

Title: Assemblages of culturable airborne fungi in a typical urban, tourism-driven center of southeast China

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1 **Abstract:** Understanding the prevalence of airborne fungi in a city or region is
2 important for ecological diagnosis and specific treatment of allergic manifestations
3 induced by inhalation of fungal allergens. The present study was conducted to assess
4 characteristics and variation of culturable airborne fungi at four selected sampling
5 sites in Hangzhou, southeast China. Results showed that concentration of culturable
6 fungi in the air ranged from <12 colony forming units (CFU)/m³ to 8767 CFU/m³
7 with mean of 848 CFU/m³. We identified a total of 352 fungal isolates from multiple
8 sampling sites and across seasons, which were distributed across 21 genera and 85
9 species of fungi. *Penicillium*, *Cladosporium*, *Alternaria*, *Aspergillus*, and
10 *Trichoderma* were the most predominant fungi based on their frequency and
11 concentration percentage, and fungal composition differed from site to site and from
12 season to season. Approximately 36.5% of the total number of isolated fungal species
13 belonged to *Penicillium*, which also represented the maximum proportion of the total
14 fungal concentration at about 29.6%. The fungal species with higher frequency in
15 Hangzhou were *P. chrysogenum* (7.7%), *C. cladosporioides* (6.3%), *Alternaria*
16 *alternata* (5.6%), *P. funiculosum* (4.3%), *Aspergillus sydowii* (4.0%). Moreover, there
17 was significant variation between sampling sites, with higher fungal concentrations
18 detected at Zhejiang Gongshang University Jiaogong Campus (ZJGSUJC) and
19 Breeze-ruffled Lotus at Quyuan Garden (BLQG), followed by Yan'an Road Business
20 Street (YRBS), while the lowest concentrations ($P < 0.05$) were found at Tianmushan
21 and Jiaogong Cross Road (TJCR). Furthermore, different patterns of seasonal
22 variation in fungal concentrations were found at different sampling sites, and the

1 mean fungal concentration at all four sites was lowest during the winter, while there
2 was no difference among summer, autumn, and spring. Our results can provide a
3 baseline for studying airborne culturable fungi in southeast China, and will enable
4 evaluation of the risks to human health from exposure to the atmosphere.

5 **Keywords:** *Penicillium*; *Cladosporium*; airborne fungi; species composition;
6 concentration distribution

7 **1. Introduction**

8 Bioaerosols, well known normal components in the air, contain microorganisms and
9 their components such as fungi, bacteria, endotoxin, mycotoxins, and allergens (Kim
10 et al., 2018). Undoubtedly, airborne fungi constitute a significant and dominant part of
11 global bioaerosols and belong to the coarse fraction of air particulate matter (Sesartic
12 and Dallafior, 2011). On one hand, airborne fungi has a potential role in regulating
13 atmospheric chemistry and modulating climate by acting as ice and cloud
14 condensation nuclei (Ariya et al., 2009; Fröhlich-Nowoisky et al., 2009; Sesartic et al.,
15 2013). On the other hand, fungi in the air can damage building materials in humid
16 conditions, and they can also invade and infect living organisms (Eduard, 2009;
17 Salonen et al., 2015). Furthermore, fungi and their by-products are significant causes
18 of adverse effects on human health such as respiratory disorders, hypersensitivity
19 pneumonitis, and toxic reactions (Gorny et al., 2002; Fracchia et al., 2006), and
20 exposure to fungi may lead to allergic sensitization and symptoms of allergies and
21 asthma (Stark et al., 2005; Park et al., 2006). Generally, more than 80 genera of fungi
22 had been associated with symptoms of respiratory tract allergies, and over 100 species

1 had been implicated in serious human and animal infections (Horner, 2004).

2 The adverse health effects of fungi have been studied globally, and many studies
3 have assessed the presence of airborne fungi in different environments (Shelton et al.,
4 2002; Adhikari et al. 2004; Fang et al., 2005; Zuraimi et al., 2009; Wang et al., 2011;
5 Salonen et al., 2015; Li et al., 2015). These results have significantly enriched our
6 baseline knowledge of fungal characteristics in the atmosphere, and supported many
7 applications related to public health and international security. However, such studies
8 face significant challenges, as the high number of factors that can influence fungi
9 (such as seasonal effects, local climate, weather patterns, and human activities) might
10 lead to enormous differences in the community of airborne fungi found in different
11 regions (Jones and Harrison, 2004; Kalyoncu, 2010). Therefore, it is necessary to
12 collect detailed information about airborne fungi from different environments with
13 typical characteristics, and to better understand fungal distributions.

14 Hangzhou, the capital and largest city of Zhejiang Province in China, has a
15 subtropical monsoon climate with four distinctive seasons, a warm winter and hot
16 summer, and abundant precipitation. Impressively, Hangzhou has one of the most
17 popular attractions in southeastern China, West Lake, and is also regarded as one of
18 the most desirable cities to inhabit in China... After hosting the G20 summit in 2016,
19 Hangzhou is attracting more and more tourist, and the number of tourists in Hangzhou
20 has increased consistently. Since there is still little known about the characteristics,
21 concentration, and distribution of airborne fungi in a typical
22 tourist city of southeastern China, we chose Hangzhou as a model location for

1 measuring airborne culturable fungi. The main objective of the study was to describe
2 the group and concentration variation pattern of airborne culturable fungi in
3 Hangzhou in a detailed, systemic manner.

4 **2. Materials and methods**

5 2.1 Description of sampling sites

6 Hangzhou, a world-famous city in southeast China, was selected as the location for
7 airborne fungal sampling in this study. Four typical sampling sites were selected for
8 this study based on their urban function (Fang et al., 2005, 2007): (1) Tianmushan and
9 Jiaogong Cross Road (TJCR), a heavily trafficked intersection located in Xihu district
10 about 3 km from the city center; (2) Zhejiang Gongshang University Jiaogong
11 Campus (ZJGSUJC), a cultural and educational area situated in Xihu district about 4
12 km from the city center; (3) Yan'an Road Business Street (YRBS), a commercial area
13 and business district located at the center of Hangzhou City and in Xiacheng district;
14 and (4) Breeze-ruffled Lotus at Quyuan Garden (BLQG), a scenic tourist area situated
15 in Xihu district near West Lake, and about 5 km from the city center. Detailed
16 information about these selected sites can be found in **Table 1**.

17 2.2 Sampling design and methods

18 We used an FA-1 sampler (imitated Andersen sampler, fabricated by the Applied
19 Technical Institute of Liaoyang, China) for the collection of culturable airborne fungi
20 (Fang et al., 2005). Each stage of the airborne fungal sampling included a plate with
21 400 holes of uniform diameter, through which air was drawn at 28.3 L min^{-1} before
22 coming into contact with nutrient agar-filled petri dishes. Airborne particles were

1 separated into six fractions: the aerodynamic cut-size diameters of the six stages were
2 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),
3 1.1-2.1 μm (stage 5), and 0.65-1.1 μm (stage 6). The FA-1 sampler was sterilized in a
4 hot air oven at 180 °C for 2 h before each 24 h measurement, and subsequently
5 washed with 5% bleach and 70% ethanol solution at the sampling site prior to next
6 collection.

7 Fungal sampling in the air was conducted at four sampling sites throughout
8 Hangzhou from Jul 2014 to Jun 2015. Sampling devices were operated at a sampling
9 flow rate of 28.3 L min^{-1} , and maintained with a platform at a height of ~1.5 m. Air
10 samples were collected for 3 min in triplicate, three times daily (09:00, 13:00, and
11 17:00 hours) for three consecutive days of each month of the year. For each air
12 sampling event, the FA-1 sampler was loaded with 9.0 cm petri dishes containing
13 Sabouraud agar with chloramphenicol to inhibit bacterial growth. Exposed culture
14 dishes were incubated for 72 h at 25 °C.

15 2.3 Enumeration of fungi

16 After incubation, the fungal colonies were counted, and the concentration of the
17 samples was expressed as CFU per cubic meter of air (CFU/m^3). However, since
18 superposition is unavoidable when the microbial particles impact the same spot
19 through the same sieve pore, the number of colonies was recalculated using Macher's
20 method (Macher, 1989; Fang et al., 2007). Fungal concentration was recorded as <12
21 if total colonies collected with the sampler was less than one.

22 2.5 Fungal identification

1 After incubation and counting, the fungal colonies growing on each dish were
2 identified microscopically to the genus level, based on the morphology of observed
3 hyphae, conidia, and sporangia. Fungal colonies that were subcultured onto malt
4 extract agar but had not developed sporing structures after 14 days were described as
5 “non-sporing isolates.” The fungal isolates were then identified further using the
6 molecular method described below. Each pure isolate was homogenized in liquid
7 culture medium, and DNA was extracted using the cetyl trimethyl ammonium
8 bromide method (Möller et al., 1992). The internal transcribed spacer (ITS) region of
9 the fungal rRNA genes was amplified using the following universal primer set: ITS₁
10 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS₄
11 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990; Wang et al., 2011). The
12 reaction mixture (50 µL) consisted of 0.3 µL Taq polymerase, 2 µL dNTP, 5 µL 10 ×
13 polymerase chain reaction (PCR) buffer, 2 µL of each primer, and 1.0 µL (ca. 10 ng
14 DNA) of template. The amplification program was as follows: initial denaturation at
15 94 °C for 5 min, 30 cycles of 94 °C for 30 s, annealing at 55 °C for 30 s, extension at
16 72 °C for 30 s, followed by a final extension at 72 °C for 10 min. The PCR products
17 were detected using electrophoresis on a 1% agarose gel. The sequences were
18 obtained by the Beijing Genomics Institute, China, and were analyzed with the
19 BLAST program of the National Center for Biotechnology Information, USA
20 (<http://www.ncbi.nlm.nih.gov/Blast.cgi>). Sequences showing the highest similarity to
21 those of the clones were extracted from GenBank.

22 2.5 Statistical analysis

1 All experimental data were analyzed with the software programs Excel 2010 and
2 SPSS Version 19.0 (SPSS. Inc., Standard Version). Descriptive statistics were
3 calculated to summarize fungal concentrations (including mean, median, and
4 geometric mean concentration). The data for airborne fungal concentration were
5 normally distributed; one-way analysis of variance (ANOVA) was used to compare
6 different sampling sites and sampling times, followed by Tukey and Duncan post hoc
7 tests.

8 **3. Results**

9 3.1 Fungal groups and characterization of variation

10 3.1.1 Description of fungal taxa

11 A total of 352 fungal isolates belonging to 21 genera and 85 species of culturable
12 airborne fungi were identified from the four selected sampling sites in Hangzhou
13 (**Fig.1**). The fungal genera that appeared with high frequency were *Penicillium*
14 (31.0 %), *Cladosporium* (13.6%), *Aspergillus* (10.5%), *Alternaria* (6.5%) and
15 *Trichoderma* (6.3%), *Phoma* (4.6%) and *Eurotium* (3.4%), and all these dominant
16 fungal genera accounted for 75.9% of the total isolates. As for fungal species, *P.*
17 *chrysogenum* (7.7%), *C. cladosporioides* (6.3%), *Alternaria alternata* (4.6%), *P.*
18 *funiculosum* (4.3%) and *Aspergillus sydowii* (4.0%) were detected prevalently,
19 followed by *C. tenuissimum* (2.3%), *Alternaria tenuissima* (2.0%) and *Aspergillus*
20 *nidulans* (2.0%) at lower rates. Numerous other fungal species identified were
21 observed less frequently. Moreover, approximately 36.5% (31 species) of the total
22 number of species belonged to the genus *Penicillium*. A further 7 species of

1 *Aspergillus* (8.2%), 6 species of each of *Cladosporium* and *Phoma* (7.1%), and 5
2 species of *Trichoderma* (5.9%) were identified in the fungal samples.

3 3.1.2 Fungal groups among sampling sites

4 **Fig.2** demonstrates the frequency and concentration percentage (%) of culturable
5 airborne fungi at different sampling sites in Hangzhou. Higher frequency of airborne
6 fungi at selected sampling sites was identified as *Penicillium*, *Mycelia sterilia*,
7 *Cladosporium*, *Alternaria* and *Aspergillus*. The maximum concentration percentage of
8 *Penicillium* detected were 32.7%, 31.0%, 28.8%, and 25.9% at the sampling sites
9 TJCR, YRBS, ZJGSUJC, and BLQG, respectively. While the higher concentration
10 percentage of *Penicillium* occurred at TJCR and YRBS, in contrast, the lower
11 concentration percentage of *Alternaria* were also observed at those sites, with 10.0%
12 at TJCR, and 9.6% at YRBS. The lowest concentration percentage of *Cladosporium*
13 was 19.0% observed at TJCR. Finally, higher concentration percentage of *Aspergillus*
14 were found at TJCR (8.1%) and YRBS (8.6%) than at ZJGSUJC (6.7%) and BLQG
15 (7.6%).

16 3.1.3 Fungal groups across seasons

17 **Fig.3** demonstrates the frequency and concentration percentage of culturable airborne
18 fungi across different seasons in Hangzhou. Higher frequency of airborne fungi across
19 different seasons was observed as *Penicillium*, *Cladosporium*, *Mycelia sterilia*,
20 *Aspergillus* and *Alternaria*. In winter, the frequency of airborne fungi was much lower
21 than those of other seasons in a year. *Penicillium* had the maximum fungal
22 concentration percentage in all for seasons, accounting for 28.5%, 29.2%, 29.4%, and

1 28.9% in spring, summer, autumn, and winter, respectively. *Cladosporium* was the
2 second most concentrated group isolated from the samples, followed by *Alternaria*,
3 no-sporing isolates, and *Aspergillus*. Their concentration percentage varied from 7.1%
4 to 24.6%. Additionally, a higher concentration percentage of *Cladosporium* was
5 observed in autumn and winter than in spring and summer ($P < 0.05$), while there
6 were no seasonal differences in concentration percentage of *Penicillium* ($P > 0.05$).
7 The highest and lowest concentration percentage of *Alternaria* were observed in
8 winter and spring ($P < 0.05$), respectively, whereas those of *Aspergillus* were higher in
9 spring and summer ($P < 0.05$).

10 3.2 Fungal concentration and variation

11 3.2.1 Overall fungal concentration

12 Fungal concentrations varied greatly among different sampling sites across Hangzhou,
13 ranging from <12 CFU/m³ to 8767 CFU/m³. The mean and median fungal
14 concentrations were approximately 848 ± 193 CFU/m³ and 550 CFU/m³ (**Fig.4**).

15 3.2.2 Spatial variation of fungal concentration

16 Fungal concentrations from the different sampling sites are demonstrated in **Fig.4**.

17 Significantly higher concentrations were measured at ZJGSUJC and BLQG, followed
18 by YRBS, and the lowest concentrations were found at TJCR ($P < 0.05$). No significant

19 differences in fungal concentrations were detected between ZJGSUJC and BLQG

20 ($P > 0.05$). The mean concentrations were as follows: ZJGSUJC (1176 ± 91 CFU/m³),

21 BLQG (939 ± 74 CFU/m), YRBS (719 ± 43 CFU/m³), and TJCR (557 ± 28 CFU/m³).

22 3.2.3 Temporal variation of fungal concentration

1 3.2.3.1 Seasonal variation of fungal concentration

2 The mean fungal concentration from all four sites was lowest during the winter (368
3 CFU/m³), with no differences among the other seasons of summer (1003 CFU/m³),
4 autumn (1068 CFU/m³), and spring (952 CFU/m³). Individually, the highest fungal
5 concentrations from TJCR and YRBS were observed in the autumn, followed by
6 spring and summer, and they were the lowest during the winter ($P<0.05$). Similarly,
7 the lowest fungal level was found during the winter at ZJGSUJC and BLQG, but there
8 was no difference in fungal concentration among the other seasons at either location
9 ($P>0.05$) (Fig. 5).

10 3.2.3.2 Monthly variation of fungal concentration

11 The highest fungal concentrations among all four sites combined were observed in
12 Jun (1641 CFU/m³), Jul (1649 CFU/m³), Oct (993 CFU/m³), and Nov (1268 CFU/m³),
13 while the lowest concentrations were in Jan (489 CFU/m³), Feb (357 CFU/m³), Mar
14 (257 CFU/m³), and Apr (342 CFU/m³). When each site was analyzed individually, the
15 fungal concentrations within TJCR were highest from Jun to Jul and from Oct to Dec
16 compared to other months of the year ($P<0.05$); the highest concentration was in Nov
17 (856 CFU/m³) and the lowest was during Mar (241 CFU/m³). For the ZJGSUJC site,
18 higher concentrations were found during Jun (2661 CFU/m³) and Jul (2674 CFU/m³),
19 while the lowest concentrations were in Mar (208 CFU/m³) and Apr (314 CFU/m³).
20 Within BLQG, the fungal concentrations during Jun (2143 CFU/m³) and Jul (2307
21 CFU/m³) were the highest, and Feb (364 CFU/m³) and Mar (260 CFU/m³) were the
22 lowest. Finally, higher fungal concentrations were detected at YRBS during Oct (1113

1 CFU/m³), Dec (1283 CFU/m³) and May (1008 CFU/m³), and the lowest were during
2 Mar (321 CFU/m³) and Apr (331 CFU/m³) (**Fig. 6**).

3 3.2.3.3 Fungal concentration of three time points in a day

4 Fungal concentration of three time points in a day at four selected sampling sites in

5 Hangzhou was demonstrated in Fig.7. Totally, significantly higher fungal

6 concentrations were recorded at 9:00 and 17:00 as compared to 13:00 ($P<0.05$).

7 However, there was no difference in fungal concentrations among times of day at

8 BLQG, while they were lowest at 13:00 ($P<0.05$) at each of the other sites (TJCR,

9 ZJGSUJC, and YRBS) .

10 3.3 Correlation between environmental parameters and fungal concentration

11 **Data from all sampling sites demonstrated that air temperature influenced**

12 **positively ($p<0.01$) the total fungal count and the genera of *Penicillium*,**

13 ***Aspergillus* ($p<0.01$), and significantly positive correlation between relative**

14 **humidity and concentration of total fungi and *Penicillium* was also found**

15 **($p<0.01$). Interestingly, air temperature and relative humidity affected**

16 **significantly and positively the concentration of total fungi, *Penicillium*,**

17 ***Alternaria*, *Aspergillus*, *Cladosporium*, No-sporing at the sampling site of BLQG**

18 **($p<0.01$) (Table 2). 4. Discussion**

19 Airborne fungi are among the most common organisms in nature and they are

20 correlated with adverse health effects of humans and plants (Shelton et al., 2002).

21 *Penicillium*, *Alternaria alternata*, and *Closporium herbarum* are likely to cause

22 allergies, and types like *Stachybotris*, *Trichoderma*, *Fusarium*, and *Aspergillus flavus*

1 can produce mycotoxins that are harmful to humans. *Mucor* and *Rhizopus* can cause
2 high rates of infection (Garrett, et al., 1998; Dillon, et al., 1999; Bush, et al., 2006). In
3 the present study, *Penicillium*, *Cladosporium*, *Aspergillus*, *Alternaria*, and
4 *Trichoderma* were determined as the predominant fungal genera in the atmosphere of
5 Hangzhou, and their relative composition appeared to differ among sampling sites and
6 seasons. Our results were basically in accordance with other reports, such as the
7 finding that *Cladosporium*, *Penicillium*, *Asperigillus* and *Alternaria* spores were
8 constantly present in the Dublin atmosphere (O’Gorman et al., 2008). In the Helwan
9 area of Egypt, *Asperigillus*, *Penicillium*, *Alternaria* and *Cladosporium* were the most
10 predominant airborne fungal genera (Abdel Hameed et al., 2009), and the dominant
11 fungal genera were *Cladosporium*, *Penicillium* and *Aspergillus* in the Austrian state of
12 Styria (Haas et al., 2014). In Turkey’s Manisa, *Cladosporium* was the most dominant
13 fungal genus, followed by *Penicillium*, *Asperigillus* and *Alternaria* (Kalyoncu et al.,
14 2008). From 1993 to 2013 in Sagamihara, the most common fungi were
15 *Cladosporium*, *Alternaria*, *Penicillium*, etc (Saito et al., 2015). Together, those results
16 suggest that the dominant culturable fungal genera in the atmosphere of different
17 regions are almost the same, whereas the fungal percentage in different districts varied
18 tremendously because of great differences in meteorological factors, geographical
19 location, air pollutants, human activity, and fungal growth substrates (Tang, 2009;
20 Abdel Hameed, et al., 2012; Gao et al., 2016; Pyrri, et al., 2017). Previously, we found
21 that *Cladosporium* was the most dominant fungal genus in the atmosphere of Beijing,
22 northern China (Fang et al., 2005). In this study, the most abundant fungi in Hangzhou,

1 southeastern China were *Penicillium*. Definitely, different kinds of fungi have
2 different environmental growth requirements. *Cladosporium* are usually found in
3 higher concentrations in dry regions due to their dry-weather spores, and *Pencillium*
4 have a passive launching mechanism of dry small conidia that can be liberated by
5 even slight air currents or vibrations (Lin et al., 2000; Abdel Hameed, et al., 2009).
6 Beijing, a typical urban center of northern China, is located within an area of
7 semi-humid continental monsoon climate in a warm temperate zone, with an average
8 annual rainfall of about 600 mm. Hangzhou, a typical urban area of southeastern
9 China, is situated in an area of subtropical monsoon climate, with an average annual
10 rainfall of 1100 to 1600 mm. These climate characteristics might be the critical factors
11 driving the higher fungal percentage of *Pencillium* in Hangzhou compared to in
12 Beijing.

13 Vegetation coverage in sampled areas is a critical factor affecting variation
14 pattern of airborne fungi in outdoor environment. Among the four sites that we
15 sampled in Hangzhou, ZJGSUJC and BLQG had significantly higher bacterial
16 concentrations, followed by YRBS, with the lowest concentration found in TJCR
17 ($P<0.05$). These results were in full accordance with our previous studies, which
18 demonstrated that the concentration of airborne fungi in greener areas was
19 significantly higher than that of densely urban and highly trafficked areas (Fang et al.,
20 2005). They also agree with other published findings that show green areas with
21 multiple trees, shrubs, and herbaceous plants have much higher fungal concentrations
22 (Ju et al., 2003), since phylloplanes (i.e. leaf surfaces) can allow for the growth of

1 several saprophytic and parasitic fungi (Picco et al., 2000). Therefore, our present
2 results further support the idea that areas with high vegetation coverage have higher
3 levels of airborne fungi.

4 Weather conditions are another important factor that strongly affects variation in
5 patterns of airborne fungal concentrations in outdoor environments. Indeed, we found
6 that the total fungal concentration from all four sites was lowest during winter, with
7 no difference among the other seasons. However in Beijing, the fungal concentration
8 was higher in summer and autumn, and lower in spring and winter (Fang et al., 2005),
9 which agreed with the study of Haas (2014). On one hand, most fungal spores in the
10 air are thought to come from vegetation rather than from soil, and phylloplanes
11 provide more habitats for fungal growth (Picco et al., 2000). In Beijing, urban plants
12 only flourish in summer and early autumn, while urban plants grow very well in
13 Hangzhou, even during early spring and late autumn. On the other hand, air
14 temperatures during summer and autumn in Beijing are conducive to the germination,
15 growth, and propagation of airborne fungi, while in Hangzhou, all seasons of the year
16 except winter support fungal growth in the atmosphere. That might lead to great
17 differences in seasonal variation patterns of fungi, between urban areas in the
18 southeast versus north of China.

19 Meteorological parameters are other critical factors affecting fungal survivability.
20 In the present study, air temperature influenced positively the concentration of total
21 fungi, *Penicillium*, *Aspergillus*, and relative humidity affected significantly and
22 positively the concentration of total fungi and *Penicillium*. Pyrri et al. (2017) reported

1 that air temperature exerted a consistently strong influence and was the single best
2 predictor of the fungal concentration in the atmosphere. Air temperature influenced
3 positively the total fungal count as well as the genera *Cladosporium*, *Aspergillus* and
4 *Alternaria*, and negatively the genus *Penicillium*, while relative humidity had negative
5 effects to the prevalent genera except *Penicillium* (Pyrri et al., 2017). In the study of
6 Abdel Hameed (2012), air temperature and relative humidity were the most predicted
7 variants for airborne fungi. Air temperature was positively and negatively correlated
8 with *Aspergillus* and *Penicillium*, respectively, while relative humidity was positively
9 correlated with total fungi, *Aspergillus* and *Cladosporium* (Abdel Hameed, et al.,
10 2012). All those results showed that air temperature and relative humidity were the
11 most important meteorological parameters strongly affecting fungal concentration of
12 the atmosphere. Notably, the mean concentration of airborne fungi was lower in
13 Hangzhou (848 CFU/m³) than in Beijing (1163 CFU/m³) (Fang et al., 2005), and a
14 much higher mean bacterial concentration of the atmosphere was also detected in
15 Beijing (2217 CFU/m³) compared to in Hangzhou (292 CFU/m³) (Fang et al., 2007;
16 Fang et al., 2016). These results suggest that microbial concentrations in the air might
17 be much lower in the typical urban landscape of south China compared to those of
18 north China. Firstly, Beijing has a continental monsoon climate with cold dry winters
19 and arid windy springs, which leads to many days of sandy and dusty weather
20 throughout the year. In contrast, Hangzhou's climate is subtropical so it seldom
21 experiences sandy or dusty weather. It was reported that sand and dust near the ground
22 is one of the main sources of airborne microbes (Polymenakou et al., 2008; Chen et al.,

1 2010; Jeon, et al., 2013; Maki, 2014). Secondly, as a whole, urban plants have better
2 annual growth in Hangzhou than in Beijing due to those climate differences, and
3 volatile secretions released by plants can disinfect bacteria in the air (Xie et al., 1999).
4 Thirdly, Hangzhou is one of the typical tourism cities of southeastern China; it is very
5 clean and is often rated as one of the most livable urban areas in the country. All of
6 these factors may directly result in the lower concentrations of airborne microbes in
7 Hangzhou as compared to Beijing.

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Lists of table titles

Table1 Detail information of the four selected sampling sites in Hangzhou

Table2 Correlation between environmental parameters and individual/total fungal species concentration at different sites

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Table1 Detail information of the four selected sampling sites in Hangzhou

Sampling sites	Functional type	Architecture type	Vehicle and personnel flow	Vegetation coverage
TJCR	Heavy traffic intersection	High and low office buildings and hotel around, main traffic road	With about 180 time min ⁻¹ flow of vehicles, and about 30 time min ⁻¹ flow of personnel	Less than 5 percent
ZJGSUJC	Cultural and educational area	Experimental buildings, classrooms, student dormitory and office buildings around	With about few flow of vehicle and about 10 time min ⁻¹ flow of personnel, and about 100 time min ⁻¹ flow of personnel off class	About 50 percent
YRBS	Commercial area and business district	Mall and many shopping buildings around	With 60 time min ⁻¹ flow of vehicles, and 80 time min ⁻¹ flow of personnel	Less than 5 percent
BLQG	Scenic tourist area	No buildings around	With few flow of vehicle and personnel	More than 95 percent

Table2 Correlation between environmental parameters and individual/total fungal species concentration at different sampling sites

Sampling sites	Fungal concentration	Air temperature	Relative humidity
ZJGSUJC	Total fungi	0.330**	0.497*
	<i>Penicillium</i>	0.350**	0.246*
	<i>Alternaria</i>	0.364**	0.156
	<i>Aspergillus</i>	0.355**	0.133
	<i>Cladosporium</i>	0.206	0.202
	No-sporing	0.362**	0.323**
TJCR	Total fungi	0.334**	0.267**
	<i>Penicillium</i>	0.316**	0.225*
	<i>Alternaria</i>	0.222	0.177
	<i>Aspergillus</i>	0.420**	0.335**
	<i>Cladosporium</i>	0.260*	0.238*
	No-sporing	0.220	0.163
YRBS	Total fungi	0.258*	0.277*
	<i>Penicillium</i>	0.265*	0.288**
	<i>Alternaria</i>	0.142	0.210
	<i>Aspergillus</i>	0.304**	0.274*
	<i>Cladosporium</i>	0.114	0.024
	No-sporing	0.271*	0.206
BLQG	Total fungi	0.359**	0.351**
	<i>Penicillium</i>	0.318**	0.370**
	<i>Alternaria</i>	0.328**	0.318**
	<i>Aspergillus</i>	0.312**	0.329**
	<i>Cladosporium</i>	0.253**	0.235*
	No-sporing	0.481**	0.322**

* represents $p < 0.05$ (2-tailed), ** represents $p < 0.01$ (2-tailed).

Lists of figure titles

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Fig.2 Spatial variation of culturable airborne fungal frequency and concentration percentage at four selected sampling sites in Hangzhou

Fig.3 Seasonal variation of culturable airborne fungal frequency and concentration percentage at four selected sampling sites in Hangzhou

Fig.4 Mean concentration of dominant airborne fungi at different sampling sites in Hangzhou

Fig.5 Seasonal variation of airborne fungal concentration at different sampling sites in Hangzhou

Fig.6 Monthly variation of airborne fungal concentration at different sampling sites in Hangzhou

Fig.7 Fungal concentration of three time points in a day at different sampling sites in Hangzhou

Fig.1 The percentage of isolated airborne fungal genera and species identified at the selected sampling sites in Hangzhou

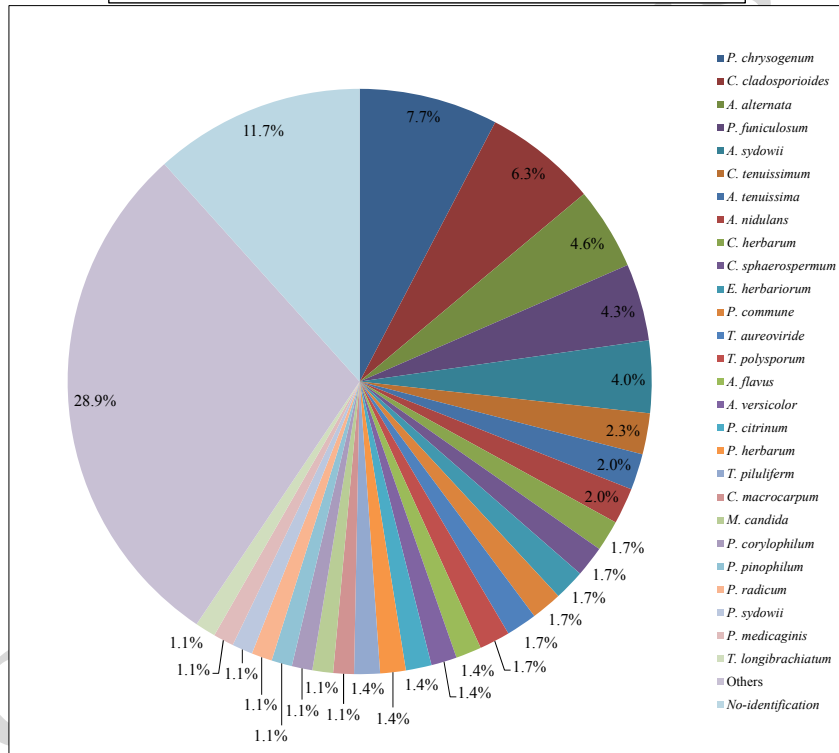
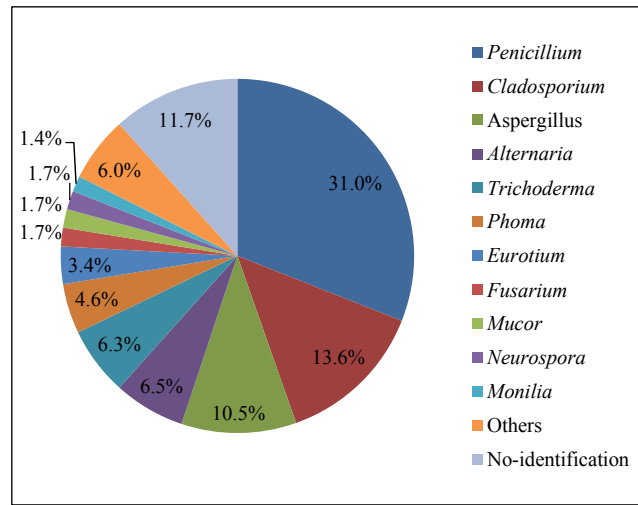
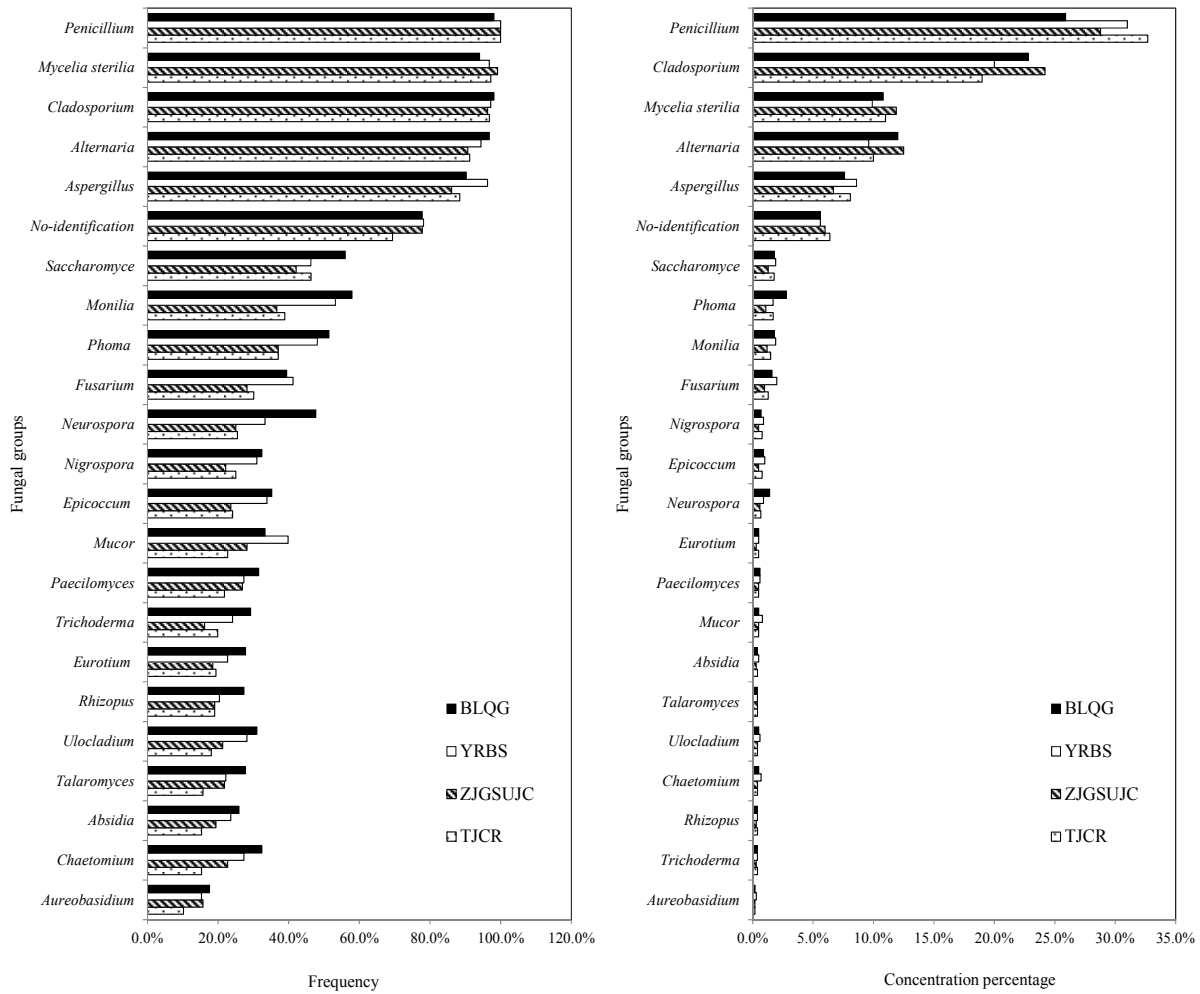


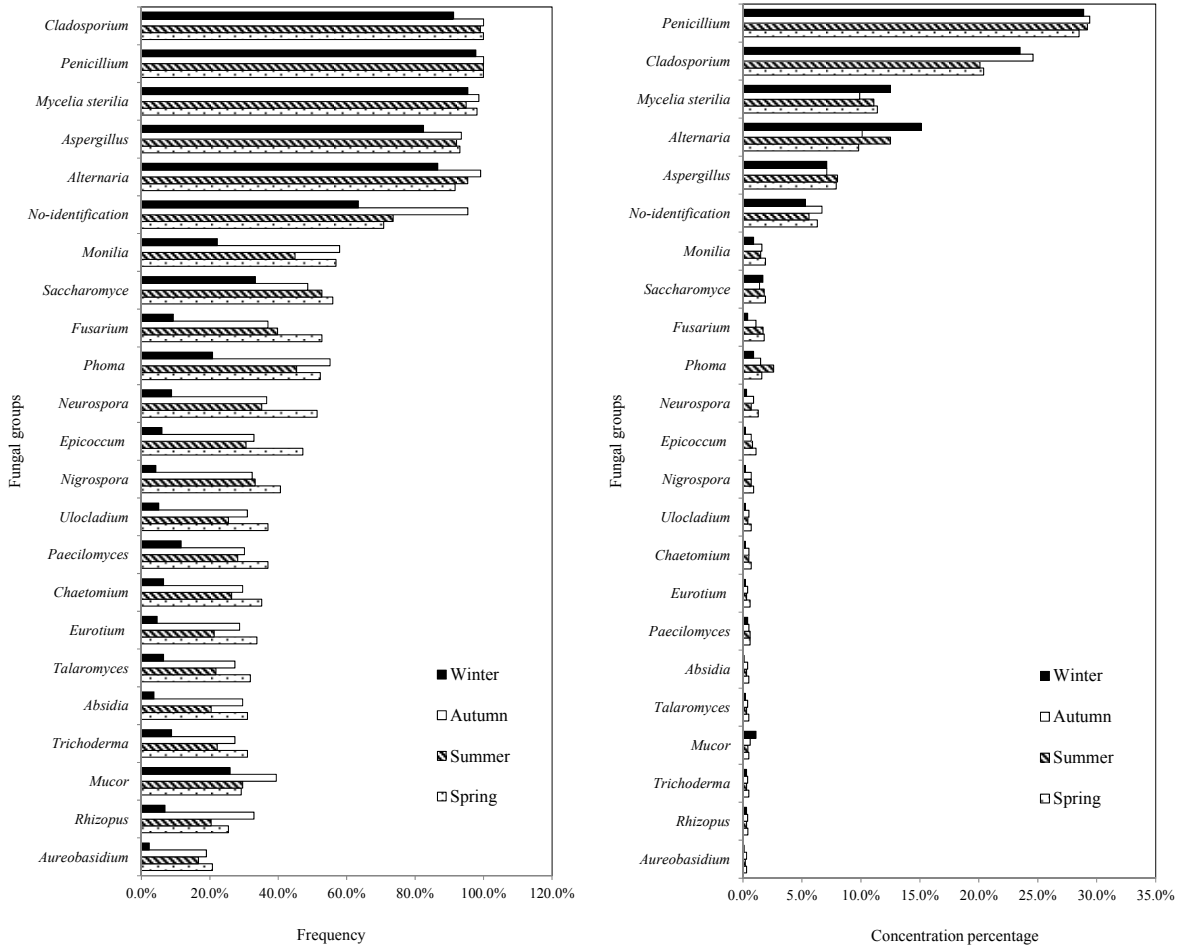
Fig.2 Spatial variation of culturable airborne fungal frequency and concentration percentage at four selected sampling sites in Hangzhou



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Fig.3 Seasonal variation of culturable airborne fungal frequency and concentration

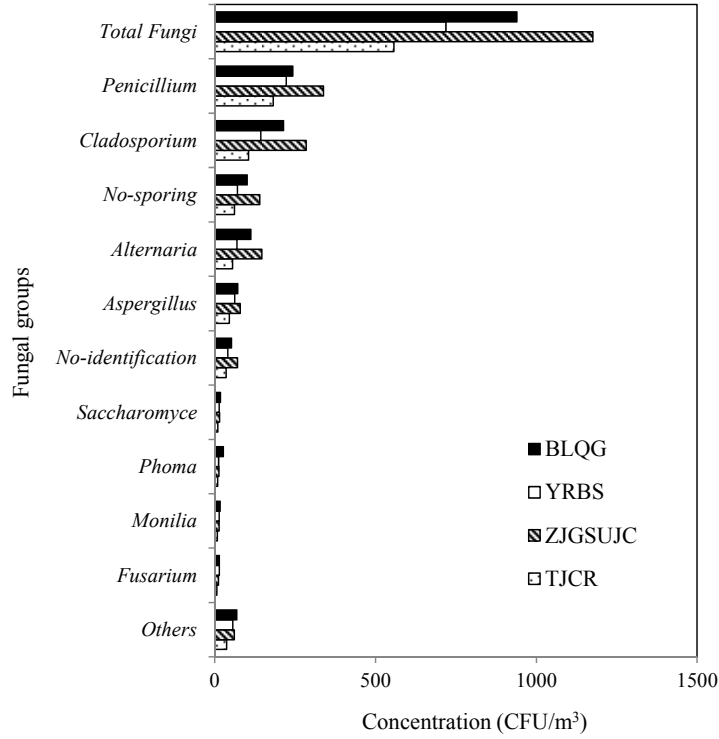
percentage at four selected sampling sites in Hangzhou



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Fig.4 Mean concentration of dominant airborne fungi at different sampling sites in

Hangzhou

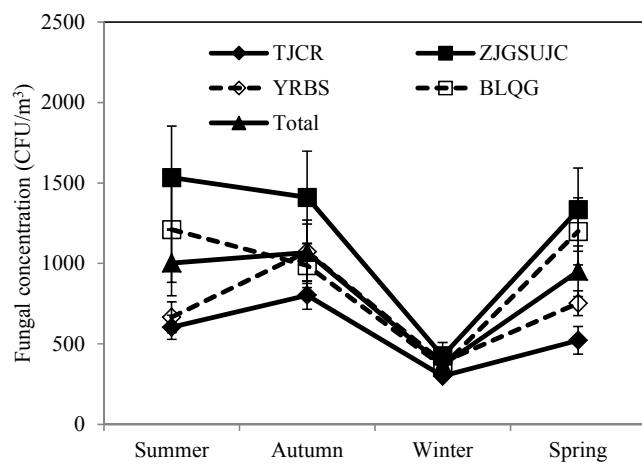


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Fig.5 Seasonal variation of airborne fungal concentration at different sampling sites in

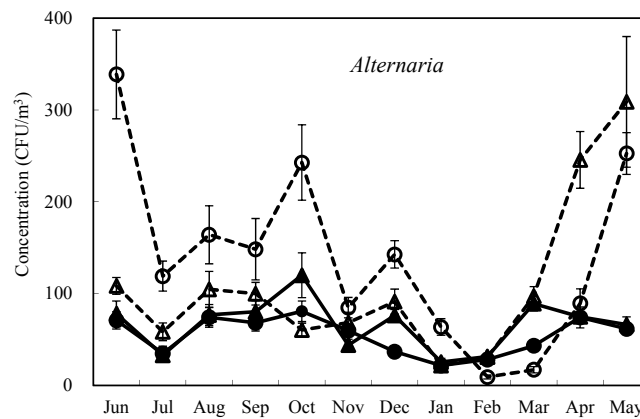
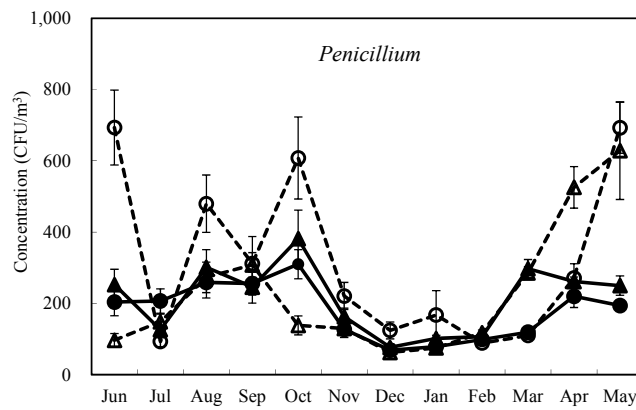
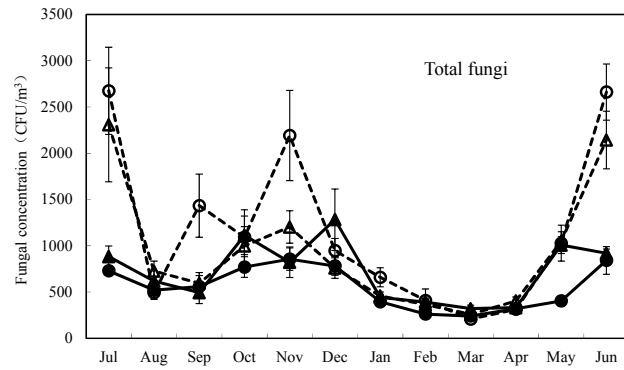
Hangzhou

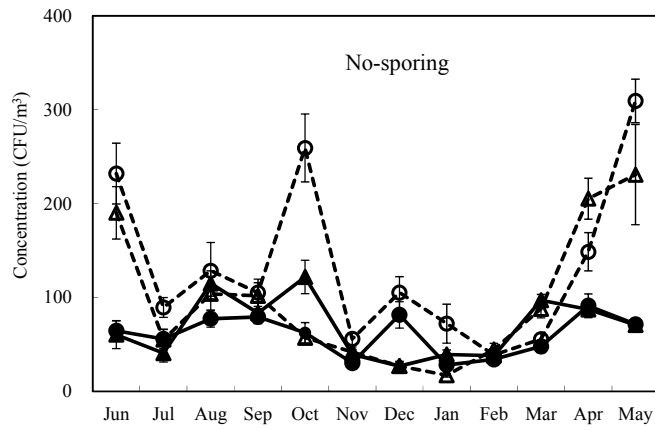
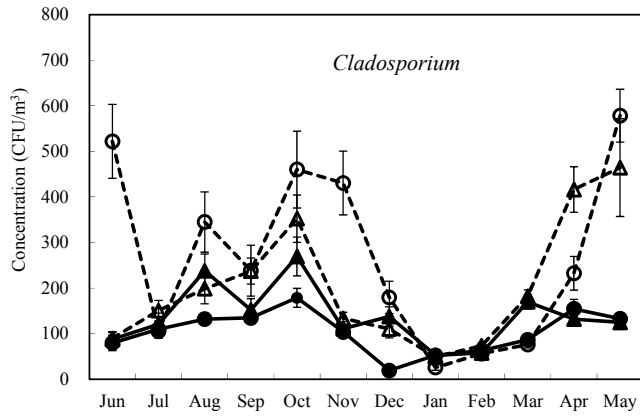
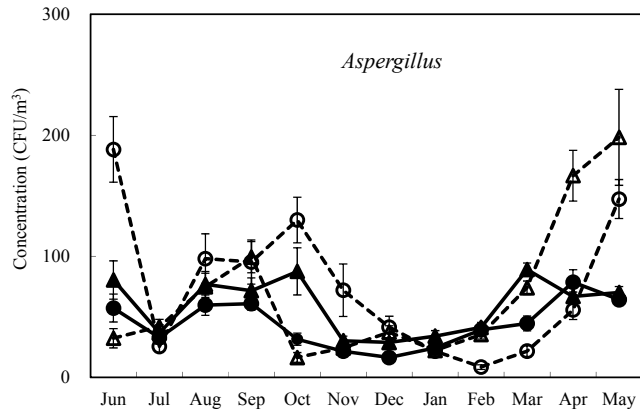


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Fig.6 Monthly variation of airborne fungal concentration at different sampling sites in

Hangzhou





● TJCR ⊙ ZJGSUJC ▲ YRBS ▲ BLQG

Fig.7 Fungal concentration of three time points in a day at different sampling sites in

Hangzhou

