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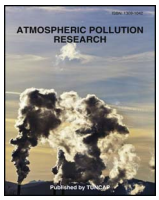

Investigation of fungal spore characteristics in $PM_{2.5}$ through organic tracers in Shanghai, China

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ABSTRACT

In order to investigate the fungal spore tracers in fine particles ($PM_{2.5}$), including mannitol and arabitol at an urban site in a Chinese megacity, $PM_{2.5}$ samples were collected in Shanghai from May 22 to June 19, 2015. The analysis results showed that the average concentration of airborne mannitol and arabitol were 5.79 and 3.86 ng m^{-3}, respectively. Mannitol and arabitol exhibited obvious positive correlations at ambient temperature, resulting from improving fungal spores formation rate and emission strength along with higher temperature. The concentrations of fungal spore tracers with Relative humidity-RH 70%–85% were higher than that RH > 85% and RH < 70%, which reflected that fungal spores released would be restrained under higher humidity. The concentrations between arabitol and mannitol showed negative correlation with wind speed, probably due to the dilution effect of wind. Three ions components (sulfate, nitrate and ammonium) exhibited poor correlations with fungal spore tracer. Based on the results, mannitol had a similar formation pathway with arabitol, resulting in strong correlation between them during our campaign. The number concentration of fungal spores was 10513.16 spores m^{-3}, while fungal spores contributed about 1.91% for organic carbon OC using conversion factors.

1. Introduction

Primary biological aerosols (PBAs) are ubiquitous particles emitted directly from the biosphere into the atmosphere, including pollen, fungal spores, bacteria, viruses, and fragments of animals and plants (Jaenicke et al., 2007). Besides their adverse health effects, PBAPs may play an important role in atmospheric processes, such as acting as ice nuclei (IN) or cloud condensation nuclei (CCN) (Christner et al., 2008; Pratt et al., 2009; Spracklen and Heald, 2014; Dumka et al., 2015).

PBAs can account for a large proportion of aerosol particle mass in ambient air and their abundance varies on a large scale. PBAs dominated in carbonaceous aerosols, accounting for up to 50% as revealed by previous studies (Jaenicke, 2005; Després et al., 2012). Hence, more observations are needed to better quantify the contribution of PBAs to aerosols on continental and global scales.

The fungi constitute an important component of PBAs. Fungal spores are produced during the life cycle of fungi, and often constitute the dominant biological component of ambient aerosols in the size range of 2–10 μm (Zhang et al., 2010). In fact, the proportion of fungi diversity detected in fine particles is comparable to that of coarse particles (Yang et al., 2012). In particular, previous studies have reported that more human pathogens and allergens were found in fine particles than in coarse particles (Fröhlichnowoisky et al., 2009). Moreover, fine particles (with aerodynamic diameters < 2.5 μm, $PM_{2.5}$) can easiler penetrate deeply into human lungs than coarse particles. Hence, these facts highlight the important of investigating the characteristics of fungal spores in fine particles in populated urban areas.

The traditional quantitative method of fungal spores was mainly based on colony counting (Colony Forming Units, CFU) (Després et al., 2012). This method could calculate the number concentration of total fungal spores and identified the morphology (Gonçalves et al., 2010). Concentrations of total fungal spores have been reported by using microscopic techniques for quantification (Bauer et al., 2002a; Winiwarter et al., 2009; Gonçalves et al., 2010). However, the method (CFU) depends on the culture of microorganisms. Furthermore, it cannot be applied to filter media (e.g. quartz filter) which are commonly used for investigations of the chemical composition of atmospheric particulate matter. A molecular tracer method was developed for identifying fungal aerosols recently (Lee et al., 2004). For example, Polysaccharides (Douwes et al., 1999), phospholipids (Womiloju et al., 2003), sugar

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alcohols (Bauer et al., 2008a; Buiarelli et al., 2013), proteins (Menetrez et al., 2007; Liu et al., 2016) and ergosterol (Burshtein et al., 2011; Di Filippo et al., 2013) had all been found to be tracers for fungal aerosols. Ergosterol in fungi and fungal spores is bound to the cell membrane in both the free form and esterified The sugar alcohols, especially arabitol and mannitol constitute an important fraction of the dry weight of fungi, as they are common storage substances in fungi (Carlile et al., 2001). Burshtein et al. (2011) observed that the poorly correlation between arabitol/mannitol and ergosterol in summer and winter, due to the fact that these sugar alcohols frequently occur in some plants and algae. Further, mannitol and arabitol were well correlated with ergosterol in spring and autumn might be attributed to decomposition of vegetation and plant. They suggested that arabitol and mannitol might not be specific biomarkers for fungi (Burshtein et al., 2011). Lang-Yona et al. (2011) found that the concentration of fungal spores based on ergosterol were pooly correlated with DNA-based assays throughout the year. They argue that ergosterol degradation may contribute to the observed differences (Lang-Yona et al., 2011). Given the potential degradation of ergosterol, the results of Burshtein et al. (2011) may not be sufficient in this case to justify the use of arabinitol and mannitol as tracers for fungal substances. Although other sources of sugar alcohols in airborne particulate matter (PM) cannot be excluded, such as plants and algae, arabitol and mannitol have been mostly associated with fungal spores (Graham et al., 2003; Ion et al., 2005). The arabitol and mannitol recently proposed as source tracers for fungal spores, were used in many studies to estimate fungal spores contributions to atmospheric aerosol. i (Claeys et al., 2010; Zhang et al., 2010; Burshtein et al., 2011; Yang et al., 2012). Zhang et al. (2010) used the polyols arabitol and mannitol as tracers for fungal spores to estimate fungal contributions to atmospheric aerosol. Yang et al. (2012) report and discuss the elevated levels of fungal molecular tracers (arabitol and mannitol) in fine particles ($PM_{2.5}$) during a biomass burning season in the Sichuan Basin at Chengdu, a megacity in southwest China (Yang et al., 2012). Fungal spores abundance depends on time, climate, and geographical location (Flores et al., 2014). In addition, it is also related to population density, activity, and air circulation. Shanghai as an important coastal city of continental pollutants flowing to the Pacific Ocean, has high annual mean temperature and humidity, which is located in the subtropical area. However, the investigations into the variation of fungal tracers in aerosol particles in Shanghai in China have been very limited (Feng et al., 2013; Li et al., 2016). Li et al. (2016) and Feng et al. (2013) identified the tracer of arabitol and mannitol for fungal spores. However, the contribution and characteristic of fungal spores were not discussed in detail.

May–June is a spring harvest period. Thus, in this study, we collected 23-h $PM_{2.5}$ samples in the central of Shanghai during May–June 2015 and measured tracers (mannitol and arabitol) together with OC and inorganic ions. The purposes of the study are 1) to investigated the levels of primary biological aerosol-fungal spores in spring-summer period in Shanghai; 2) to examine the influence of meteorological factors on fungal spores tracers in the real atmosphere; 3) to estimate fungal spores contributions to OC in fine particles.

2. Experiments

2.1. Sampling

$PM_{2.5}$ samples were collected on quartz filters by a high-volume sampler (Ecotech, Hivol 3000) that was operated at an air flow rate of 1.13 $m^3\,min^{-1}$ from May 22 to June 19, 2015 at an urban site in Shanghai. As showed in Fig. 1, the sampling site (Xu Jia Hui, E121° 25′47″, N31°10′42″) was located in the central of Shanghai, representing the typical urban environment. The sampler was placed on the roof of a 20 m-high building on the XJH campus of Shanghai Jiao Tong University. The sampling continued 23 h periods for each sample. Field blanks were obtained every ten days by mounting the filters in the sampler for 5 min without airflow during the study period. All quartz fiber filters (8 × 10 inch) were baked at 550 °C for 5.5 h to remove organic material before used. All sample filters stored at −20 °C after sample collection in order to prevent loss of volatile components due to evaporation and degradation. A total of 24 air $PM_{2.5}$ samples were obtained including three field blanks during the study period.

2.2. Chemical analysis

The quartz fiber filters were analyzed for OC and EC by the IMPROVE method with a DRI Model 2001A Thermal/Optical Carbon Analyzer. A punch (4.9 cm^2) of each quartz filter was sonicated with 20 mL of ultra-pure deionized water (> 18.2 MΩ resistivity) in an ice-water bath for extracting nitrate (NO_3^-), sulfate (SO_4^{2-}) and ammonium (NH_4^+). It is worth noting that the water temperature should be maintained at a temperature of 20–30 °C during the ultrasound. Then the filtered extract was analyzed with IC (ion chromatography).

The extraction procedures were used in this study were drawn from a previously published study (Bauer et al., 2008a). Briefly, about 20 cm^2 of each filter was extracted three times in 20 mL of dichloromethane/methanol (1:1, v/v) and sonicated for 25 min each time, the combined extracts evaporated to approximately 1 mL by rotary evaporator, and further concentrated dryness under a gentle stream of nitrogen. Then the sample derivatized with 100 mL N, O- bis-(trimethylsilyl) – tri-fluoroacetamide (BSTFA) and 20 mL pyridine at 75 °C for 45 min. Each derivatized sample was injected into the GC/MS for identification and quantification. The gas chromatography conditions: constant temperature for 10 min at 60 °C, increased to 300 °C for 20 min at 10 °C·min^{-1}, the interface temperature of GC/MS was 300 °C. The carrier gas He flowing rate was 1.0 mL min^{-1}. The ionization of the organic tracer through the interface was by electron impact (70 eV), and scanned from 50 to 500 amu. Arabitol and mannitol were quantified by authentic standards, including D-(+)-arabitol (> 99%) and D-mannitol (> 99%). The determination of the limits of detection (LODs) and quantification (LOQs), respectively, were based on three and ten times the signal-to-noise ratio. The method detection limits for arabitol and mannitol, were 0.04, 0.06 ng m^{-3}, respectively. Limit of quantification of species for arabitol and mannitol were 0.135, 0.187 ng m^{-3}.

2.3. Quality assurance/quality control

The extraction and analysis method of field blanks and laboratory blanks was the same with that in ambient samples. The target compound (mannitol and arabitol) in the blank field and laboratory samples were below the detection limit. The extraction recoveries of mannitol and arabitol were 64–105%. Recoveries of the target compounds in six spiked samples (authentic standards spiked into solvent with prebaked quartz filter) were 73 ± 5% for arabitol, 79 ± 14% for mannitol. And the relative standard deviation (RSD) for the quantification of standards were 4.8%, 5.6%, for arabitol and mannitol, respectively.

3. Results and discussion

3.1. Ambient concentrations of fungal spore tracers

The average concentration of three secondary inorganic acid ions, sulfate, nitrate and ammonium (SNA) was 22.17 μμg m^{-3}, accounting for 86.23% of the total amount of water-soluble ions. SNA is the dominant component of water-soluble inorganic ions in aerosol in Shanghai. The proportion of other ions is less than 15% of the total amount of water-soluble ions. The measured results during our campaign were listed in Table 1. The average concentrations of OC and EC were 7.17 ± 3.91 μg m^{-3}, 2.56 ± 1.38 μg m^{-3} (Table 1), in the range of 3.12–16.28 μg m^{-3}, 1.23–4.90 μg m^{-3}, respectively (Fig. 3a, Table 1). The daily average concentrations of mannitol and arabitol in $PM_{2.5}$ in the campaign were shown in Fig. 3b.