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The spatiotemporal distributions and determinants of ambient fungal spores in the Greater Taipei area

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ABSTRACT

Airborne fungal spores, a type of bioaerosols, are significant air pollutants. We conducted a study to determine the spatiotemporal distributions of ambient fungi in the Greater Taipei area and develop land use regression (LUR) models for total and major fungal taxa. Four seasonal sampling campaigns were conducted over a year at 44 representative sites. Multiple regressions were performed to construct the LUR models. Ascospores were the most prevalent category, followed by *Aspergillus/Penicillium*, basidiospores, and *Cladosporium*. The highest fungal concentrations were found in spring. According to the LUR models, higher concentrations of *Aspergillus/Penicillium* and basidiospores were respectively present in residential/commercial areas and in areas with shorter road lengths. Various meteorological factors, particulates with aerodynamic diameters of ≤10 μm, and elevation also had significant relationships with fungal concentrations. The LUR models developed in this study can be used to assess spatiotemporal fungal distribution in the Greater Taipei area.

1. Introduction

Ambient bioaerosols include microorganisms, particles originating from plants and animals, toxins originating from microorganisms, etc. Bioaerosols are abundant in nature, especially fungi and pollen (Macher, 1999; Monn and Koren, 1999; Jones and Harrison, 2004). The associations between exposure to ambient bioaerosols and adverse health effects, such as exacerbation of asthma, allergies, and respiratory diseases, have been reported (Anderson et al., 2001; Burge, 2001; Burge and Rogers, 2000; Cakmak et al., 2002; Peden and Reed, 2010).

Fungal spores are one of the most prevalent bioaerosols in the ambient environment (Burge and Otten, 1999) and account for up to 60% of organic particulate matter with aerodynamic diameters of ≤10 μm (PM_{10}) (Bauer et al., 2008). Many studies have investigated the distributions of fungal spores and the associations between airborne fungi and air pollutants, meteorological factors, and other environmental events such as Asian dust storms (Pasanen et al., 1991; Jones and Harrison, 2004; Madsen et al., 2009). However, the number of sampling sites in those studies was very limited. Although the variations of bioaerosol distributions within a city are not clear yet, studies have indicated that air pollutant levels (e.g., NO_2) had significant within-city variations, that were sometimes even higher than between-city variations (Hoek et al., 2002; Jerrett et al., 2005; Hoek et al., 2008).

In recent years, researchers began to utilize land use regression (LUR) analyses combined with geographic information system (GIS) to improve the spatial distribution of air pollutants and personal exposure assessment (Hoek et al., 2008; Merbitz et al., 2012; Rivera et al., 2012), but very few studies focused on bioaerosols. One study conducted in Fresno, CA, USA found positive associations between the ambient endotoxin levels and the animal feeding operation areas, cropland, and pasture land (Tager et al., 2010). Similar results were reported by Bowers et al. (2010). Their results showed that the

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level of airborne bacteria was positively correlated with agricultural land uses. However, land use regression has not been applied to predict the spatiotemporal distributions of ambient fungal spores.

In order to understand the spatiotemporal distribution of ambient fungal spores and to evaluate the feasibility of applying LURs to ambient fungi, we conducted a one-year study to monitor ambient bioaerosols at multiple sites in the Greater Taipei area. Land use regressions and GIS were utilized to evaluate the characteristics and the determinants of ambient fungal spores in the Greater Taipei area.

2. Materials and methods

2.1. Sampling locations

According to Hoek et al. (2008), at least 40 sites are needed for LUR analyses in atmospheric pollution monitoring studies, and one to four 7–14 day sampling campaigns are usually performed. Therefore, we selected 44 sampling locations for bioaerosol sampling campaigns. Because of the limited human and equipment resources, the sampling sites were divided into 4-8 groups for each sampling campaign.

To select representative sampling sites, the Greater Taipei area (Taipei City and New Taipei City) was divided into 5 homogenous areas using principle component analysis (PCA) and cluster analysis according to land-use information (e.g., household density, population density, and vegetation distribution) and environmental factors (e.g., meteorological and air pollution factors), which are likely to be related to the distribution of bioaerosols (Chen, 2011). We randomly selected 100 schools in the Greater Taipei area to make telephone interviews to obtain preliminary information such as nearby environmental conditions, availability of space and power for sampling, school consent, etc. After considering the criteria for proper sampling and school consent, we chose 65 candidate sites across the 5 homogenous areas. Then site visits were conducted to evaluate the appropriateness for longitudinal bioaerosol sampling. Finally, we selected 44 sampling sites for bioaerosol monitoring with 8–11 sampling sites in each homogeneous area (Fig. 1).

2.2. Air sampling

Four sampling campaigns were performed from November 2011 to August 2012, 1 in each season. Every sampling campaign lasted 1–2 weeks, and 6–9 sampling sites were sampled each day. Sampling periods were chosen to avoid the effects of events in Taiwan on study results (Hoek et al., 2008), such as the Lunar New Year and Tomb-Sweeping Festival. Samples were collected for 24 h at each sampling location every season. A central sampling location was continuously monitored to adjust for variations of different sampling dates within a season (Hoek et al., 2002). The central monitoring site was located on the roof platform of a 10-story building at Taipei Medical University.

Air samples were collected using 37-mm polycarbonate filters (pore size 0.8 μm) and 3-piece plastic cassette holders, coupled with personal pumps (224-PCXR series, AirChek XR5000, SKC Inc., Eighty Four, PA, USA) with a flow rate of 5 L/min. After sampling, the filters were stored in pyrogen-free tubes with an aseptic technique and immediately shipped back to the laboratory. Samples were stored at 4 °C and were processed and extracted within 1 week.

2.3. Sample analysis

Samples were extracted using 3 mL of extraction buffer (pyrogen-free water + 0.01% Tween 80), vortexed for 2 min, and sonicated for 15 min. The extracted solutions were used for the subsequent analyses.

One milliliter of the eluted samples was transferred to a 1.5-mL Eppendorf tube, centrifuged for 10 min at 8000 rpm and 25 °C, and then 0.9 mL of the supernatant was removed. The remaining elute was vortexed, and then 0.05 mL of elute was transferred to a glass slide and allowed to dry on a slide warmer plate at 50 °C. Slides were stained using glycerin jelly (Rogers and Muilenberg, 2001), covered with cover slips, and sealed at the edges with nail polish. Slides were examined under a microscope at a magnification of 800×. Fungal spores were counted and identified based on their morphologies according to the classification of the American Academy of Allergy, Asthma & Immunology (AAAAI) Aeroallergen Network (Muilenberg, 1999; Smith, 2000; Lin et al., 2004). Spores were identified into 26 categories (see Supplemental Material Table S1). Fungal spore concentration was calculated and reported as spores/m^3 of the air volume sampled (see Supplemental Material Formula S1).

2.4. Social and environmental factors

Social factors included population density, types of land use, and points of interest (POIs). These POIs are the places or landmarks such as markets, pet shops, schools, and gas stations. The POIs included in this study were chosen from the categories that may affect bioaerosol concentrations and distributions. POI data of the Greater Taipei area were obtained from Kingwaytek Technology Co., Ltd. (Taipei, Taiwan). The remaining social factors were provided by the Taiwan National Land Surveying and Mapping Center. The land use data included agricultural areas, residential areas, forests, etc. (Supplemental Material Tables S2 and S3). In this study, a village was the smallest data unit.

Environmental data included terrain, vegetation cover, meteorological data, and atmospheric pollutants. We obtained terrain data from the Aerial Survey Office, Forestry Bureau, Council of Agriculture (AFASI), Taiwan. A Normalized Difference Vegetation Index (NDVI) was used as an indicator of the vegetation cover which calculated the amount of green vegetation cover based upon the light scatter/absorption characteristics of live green plants. NDVI data of this study were obtained from NASA MODIS satellite images, with a spatial resolution of 500 × 500 m (Tucker et al., 1991).

Meteorological data were obtained from 49 meteorological stations of the Taiwan Central Weather Bureau (CWB) in the Greater Taipei area, and included temperature (°C), rainfall (mm), relative humidity (RH; %), and wind speed (m/s). Data of atmospheric pollutants were obtained from 18 Taiwan Environmental Protection Administration (EPA) monitoring stations located in the Greater Taipei area. Atmospheric pollutants included carbon monoxide (CO), suspended particulate matter (PM_{10} and $PM_{2.5}$), nitrogen oxides (NO_x, NO, and NO_2), ozone (O_3), and sulfur dioxide (SO_2). For subsequent analysis, the 24-h average concentrations of the meteorological factors and air pollutants were calculated according to the sampling period of the fungal spores.

2.5. Data analysis

Statistical analyses were performed using SAS statistical package (v. 9.2, SAS Institute, Cary, NC, USA), and geographic information system (GIS) ArcGIS (v.9.3, Esri, Redlands, CA, USA). The general Kriging interpolation method (Ordinary Kriging) in ArcGIS was used to estimate the meteorological and air pollution data at each sampling location. For social parameters (e.g., type of land use and POI) and other environmental factors (e.g., vegetation cover), values were calculated within a designated radius (100, 200, 400,