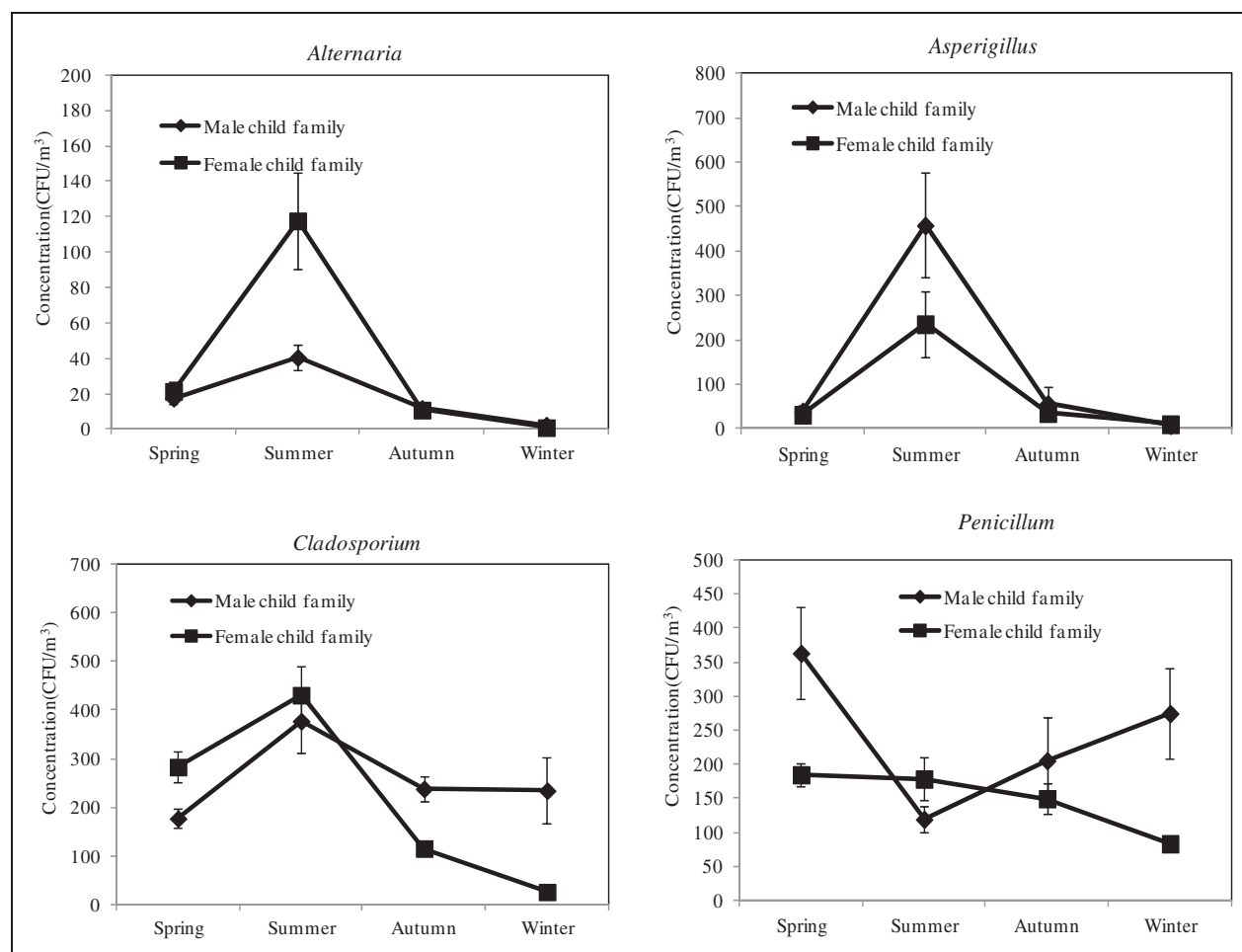


Figure 3. Seasonal variation pattern of the most common fungal concentration in family homes with children in Beijing. (a) *Alternaria*, (b) *Aspergillus*, (c) *Cladosporium*, and (d) *Penicillium*.

in homes with a male child. In homes with a female child, the concentration was also the highest in summer (431 CFU/m³), followed by spring and autumn, and lowest in winter (27 CFU/m³). However, different seasonal variations in *Penicillium* concentration were observed between the two types of home. The highest and lowest concentrations of *Penicillium* were recorded in spring (363 CFU/m³) and summer (119 CFU/m³) in homes with a male child, whereas no seasonal difference in *Penicillium* concentration was observed in homes with a female child (Figure 3).

Discussion

Understanding the composition of airborne fungi assemblages in indoor and outdoor environments is essential for risk assessments related to human health, since fungi taxa cause different adverse health effects. For example, *Mucor* and *Rhizopus* cause high rates of infection, fungi such as *Penicillium*, *Alternaria alternata*, and *Cladosporium herbarum* are likely to cause

allergies, and types like *Stachybotrys*, *Trichoderma*, *Fusarium*, and *Aspergillus flavus* can produce mycotoxins that are harmful to humans.^{29–31} In this study, a total of 24 genera including 65 species of culturable airborne fungi were detected, and this is one of the highest reported numbers of fungi genera and species identified in studies of indoor air.^{32–34} The most common fungal groups recovered were *Penicillium* (32.2%), *Cladosporium* (22.7%), *Aspergillus* (7.7%), non-sporing fungi (3.1%), and *Monilia* (2.0%). Comparing with results of other published studies demonstrates that fungal composition varies among different indoor environments. In Austrian apartments with and without visible mould growth, the two most frequent genera were *Penicillium* (53%) and *Aspergillus* (28%), respectively.³⁵ In the clinical units of a French hospital, *Penicillium* and *Aspergillus* were the two most frequently found fungi, followed by *Bjerkandera*, *Alternaria*, and *Cladosporium*.³⁶ In the indoor air of a Russian underground train station, *Acremonium*, *Aspergillus*, *Cladosporium*, and *Penicillium* were the

prevailing genera.¹¹ However, compared with indoor environments, the most dominant fungi in some Beijing outdoor environments were *Cladosporium* (36.6–43.0%), *Alternaria* (11.7–14.5%), *Penicillium* (11.0–15.5%), and *Aspergillus* (6.5–8.8%).²⁴ We found that *Aspergillus* and *Penicillium* were more common in indoor air than has been reported for outdoor air, whereas *Alternaria* and *Cladosporium* were mainly found outdoors, consistent with other reports.^{32,33} The elevated indoor concentrations of *Aspergillus* and *Penicillium* could be due to potential indoor sources such as plants, soil and planting activity, whereas the outdoor levels of *Alternaria* and *Cladosporium* that penetrated into the apartment interiors could be significant enough to outweigh the strength of indoor sources.³⁶ All of these common airborne fungi have been reported as dominant fungal genera in air environments studied in various countries.^{6,29,33,36}

Our study analysed in detail the relationship between indoor fungal concentrations and the factors that may influence the composition and relative abundance of species assemblages, such as apartment features, sampling locations, seasons and the gender of children living in households. Interestingly, fungal concentrations in the air were affected by child gender, and significantly higher concentrations were detected in homes with a male child versus those with a female child ($**P < 0.01$). Our results also demonstrated significant differences in the fungal concentration proportion and frequency between homes with a different gender of their child. The concentration proportion of *Penicillium* was significantly higher in homes with a male child ($*P < 0.05$), whereas those of *Cladosporium* were higher in homes with a female child ($*P < 0.05$). Generally, females are less likely to take vigorous exercise than their male counterparts, and girls took part in 6% less vigorous playtime activity than boys. Females were reported as almost half as physically active as males well before they reach their teens (Girls less sporty 'from early age', BBC News, 25 July 2001, 23:46 GMT 00:46 UK). Moreover, parents in China tend to be overprotective of their children because of the one-child family policy, and child activities are heavily cantered in residential homes. Residential homes with female children tend to be more clean and orderly. All of these reasons could influence the higher fungal concentration in residential homes with a male child, along with the gender-related difference in fungal compositions. Additionally, elevated concentrations of fungi in homes might result from outdoor sources that enter the home (e.g. from family outdoor activities, or open windows doors and vents), from sources within the home resulting from high moisture conditions (cooking, bathing, poor ventilation), or from the disturbance of settled dust (indoor activities)

– all these potential sources require further investigation.

Weather conditions are another important factor that strongly affects variation in patterns of assembly of airborne fungi in indoor environments. Indeed, we found that fungal concentrations were highest in summer, followed by spring and autumn, and lowest in winter ($**P < 0.01$) in Beijing homes. This pattern of variation is consistent with what has been found in outdoor environments in Beijing,²⁴ and it can be attributed to the strong relationship between indoor and outdoor airborne fungal communities, since outdoor air is the major source of indoor fungi.^{36–38} First, most fungal spores in the air are thought to come from vegetation rather than from soil. In summer, phylloplanes (i.e. leaf surfaces) provide more habitats for microorganisms due to the vigorous vegetation growing in Beijing's residential district, enabling the growth of several saprophytic and parasitic fungi.³⁹ This increased growth could result in higher fungal concentrations outside of dwellings, and fungi outdoors might penetrate into household interiors via the wind or air conditioning systems. Second, summer air temperature and moisture levels in the microenvironment of buildings are conducive to the germination, growth, and propagation of airborne fungi. In contrast, the outdoor plants do not thrive in Beijing winters, so fewer substrates are available for fungal growth at that time of year. Moreover, the lower air temperatures and moisture levels in winter can limit fungal growth.

Further analysis showed that different types of airborne fungi displayed different patterns of seasonal variation. The highest indoor concentrations of *Alternaria* and *Cladosporium* occurred in summer. The results support the study of Buzina et al.,⁴⁰ who reported that the dematiaceous genera *Alternaria* and *Cladosporium* were found mainly between May and November. Additionally, the pigmented spores of *Cladosporium* and other dematiaceous fungi, which are ubiquitous on rotted organic material, dominate during the warm growing season of plants.⁴¹ However, *Penicillium* is found more frequently in outdoor air during spring, autumn and winter; sources of it are scarcer in summer, due to the extremes of temperature and humidity, and the fungicidal effect of ultraviolet (UV) light.³⁴ Therefore, low indoor concentrations of *Penicillium* were detected during summer in this study. Reiss reported that the thin-walled colourless spores of *Aspergillus* are quickly eliminated by sunlight's UV radiation, so low concentrations should be observed in summer.⁴² In contrast, we found high *Aspergillus* concentrations in summer, suggesting that other factors may be influencing fungal assemblages. Our results provide valuable, fundamental data on

airborne fungal concentrations and species assemblages in indoor environments. They will also augment the scientific basis for prevention of microbial pollution, improvement of indoor environmental quality and reduction of health risks due to air contaminants, especially for infants and children in China.

Authors' contribution

ZF and ZO designed the research; QT and CG performed the experimental study; PL and LS performed the data analysis; XW performed the statistical analysis of all data, and ZF wrote the paper.

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