Ambient Fungal Spore Concentration in a Subtropical Metropolis: Temporal Distributions and Meteorological Determinants

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ABSTRACT

Ambient particles comprise approximately 25% of fungal spores, which cause adverse health outcomes such as respiratory diseases, allergy, and infection. In this study, we investigated temporal variations and distributions of ambient fungal spores in an urban area of the Taipei metropolis for over 1 year. A Burkard 7-day volumetric spore trap was used to collect air samples. Samples collected daily were stained, counted, and identified on the basis of morphological characteristics. The associations between fungal spores and environmental parameters were then evaluated through multiple regression analysis. Daily monitoring data revealed a large variation in fungal spore concentrations. Specifically, fungal spores peaked during summer months (June–August) and declined during winter months (December–early March); moreover, the average concentration of total fungal spores was 3,607.97 ± 3,181.81 spores m⁻³. Ascospores were the most prevalent taxon that was recovered from the samples, followed by basidiospores, Aspergillus/Penicillium, and Cladosporium. Multiple regression analysis revealed that meteorological parameters were the main predictors of fungal concentrations. Temperature, wind speed, and humidity were consistently correlated with total fungi and major fungal taxa, and sunlight had a negative association with ascospores. Among the atmospheric pollutants, particulate matter with an aerodynamic diameter ≤ 10 µm (PM₁₀) and ozone were positively associated with fungal spores. Carbon monoxide (CO) at lag day 1 had a negative association with basidiospores. This is the first study to characterize daily concentrations and determinants of ambient fungal spores in an urban area of Taipei metropolis. The obtained data can be used to evaluate the health impact of fungal spore exposure on the residents of the Taipei metropolitan area.

Keywords: Fungal spores; Bioaerosols; Temporal distribution; Air pollution.

INTRODUCTION

Recently, airborne bioparticles have attracted considerable attention because of their potency in causing various adverse health outcomes (e.g., allergic respiratory and skin diseases, asthma, and infection) (Burge and Rogers, 2000; Burge, 2001; Bush and Portnoy, 2001; Atkinson et al., 2006; Harley et al., 2009; Rimac et al., 2010; Knutsen et al., 2012). A quarter of the suspended particles in the ambient environment were reported to be bioparticles (Matthias-Maser and Jaenicke, 2000). One study conducted at two sampling sites (i.e., urban Rinnböckstrasse and suburban Schafberg) in Vienna, Austria indicated that 60% of the detected organic carbon in particulate matter (PM) with aerodynamic diameter ≤ 10 µm (PM₁₀) was contributed by fungal spores (Bauer et al., 2008). This contribution may be influenced by numerous factors, such as meteorological parameters, atmospheric pollutants, land utilization, and geographical location (Lin and Li, 2000; Adhikari et al., 2006; Degobbi et al., 2011; Abdel Hameed et al., 2012; Haas et al., 2013).

Ambient fungal spore distributions have been investigated in many countries. These data provide information on temporal variation (i.e., daily, diurnal, and seasonal variations) (Liao and Luo, 2005; Yamamoto et al., 2012; Fernández-
Rodríguez et al., 2014), spatial variation (Chow et al., 2015), and the predictive land-use regression (LUR) models (Kallawicha et al., 2015). In the United States, the concentrations of ambient fungal spores and other aeroallergens (e.g., grass, weed, and pollen) are provided by the National Allergy Bureau of the American Academy of Allergy, Asthma & Immunology on a daily basis as open access data on the Internet, which can be used as a guideline by the residents in the monitored areas, particularly the susceptible group, for avoiding or minimizing exposure (National Allergy Bureau, 2015).

In Taiwan, the distribution of fungal spores has been reported by several studies conducted in different geographical regions, including Hualien (Ho et al., 2005), Tainan (Wu et al., 2004), and Taipei (Han and Chuang, 1981), which are in the eastern, southern, and northern regions, respectively, of Taiwan. However, the concentrations, sampling time, and study period from each geographical location differed among these studies, and their data are relatively outdated (> 10 years). The most recent data for the Taipei urban area were reported by Chen et al. (2011, 2014), who used data from 2007 from the Sinjhuang monitoring station in New Taipei City. In that study, sampling was conducted only 1 week per month; no daily data were available. Furthermore, the climate change patterns over the past few years may have influenced the temporal distributions of ambient fungal spores.

To investigate understand the current temporal variation in fungal spores and their relationship with other environmental parameters, we monitored daily concentrations of fungal spores in an urban area in the Taipei metropolis for over 1 year at a fixed site-monitoring location.

MATERIALS AND METHODS

Fungal Spore Sampling and Analysis

Daily fungal spore concentration was monitored from April 2013 to March 2014 (except during Chinese New Year) using a Burkard 7-day Recording Volumetric Spore Trap (Burkard Manufacturing, Rickmansworth, England). The sampler was fixed on the rooftop of a 4-story building of Guting Elementary School in the Da’an district of Taipei City. This school is located in an urban area with various commercial and residential buildings, green parks, and heavy traffic, which is characteristic of the study area. The sampler was arranged adjacent to the Environmental Protection Administration (EPA) monitoring station. The sampling location and land utilization around the sampling site are illustrated in Fig. 1.

Airborne fungal spores were collected on Melinex tape that was coated with a thin layer of Lubrisal Stopcock Grease (Thomas Scientific, NJ, USA) and mounted on a

Fig. 1. Sampling site and surrounding major land utilization.
rotating drum inside the sampler. The drum was changed weekly, and the exposed tape was removed and cut into seven 48-mm segments. Each tape segment is equal to a 24-h interval. The tape samples were then stained using glycerin jelly (Rogers and Muilenberg, 2001) and fungal spores were counted and identified on the basis of morphological characteristics (Muilenberg, 1999; Smith, 2000; Lin et al., 2004). Spores were identified in the following categories: Ascospores, Basidiospores, Alternaria, Arthrinium, Aspergillus/Penicillium, Botrytis, Cercospora, Cladosporium, Curvularia, Drechslera/Helmithosporium, Epicoccum, Fusarium, Nigrospora, Oidium/Erysiphe, Periconia, Peronospora, Pithomyces, Polythrix, Rusts, Smuts, Stemphylium, Tetraploa, Torula, and Ulocladium. Spores that did not belong to any of these categories were classified as “other” and those that could not be recognized (e.g., broken or covering) were classified as “unidentified.” Final concentrations were reported in spores $\text{m}^{-3}$ of the air sampled.

**Environmental Parameters**

Meteorological parameters were obtained from the Central Weather Bureau at the Taipei monitoring station (approximately 3 km from Guting Elementary School) including temperature, relative humidity (RH), rainfall, atmospheric pressure, wind speed, and hour of sunlight. Daily atmospheric pollutants were obtained from the Taiwan EPA at the Guting Elementary school station including carbon monoxide (CO), suspended PM (PM$_{10}$ and PM$_{2.5}$), nitrogen oxides (NO and NO$_2$), ozone (O$_3$), and sulfur dioxide (SO$_2$). The average 24-h concentration was used for further statistical analysis.

**Statistical Analysis**

Data were analyzed using SAS statistical package (v.9.2, SAS Institute, Cary, NC, USA). The environmental parameters were non-normally distributed, and their distributions among different seasons were compared using the Kruskal-Wallis test. The correlations between fungal spore concentrations and each environmental parameter were assessed using the Spearman correlation coefficient ($\rho$). Additionally, a univariate regression analysis was used to determine the association between each potential predictor variable and fungal spore taxon. Variables with $p < 0.2$ were selected for further multivariate analysis. Multiple regression models were constructed for total fungal spores and major fungal taxa and an autoregressive model was used to evaluate the serial correlation of the fungal spore measurements. The autoregressive order was automatically selected and corrected for the error by using the stepwise autoregressive option in SAS PROC AUTOREG. Considering the growth and sporulation period of fungi in the environment, the 3-day lag effect of each environmental parameter was considered (Grimm-Gofron, et al, 2011, Sadýs et al, 2016). The seasonal effect was also tested using the monitoring seasons (i.e., spring, summer, fall, and winter) as a categorical variable in the multiple regression analysis. The final regression models included significant variables with $p < 0.01$. To approximate normality for the regression analysis, fungal spore concentrations were transformed using base-10 logarithms. Moreover, fungal spore concentrations with a value of 0 were replaced with half of the lowest detection limit (1/2 LOD) before transformation to avoid zero values in the data set (US EPA, 2000).

**RESULTS**

From April 2013 to March 2014, 351 samples of fungal spores were collected at the Guting monitoring station. Because of Chinese New Year, samples were not collected from January 28 to February 10, 2014. Table 1 presents the distributions of total fungal spore and fungal taxon concentrations. The concentration of fungal spores ranged from 241.1 to 19,987.9 spores m$^{-3}$ (mean ± SD: 3,608.0 ± 3,181.8 spores m$^{-3}$). The most prevalent fungal spore taxa were ascospores, basidiospores, Aspergillus/Penicillium, and Cladosporium (mean concentrations: 1130.7, 763.1, 711.1, and 590.8 spores m$^{-3}$, respectively). These taxa were present in >99% of the total samples. The environmental parameters for each season are listed in Table 2; notably, each parameter differed significantly among the four seasons ($p < 0.05$).

The daily and monthly distributions of total fungal spores and major fungal spore taxa are shown in Figs. 2 and 3. The mean concentrations of each fungal taxon in the present study were compared with those reported by Chen et al. (2011), who monitored fungal spores in Sinjiang district, another urban location in the Taipei metropolitan area (Fig. 3). Both daily (Fig. 2) and monthly (Fig. 3) monitoring data revealed that the temporal distributions of fungal spores obviously fluctuated. The results of the Kruskal-Wallis test indicated that the levels of total fungal spores and major fungal taxa exhibited significant monthly and seasonal effects during the study period ($p < 0.05$). Specifically, the concentrations of total fungal spores and major fungal taxa were elevated during the summer months (June–August), with a peak in total spores of approximately 20,000 spores m$^{-3}$. By contrast, the concentrations of fungal spores declined during the winter months (December–early March), with the lowest concentration of 241 spores m$^{-3}$. This winter effect was also observed in other fungal taxa.

The correlations between the concentrations of total fungal spores and major fungal taxa and the environmental parameters up to lag day 3 are presented in Table 3. Temperature and dew point were positively associated with total fungal spores and all fungal taxa from lag day 0–3. In addition, sunlight had positive relationships with most fungal categories from lag day 0–3. Conversely, atmospheric pressure was negatively correlated with total fungal spores and all fungal taxa from lag day 0–3. Wind speed exhibited negative associations with most fungal categories on lag days 0 and 1, as well as basidiospores on lag days 2 and 3. RH and rainfall were positively related to ascospores from lag day 0–2, but negatively associated Cladosporium on lag day 0. Most atmospheric pollutants were negatively correlated with fungal spores up to lag day 3, except PM$_{2.5}$ and SO$_2$. PM$_{2.5}$ was positively correlated with Aspergillus/Penicillium and Cladosporium on lag day 0. SO$_2$ had positive associations with total fungal spores and most fungal taxa on lag day 0.
Table 1. Distributions of fungal spore taxa (spore m⁻³) in an urban area of the Taipei metropolis during April 2013–March 2014.

| Fungal categories      | Freq (%) | Mean   | Median | SD    | Min   | Max   | IQR  |
|------------------------|----------|--------|--------|-------|-------|-------|------|
| Ascospores             | 100.0    | 779.8  | 1283.1 | 15.6  | 9607.5| 1248.3|      |
| Aspergillus/Penicillium| 100.0    | 438.9  | 710.2  | 42.8  | 4131.9| 720.2 |      |
| Basidiospores          | 99.7     | 404.4  | 870.9  | 0.0   | 4821.6| 920.1 |      |
| Cladosporium           | 99.1     | 482.2  | 542.1  | 0.0   | 4354.8| 545.5 |      |
| Smuts                  | 80.3     | 11.7   | 23.6   | 0.0   | 160.0 | 24.6  |      |
| Periconia              | 80.1     | 12.3   | 12.2   | 0.0   | 72.9  | 16.1  |      |
| Arthrinium             | 74.6     | 7.8    | 25.3   | 0.0   | 395.0 | 16.9  |      |
| Fusarium               | 72.9     | 10.1   | 167.8  | 0.0   | 1772.4| 35.0  |      |
| Botrytis               | 65.5     | 6.8    | 34.5   | 0.0   | 224.1 | 23.6  |      |
| Nigrospora             | 63.2     | 3.9    | 28.8   | 0.0   | 272.2 | 16.0  |      |
| Curvularia             | 58.7     | 3.4    | 13.4   | 0.0   | 151.9 | 10.1  |      |
| Torula                 | 54.7     | 3.4    | 10.5   | 0.0   | 85.6  | 10.1  |      |
| Other fungi b          | 100.0    | 13.7   | 296.2  | 3.9   | 2300.5| 207.9 |      |
| Unidentified fungi     | 78.9     | 16.9   | 35.0   | 0.00  | 213.9 | 37.1  |      |
| Total spores           | 100.0    | 2819.4 | 3181.8 | 241.1 | 19987.9| 3596.9|      |

Freq., frequency; SD, standard deviation; Min, minimum; Max, maximum; IQR, interquartile range.
a Frequency is the percentage of a specific fungal taxon present in the total sample (n = 351), b “Other fungi” are the identifiable spore that does not belong to any identified category.

Table 2. Distribution of environmental parameters for each season in an urban area of the Taipei metropolis during April 2013–March 2014.

| Parameters*          | Spring (Mar–May) | Summer (Jun–Aug) | Fall (Sep–Nov) | Winter (Dec–Feb) |
|----------------------|------------------|------------------|----------------|------------------|
| (mean ± SD)          | (n = 92)         | (n = 92)         | (n = 91)       | (n = 76)         |
| Temperature (°C)     | 22.0 ± 4.5       | 29.5 ± 1.5       | 24.4 ± 3.7     | 16.4 ± 3.5       |
| Relative humidity (%)| 72.8 ± 6.6       | 68.0 ± 5.8       | 67.5 ± 5.8     | 67.7 ± 2.9       |
| Wind speed (m s⁻¹)   | 2.11 ± 1.12      | 1.73 ± 0.70      | 2.98 ± 1.01    | 2.33 ± 8.34      |
| Rainfall (mm)        | 9.12 ± 19.28     | 14.39 ± 34.06    | 3.79 ± 9.56    | 4.69 ± 0.88      |
| Pressure (hPa)       | 1013 ± 5         | 1006 ± 4         | 1013 ± 5      | 1013 ± 5         |
| Sunlight (h day⁻¹)   | 2.15 ± 2.82      | 4.61 ± 3.22      | 4.20 ± 3.78    | 2.97 ± 3.46      |
| Dew point (°C)       | 16.8 ± 4.4       | 22.8 ± 0.7       | 17.9 ± 3.9     | 10.2 ± 2.9       |
| PM2.5 (µg m⁻³)       | 28.1 ± 11.5      | 22.7 ± 6.7       | 22.0 ± 9.6     | 27.8 ± 16.6      |
| PM10 (µg m⁻³)        | 51.0 ± 18.0      | 37.4 ± 9.7       | 46.7 ± 21.8    | 56.5 ± 27.1      |
| O₃ (ppb)             | 27.9 ± 11.2      | 22.2 ± 7.8       | 26.4 ± 8.5     | 23.5 ± 7.9       |
| CO (ppm)             | 0.66 ± 0.22      | 0.51 ± 0.13      | 0.43 ± 0.14    | 0.61 ± 0.21      |
| SO₂ (ppb)            | 2.60 ± 1.02      | 3.00 ± 1.02      | 2.27 ± 1.01    | 3.11 ± 1.69      |
| NO₂ (ppb)            | 24.1 ± 7.0       | 18.2 ± 4.6       | 16.3 ± 4.7     | 24.1 ± 7.2       |

* All environmental parameters in the 4 seasons differed significantly (p < 0.05 using ANOVA or Kruskal-Wallis test). (n = 351).

Table 4 presents the multiple regression models after adjustment for autoregressive errors. The results revealed that meteorological parameters on the sampling day were important determinants of fungal spore concentrations, and that temperature and wind speed were the most consistent predictors for fungal spore levels. Temperature was positively associated with the concentrations of total fungal spores and all major fungal spore taxa, whereas wind speed was negatively associated with all fungal categories. RH was associated with total fungi and ascospores, whereas rainfall was positively correlated with Aspergillus/Penicillium. Dew point was another meteorological predictor for fungal spores, which had a positive association with basidiospores on lag day 1. Sunlight was negatively associated with ascospores in the multivariate model; however, a non-significant positive correlation was observed in the Spearman correlation analysis (Table 3). Several atmospheric pollutants were associated with fungal spore concentrations. Specifically, the concentrations of total fungal spores, Aspergillus/Penicillium and Cladosporium increased with the increase in PM₁₀ levels, whereas the concentration of ascospores increased at higher O₃ levels. By contrast, the CO level on the previous day (lag day 1) was negatively associated with basidiospores.

DISCUSSION

In this study, concentrations of total fungal spores and major fungal spore taxa were monitored daily over a 1-year period in an urban area of Taipei metropolis. According to our review of the relevant literature, this is the first study to
Fig. 2. Daily monitoring data of total fungal spores and the four major fungal spore taxa in an urban area of the Taipei metropolis during April 2013–March 2014. The discontinuing line indicates missing data due to no sampling.

be conducted in a metropolitan area in Asia for examining the effects of meteorological and other factors on ambient spore concentrations. Ascospores, Aspergillus/Penicillium, basidiospores, and Cladosporium were the most prevalent fungal taxa, and observed in > 99% of the samples. Significant monthly and seasonal variations in fungal spore concentrations were observed, with a peak during the summer months and trough in winter.

Fungal spores are critical air pollutants with adverse health effects. Lung function decline has been associated with fungal spore concentrations in school children (Chen et al., 2011, 2014) when the average ambient fungal spore concentration exceeded 1,500 spores m⁻³ and early childhood wheezing was aggravated by ascospores and basidiospores in the participants of a birth cohort (Harley et al., 2009). Furthermore, the presence of specific fungal taxa (i.e., Botrytis and Mildews) was reported to increase emergency visits and hospital admissions (Atkinson et al., 2006). The mean monthly concentrations of total fungal spores, the concentrations of ascospores and basidiospores, and the
Fig. 3. Temporal distributions of total fungal spores and the four major fungal spore taxa in an urban area of the Taipei metropolis during April 2013–March 2014. The box plots indicate the mean (dotted line) and median values (solid line in the box), including 10th (lower bar), 25th (lower bottom line of the box), 75th (top line of the box), and 90th (upper bar) percentiles and outliers (o) of the daily monitoring data for each month. The markers (●) indicate average concentrations of each fungal spore taxa in each month of monitoring data from October 2007–June 2008. Data from the same month was plotted for comparison.
### Table 3. Spearman’s correlation coefficients between the concentrations of ambient fungal spores and environmental parameters.

| Fungal taxa          | Lag-0-day          | Lag-1-day          | Lag-2-day          | Lag-3-day          |
|----------------------|--------------------|--------------------|--------------------|--------------------|
|                      | Temp | RH  | Wind speed | Rainfall | Dew point | Sunlight | Pressure | PM$_{2.5}$ | PM$_{10}$ | CO  | O$_3$ | NO$_2$ | SO$_2$ |
| Total fungal spores  | 0.696*** | 0.052 | -0.304*** | 0.083 | 0.733*** | 0.181** | -0.702*** | 0.069 | -0.139** | 0.078 | -0.106* | -0.0766 | 0.122* |
| Ascospores           | 0.613*** | 0.257*** | -0.212*** | 0.273*** | 0.695*** | 0.003 | -0.694*** | -0.025 | -0.236*** | 0.056 | -0.124* | -0.087 | -0.031 |
| Aspergillus/Penicillium | 0.412*** | 0.028 | -0.191** | 0.064 | 0.452*** | 0.091 | -0.473*** | 0.169** | 0.036 | 0.152** | -0.003 | 0.009 | 0.156** |
| Basidiospores        | 0.770*** | -0.000 | -0.339*** | -0.052 | 0.786*** | 0.276*** | -0.715*** | 0.019 | -0.176** | 0.043 | -0.143** | -0.010 | 0.143** |
| Cladosporium         | 0.554*** | -0.176** | -0.264*** | -0.163** | 0.512*** | 0.346*** | -0.465*** | 0.181** | 0.034 | 0.073 | -0.036 | -0.049 | 0.238*** |
|                      | 0.689*** | 0.073 | -0.182*** | 0.089 | 0.729*** | 0.195*** | -0.702*** | -0.045 | -0.247*** | -0.050 | -0.126* | -0.180*** | 0.017 |
| Ascospores           | 0.624*** | 0.186*** | -0.088 | 0.208*** | 0.684*** | 0.075 | -0.693*** | -0.081 | -0.273*** | -0.069 | -0.109* | -0.199*** | 0.070 |
| Aspergillus/Penicillium | 0.409*** | 0.073 | -0.099 | 0.093 | 0.455*** | 0.119* | -0.486*** | 0.044 | -0.090 | 0.023 | -0.047 | 0.078 | 0.047 |
| Basidiospores        | 0.727*** | 0.043 | -0.185*** | 0.016 | 0.762*** | 0.245*** | -0.690*** | -0.073 | -0.265*** | -0.082 | -0.103 | -0.209*** | 0.019 |
| Cladosporium         | 0.521*** | -0.047 | -0.231*** | -0.032 | 0.522*** | 0.247*** | -0.462*** | 0.012 | -0.133 | 0.013 | -0.107* | -0.078 | 0.082 |
|                      | 0.677*** | 0.046 | -0.071 | 0.079 | 0.708*** | 0.184*** | -0.691*** | -0.071 | -0.273*** | -0.134* | -0.097 | -0.251*** | 0.005 |
| Ascospores           | 0.626*** | 0.127* | -0.023 | 0.139* | 0.665*** | 0.107* | -0.678*** | -0.063 | -0.254*** | -0.132* | -0.064 | -0.255*** | 0.045 |
| Aspergillus/Penicillium | 0.410*** | 0.075 | -0.012 | 0.090 | 0.450*** | 0.093 | -0.495*** | -0.003 | -0.129* | -0.022 | -0.050 | -0.115* | 0.018 |
| Basidiospores        | 0.703*** | 0.043 | -0.136* | 0.042 | 0.739*** | 0.193*** | -0.678*** | -0.087 | -0.293*** | -0.111* | -0.107* | -0.238*** | 0.028 |
| Cladosporium         | 0.490*** | -0.034 | -0.038 | 0.034 | 0.508*** | 0.193*** | -0.468*** | -0.058 | -0.203*** | -0.133* | -0.042 | -0.192*** | 0.004 |
|                      | 0.668*** | 0.038 | -0.062 | 0.052 | 0.693*** | 0.148*** | -0.684*** | -0.044 | -0.248*** | -0.138** | -0.072 | -0.242*** | 0.018 |
| Ascospores           | 0.634*** | 0.103 | -0.056 | 0.093 | 0.662*** | 0.113* | -0.678*** | -0.042 | -0.238*** | -0.118* | -0.041 | -0.235*** | 0.035 |
| Aspergillus/Penicillium | 0.400*** | 0.089 | 0.008 | 0.075 | 0.444*** | 0.083 | -0.483*** | 0.003 | -0.126* | -0.051 | -0.052 | -0.129* | 0.027 |
| Basidiospores        | 0.691*** | 0.059 | -0.145** | 0.051 | 0.726*** | 0.130* | -0.688*** | -0.079 | -0.280*** | -0.096 | -0.123* | -0.213*** | 0.033 |
| Cladosporium         | 0.478*** | -0.019 | 0.013 | 0.028 | 0.490*** | 0.122* | -0.464*** | -0.027 | -0.180*** | -0.145** | -0.009 | -0.192*** | 0.001 |

*p < 0.05, **p < 0.01, ***p < 0.001 (n = 351).
Table 4. Multiple regression models for total fungal spores and major fungal spore taxa in an urban area of the Taipei metropolis during April 2013–March 2014.

| Variables                  | β Coef. | Std. Error | p value | R²  |
|----------------------------|---------|------------|---------|-----|
| Total fungal spores        |         |            |         |     |
| Intercept                  | 1.4527  | 0.2608     | <0.0001 | 0.73|
| Temperature                | 0.0473  | 0.0045     | <0.0001 |     |
| Relative humidity          | 0.0110  | 0.0024     | <0.0001 |     |
| Wind speed                 | -0.0406 | 0.0138     | 0.0034  |     |
| PM₁₀                       | 0.0034  | 0.0007     | <0.0001 |     |
| Ascospores                 |         |            |         |     |
| Intercept                  | 0.4240  | 0.3427     | 0.2168  | 0.76|
| Temperature                | 0.0407  | 0.0076     | <0.0001 |     |
| Relative humidity          | 0.0216  | 0.0031     | <0.0001 |     |
| Wind speed                 | -0.0782 | 0.0174     | <0.0001 |     |
| Sunlight                   | -0.0151 | 0.0054     | 0.0057  |     |
| O₃                         | 0.0057  | 0.0020     | 0.0044  |     |
| Aspergillus/Penicillium    |         |            |         |     |
| Intercept                  | 2.1746  | 0.1793     | <0.0001 | 0.58|
| Temperature                | 0.0198  | 0.0063     | 0.0019  |     |
| Rainfall                   | 0.0018  | 0.0007     | 0.0107  |     |
| Wind speed                 | -0.0573 | 0.0165     | 0.0006  |     |
| PM₁₀                       | 0.0030  | 0.0009     | 0.0006  |     |
| Basidiospores              |         |            |         |     |
| Intercept                  | 1.4348  | 0.1820     | <0.0001 | 0.75|
| Temperature                | 0.0436  | 0.0084     | <0.0001 |     |
| Wind speed                 | -0.1302 | 0.0191     | <0.0001 |     |
| Dew point lag-1-day        | 0.0331  | 0.0086     | 0.0001  |     |
| CO lag-1-day               | -0.3360 | 0.1048     | 0.0015  |     |
| Cladosporium               |         |            |         |     |
| Intercept                  | 1.4571  | 0.1345     | <0.0001 | 0.48|
| Temperature                | 0.0456  | 0.0044     | <0.0001 |     |
| Wind speed lag-1-day       | -0.0556 | 0.0175     | 0.0016  |     |
| PM₁₀                       | 0.0047  | 0.0009     | <0.0001 |     |

*Forward stepwise approach was applied to select the significant variable (p < 0.01) from total 13 candidate variables (i.e., temperature, relative humidity, wind speed, rainfall, dew point, sunlight hour, atmospheric pressure, PM₂.₅ &₁₀, CO, O₃, NO₂, and SO₂). Unless indicated otherwise, all variables in the final regression models are lag day 0 data. (n = 351).

Concentrations of Botrytis and mildews in the present study all exceeded the concentrations observed by Chen et al. (2011, 2014) (Fig. 3), Harley et al. (2009), and Atkinson et al. (2006), respectively. These results indicate the potential adverse health effects of fungal spores on the residents of Taipei metropolis. However, further investigation is required to verify this inference.

A strong daily variation related to meteorological parameters was also observed in the present study. Temperature, RH, rainfall, and dew point were all positively associated with the concentrations of fungal spores by up to lag day 1. Similar results have been reported in many studies in various geographical areas worldwide (Sousa et al., 2008; Ho et al., 2005; Quintero et al., 2010; Grimm-Gofron et al., 2011; O’Connor et al., 2014). These relationships are most likely a result of the growth requirement of fungi through either a direct or indirect effect on water availability (aw) (Burge and Otten, 1999). The observed delay in effects was probably caused by the time required for the fungi to sporulate and release spores into the air. However, the regression models revealed that most parameters were significantly associated with fungal concentration on lag day 0; it is likely that these parameters primarily influenced the releasing mechanism of fungal spores rather than the sporulation process.

Additionally, we observed a negative relationship between wind speed and fungal spore concentrations, similar to that found in previous studies (Sabariego et al., 2000; Ho et al., 2005; Quintero et al., 2010; Filali Ben Sidel et al., 2015). Wind speed affected the dispersion process of fungal spores, resulting in atmospheric dilution. Moreover, we observed a negative relationship between sunlight and ascospores in the multivariate analysis (Table 4). Although sunlight exhibited a weak and insignificant positive association (r = 0.003) with ascospores in the Spearman correlation analysis, it had a significant positive association in the univariate analysis after adjustment and correction for the autoregressive error, and it remained significant in the multivariate analysis. The diverse effects of sunlight have been reported in previous studies. In particular, some fungal taxa require sunlight to activate the growth and sporulation processes (Macher, 1999; Sabariego et al., 2000); however, high light intensity...
and longer exposure duration can damage fungal cells and lead to cell death (García-Fernández et al., 2012).

Other meteorological parameters, such as rainfall and dew point, exhibited positive and significant relationships with Aspergillus/Penicillium and basidiospores, respectively, in the multivariate analysis (Table 4). Similar results have been reported in some other studies (Heo et al., 2014; Kallawicha et al., 2015). However, Burge and Roger (2000) noted that rainfall involves both dispersion of fungal spores into and their removal from the ambient environment; therefore, negative associations have also been reported previously (Ho et al., 2005; Pakpour et al., 2015). Dew point was demonstrated to increase basidiospore concentrations in the air (Quintero et al., 2010). This could result from the characteristic of the spore-bearing cell, which actively releases spores by absorbing water from the air as humidity increases. This active mechanism occurs through a combination of high RH and high dew point in the ambient air, usually at dawn or after a period of light rain (Burge and Rogers, 2000; Jones and Harrison, 2004).

According to the projected data of the Taiwan Climate Change Projection and Information Platform (TCCIP, 2016), the temperature and rainfall in Taipei are expected to increase by a maximum of 1.71°C and 64.24 mm, respectively, between 2021 and 2040. Considering these projected data for our final regression models, we inferred that the increased temperature will contribute to a higher concentration of Cladosporium (specifically, an increase of 34 spores m⁻³), which also leads to a higher concentration of total fungal spores. Moreover, the number of days for which the Cladosporium concentration exceeds 1500 spores m⁻³ and the threshold concentration causing decreased lung functioning will also increase from 18 to 20 days in the future (0.6% increase) (Chen et al., 2014). More adverse health outcomes can also be expected. Furthermore, the increasing temperature and rainfall in the projected data will affect the concentrations of other fungal taxa associated with these parameters. According to the regression model of each fungal taxon, the concentration of ascospores and basidiospores are expected to increase by 3 and 32 spores m⁻³, respectively, when temperature is increased, while 211 spore m⁻³ of Aspergillus/Penicillium is expected to increase when both temperature and rainfall are increased. However, further investigation is warranted to estimate the potential adverse health outcomes of these increasing concentrations.

Atmospheric pollutants were also associated with fungal spore concentrations in the present study. In our multivariate analysis, PM₁₀ exhibited positive associations with total fungal spores, Aspergillus/Penicillium, and Cladosporium (Table 4), which is similar to the results that were reported in Hualien, on the east coast of Taiwan (Ho et al., 2005). Aspergillus/Penicillium and Cladosporium spores are easily adsorbed on the larger-sized particle surface and re-suspended in the air because of their small size (approximately 2–10 μm) (Burge and Rogers, 2000). These adsorbed spores can disperse across the continents during a dust event or through the prevailing wind (Brown and Hovmøller, 2002; Wu et al., 2004; Chao et al., 2012; Jeon et al., 2013). In addition to long-range transportation, anthropogenic sources, such as industrial activities, traffic combustion, soil re-suspension, and agricultural activities, can disperse substantial amount of PM in the air (Lin et al., 2008; Mazzei et al., 2008; Chuang et al., 2016). These PM are potential sources and companions of fungal spores in the ambient environment (Womilolu et al., 2003; Bauer et al., 2008). Considering the common size range of fungal spores, we further analyzed the relationship between PM₁₀ and fungal spores. The univariate results suggested that PM₁₀ had positive associations with fungal spores same as PM₁₀. However, PM₁₀ was not included in the multivariate models because of its strong correlation with PM₁₀. We decided to include only PM₁₀ instead of PM₂.₅₄ because PM₁₀ contained the spores that has an aerodynamic diameter < 2.5 μm which were often observed in our samples (i.e., some species of Aspergillus/Penicillium and the apical cell of Cladosporium). Another reason is that the models with PM₁₀ provided slightly higher R² compared to ones with PM₂.₅₄, and thus only PM₁₀ was included.

An experimental study by Sommer et al. (1981) has demonstrated that CO can suppress the growth of fungi in the environment, which is similar to the negative effect of O₃ on the viability of ambient culturable fungi (Wu et al., 2007). Therefore, we hypothesized that high levels of O₃ and CO were negatively correlated to fungal spore concentrations. However, our results revealed that only CO on lag day 1 had a significant negative relationship with basidiospores in the final regression model. This negative association probably results from the CO that inhibits the growth of mushrooms (which were the main sources of basidiospores observed in the present study), resulting in decreased basidiospore production. CO may also be an indicator for traffic heavy traffic leaves a small habitat wherein mushrooms can grow (Newbound et al., 2010; Kallawicha et al., 2015), resulting in a low concentration of basidiospores. Additional experimental studies are required to confirm the causal relationship between fungal spores and these environmental pollutants.

Research conducted in the United States (Adhikari et al., 2006), Portugal (Sousa et al., 2008), and Poland (Grinn-Gofron and Strzelczak, 2013) has reported positive associations between low O₃ levels (median = 29 ppb) and fungal spores, which is similar to our findings (Table 4); however, the mechanism of the association remains unclear. Another experimental study reported that a low level of O₃ (0.1 ppm) with short exposure time (1h) can induce the germination of certain fungi (James et al., 1982); however, most studies used O₃ to control or delay the growth of fungi. Significant inhibition effects were observed at a high concentration of O₃ (0.3 ppm), with the synergistic effects of temperature and exposure time (Palou et al., 2001; Antony-Babu et al., 2009). However, the ambient O₃ levels were relatively low (usually < 0.05 ppm) with significant spatiotemporal variations in our study. Therefore, the fungicidal effect was not observed. We only observed a positive correlation between O₃ and ascospores (Table 4), but the mechanism of this association is unclear. It might result from covariation of ambient air pollutants rather than intracellular mechanism. The actual relationship needs to be further investigated.
The daily average total spore concentration of 3,608 ± 3,182 spores m⁻³ noted in the present study is comparable to that described in some European studies (Docampo et al., 2011; Fernández-Rodríguez et al., 2014). However, our recorded concentrations were lower than those found in a study conducted in Puerto Rico (Quintero et al., 2010), where the mean concentration in the peak month (September) reached to nearly 30,000 spores m⁻³, (our peak data was 8,126 spores m⁻³ in June). Moreover, the lowest mean concentration collected by Quintero et al. was approximately 10,000 spores m⁻³ in January, whereas that in the present study was 790 spores m⁻³ in December which is winter period. The winter effect has also been observed in a study conducted in Finland where the researchers found that the fluorescent bioaerosol concentrations declined in winter which might result from the snow cover and lower biological activities (Saari et al., 2015). Various fungal taxa that were prevalent in the air in the Taipei urban area contributed to the total fungal spore number; ascospores contributed the most, followed by basidiospores, Aspergillus/Penicillium, and Cladosporium. These four taxa were present in >99% of all samples and their concentrations were higher than that of the other two most prevalent taxa (i.e., Smuts and Periconia), which were observed in >80% of all samples.

These findings are similar to those by Chen et al. (2011). However, the concentrations of each category varied between their study and the present study. For example, We observed higher fungal concentrations than did Chen et al., who reported an average total fungal spore concentration of 1,548.4 spores m⁻³ during the study period (September 2007–June 2008) and a highest concentration of 3,567 spores m⁻³ in February. According to the comparison data (Fig. 3), a large temporal variation was observed in the daily monitoring data, but could not be recorded because of to the sampling strategy (i.e., 1 week per month) used in that study; temporal variation was also observed in other major fungal taxa. These results reveal the importance of daily monitoring, which can reflect the temporal variation in airborne fungal spores more accurately.

Compared with the data collected by Chen et al. (2011), the average monthly concentrations of Aspergillus/Penicillium in the current study were 3–40 times higher, and those of other major fungal taxa were approximately 3 times higher (Fig. 3). After referring to Khattab and Levetin (2008) regarding sampling height difference and fungal spore concentration, the Aspergillus/Penicillium concentration in the present study was expected to be lower because of the higher sampling height (15 m above the ground) than that used by Chen et al. (2.5 m above the ground); however, our results revealed a higher Aspergillus/Penicillium concentration. The possible reasons for this result include different sampling locations, nearby land utilization, and changes in meteorological and environmental conditions over the past few years, as has been reported by many studies (Ding et al., 2016; Kuo et al., 2016; TCCIP, 2016; Tung et al., 2016).

Moreover, the daily concentrations of fungal spores in Hualien (mean ± SD: 4,844 ± 4,428 spores m⁻³), and Tainan (mean ± SD: 28,684 ± 4,975 spores m⁻³) were much higher than that recorded in the Taipei urban area here. However, in Hualien, the “other” fungal spore category had the highest concentrations, followed by ascospores, Cladosporium, Aspergillus/Penicillium, and Ganoderma (basidiospores) (Ho et al., 2005). In Tainan, Cladosporium was the most prevalent fungi (Wu et al., 2004). These results indicate the spatial variation in fungal spores: specifically, fungal spores in Hualien and Tainan generally originate from natural sources through local climate conditions, whereas the spores in the Taipei metropolis mainly originate from the nearby residential areas and human activities. This variation is corroborated by the high concentrations of Aspergillus/Penicillium in the Taipei metropolis, where the residential and commercial areas are major determinants of this fungal category (Kallawicha et al., 2015).

In short, our results provide timely daily ambient fungal composition and concentrations in an urban area of Taipei metropolis. Although the $R^2$ of some fungal taxon models (i.e., Aspergillus/Penicillium and Cladosporium) were not optimal ($R^2 < 0.7$), they are comparable to those in other air pollution studies, in which the $R^2$ ranged from 0.11 to 0.86 (Rivera et al., 2012; Saraswat et al., 2013; Adam-Poupart et al., 2014). Furthermore, these models are also comparable to other bioaerosol studies (i.e., fungal spores and pollen) in which the $R^2$ ranged from 0.27 to 0.77 (Ho et al., 2005; Kallawicha et al., 2015; Silva-Palacios et al., 2015). Moreover, these constructed regression models can satisfactorily predict fungal spore concentrations by using only meteorological and environmental parameters, which can enable residents to avoid or minimize exposure to high fungal spore concentrations. In addition, the Burkard 7-day spore trap used in our study is effective for long-term monitoring. One limitation of this study is that fungal spores were identified using morphological characteristics, which may lead to the misclassification of fungal spore categories; this is particularly true for those spores with common characteristics, such as hyaline ascospores and Fusarium spp., which have similar size, shape, and color. These samples could not be processed for further confirmation analysis because the spores were fixed and stained on slides. However, the Burkard 7-day spore trap helped capture both culturable and non-culturable fungal spores, which overcame the limitation of the culture-based method.

**CONCLUSIONS**

By monitoring daily fungal spore concentrations in an urban area of the Taipei metropolis, we demonstrated temporal variation in fungal spores over 1 year. Peak concentrations were observed during summer months, and lowest concentrations were observed in the winter. Temperature and wind speed were the most consistent predictors of total and major fungal taxa. In addition, RH was positively associated with total fungal spores and ascospores, while rainfall and dew point at lag day 1 were positively associated with Aspergillus/Penicillium and basidiospores, respectively. Similar to the positive association of O₃ with ascospores, PM₁₀ was also positively associated with total fungal spores, Aspergillus/Penicillium, and
Cladosporium. By contrast, sunlight had a negative relationship with ascospores, and CO was negatively associated with basidiospores at lag day 1. To obtain the actual temporal variation in fungal spores, daily monitoring is suggested. The findings from this longitudinal study can serve as the baseline information for fungal spore distributions in the Taipei metropolitan area and can be used in further investigations of health effects of fungal spore exposure, enabling residents to avoid or minimize high fungal exposure.

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