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Carbon content of common airborne fungal species and fungal contribution to aerosol organic carbon in a subtropical city

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ABSTRACT

Interest in the role and contribution of fungi to atmospheric aerosols and processes grows in the past decade. Substantial data or information such as fungal mass or carbon loading to ambient aerosols is however still lacking. This study aimed to quantify the specific organic carbon content (OC per spore) of eleven fungal species commonly found airborne in the subtropics, and estimated their contribution to organic carbon in aerosols. The specific OC contents showed a size-dependent relationship ($r = 0.64$, $p < 0.05$) and ranged from 3.6 to 201.0 pg carbon per spore or yeast cell, giving an average of 6.0 pg carbon per spore (RSD 51%) for spore or cell size less than 10 μm. In accounting for natural variations in the composition and abundance of fungal population, weighted-average carbon content for field samples was adopted using the laboratory determined specific OC values. An average of 5.97 pg carbon per spore (RSD 3.8%) was enumerated from 28 field samples collected at the university campus. The mean fungal OC concentration was 3.7, 6.0 and 9.7 ng m^{-3} in $PM_{2.5}$, $PM_{2.5-10}$ and PM_{10}, respectively. These corresponded to 0.1%, 1.2% and 0.2% of the total OC in $PM_{2.5}$, $PM_{2.5-10}$ and PM_{10}, respectively. In the study period, rain provided periods with low total OC but high fungal prevalence and fungi contributed 7–32% OC in $PM_{2.5-10}$ or 2.4–7.1% OC in PM_{10}. More extensive studies are deserved to better understand the spatial-, temporal- and episodic dependency on the fungal OC contribution to the atmospheric aerosols.

1. Introduction

Organic carbon (OC) constitutes a significant fraction of atmospheric aerosols. Depending on geographical locations, OC accounts for 10–20% of the total PM_{10} mass (Offenberg and Baker, 2000; Etyemezian et al., 2005; Lin and Tai, 2001; Kim et al., 2000; Viidanoja et al., 2002), and as high as 40% of both $PM_{2.5}$ and PM_{10} aerosols in the Amazonian area (Graham et al., 2003). In Hong Kong, OC contributes about 7–26% of the mass of PM_{10} materials (Cao et al., 2003; Ho et al., 2002; Yu et al., 2004). The OC fraction of $PM_{2.5}$ materials is even higher and levels as high as 80% have been reported (Ho et al., 2002). The identification and quantification of the OC provides essential information on sources (anthropogenic and biogenic) and profiles of emissions. Detailed speciation of the organic compounds and their size distribution provides invaluable information on health implications and roles of OC in the atmospheric processes and reactions. However, substantial amounts of atmospheric OC remain uncharacterized and unidentified (Neusüss et al., 2000; Saxena and Hildemann, 1996).

Microbiological substances are conventionally considered as a minor OC source of aerosols. However, recent studies reported that primary biological aerosol particles (PBAP), which include pollen, bacteria, fungal spores, algae, and protozoa, can contribute a significant fraction in number concentrations of total aerosol particles (Mattias-Maser et al., 1999). It can amount to 20% and 30% depending on locations (Mattias-Maser and Jaenicke, 1995). Jaenicke (2005) even reported that cellular particles compose up to about 50% of total particles in a semi-rural location. Elbert et al. (2007) calculated that fungal spores accounted for an average of 35% of the PM_{1-10} mass in Amazonia. Therefore, PBAP can be a significant source of OC. Up to date, only one study has reported on the bacterial contribution (0.03%) to total organic aerosol in the atmosphere by measuring the number concentration and applying the carbon conversion factor of 17 fg carbon per cell derived from limnological studies (Bauer et al., 2002a). Fungi and fungal spores are believed to make a higher contribution to the OC due to their larger size relative to bacteria. Bauer et al. (2002b) reported an average of 13 pg carbon per fungal spore. Using this value, Bauer et al. (2002b) estimated a 2.9–5.4% contribution of the fungal OC to

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the total OC in wintry season at Mt. Rax, Austria. Indirect estimation of fungal carbon was also reported by Womiloju et al. (2003) through analyzing the profile characteristics of glycerophospholipids and their concentrations. They estimated that fragments of fungi and pollens collectively accounted for 12–22% of the total OC in the fine particulates ($PM_{2.5}$). Intact fungal spores and pollens may therefore have higher contribution to atmospheric organic carbon compared with bacteria. Due to the larger sizes of pollen (20–100 μm), most pollens will be selectively excluded by standard aerosol sampler inlets in the field. Fungal spores will be dominant over pollen in particulates of inhalable range (<10 μm) (Glikson et al., 1995). Although aerodynamic sizes of common airborne fungal spores were greater than 2.6 μm (Lee et al., 2006a) and associated with the coarse particulates ($PM_{2.5-10}$), smaller spores and fragments of spore or fungal mycelia are also present in a significant fraction (~30%) in the fine particulates ($PM_{2.5}$) (Cheng et al., 2008b; Fang et al., 2005). Understanding fungal contribution to the OC and their size distribution will certainly facilitate better comprehension on their role and impact on atmospheric processes. Both studies of Bauer et al. (2002b) and Womiloju et al. (2003) based their estimates of fungal carbon on fungal spore count collected from the field. However, only three impinger samples and one composite filter sample for each site (total three sites) were analyzed in Bauer et al. (2002b) and Womiloju et al. (2003) study, respectively. The sampling in both studies was yet insufficient for conclusive statement. Moreover, both studies were conducted in temperate zone; no study has been reported in the subtropics, where the climatic conditions favor fungal proliferation all year-round. The aim of this study was to assess the in situ fungal loading in terms of fungal carbon contribution with the weighted-average conversion factor approach demonstrated in our previously reported ergosterol studies (Cheng et al., 2008a,b). This paper reports the quantification of the specific organic carbon content (OC content of a spore or yeast cell) of common airborne fungi collected in the subtropics and estimates the fungal contribution to the OC in both $PM_{2.5}$ and PM_{10} in a subtropical city, Hong Kong.

2. Materials and methods

2.1. Determination of the specific organic carbon content

The carbon contents of the common spores/yeast cells collected were analyzed. These covered *Alternaria* sp., *Aspergillus niger*, *Candida* sp., *Cladosporium* sp., *Paecilomyces* sp., *Rhodotorula* sp. and five species of *Penicillium*, including *Penicillium chrysogenum*. Fungal spore and yeast cell suspensions were first prepared after their parental colonies had been cultured on Malt Extract Agar (MEA) at 28 °C for 14 days as described in Cheng et al. (2008a). Aliquots of suspensions were dried at 50 °C in ceramic sample boats, which were thermally pretreated at 900 °C to remove any residual carbon.

Each sample boats were then combusted at 900 °C in a total carbon content (TOC) analyzer (Shimadzu, TOC-5000), equipped with a solid sample module (SSM-5000A). Pure oxygen was used as the carrier gas with a flow-rate of 500 ml min^{-1} and a pressure at 2 kg cm^{-2}. Carbon dioxide resulting from the combustion was dehumidified and sent to a non-dispersive infrared (NDIR) detector, which output an analog signal to generate a peak. The areas of peaks were calibrated with the carbon content of D-(+)-glucose (anhydrous, RDH) standards.

To compensate for intrinsic biological variations within individual species or strains, the specific OC content was determined with a linear regression between the carbon content and the number of spores or cells used. At least five independent batches of each type of spore or cell were prepared for the analyses. In each batch, the spore or cell number concentration was first determined by microscopic counting with hemacytometer (Cheng et al., 2008a). The amount of spores was controlled by applying different volumes from the batch for the OC measurement. Four different aliquots of spores were measured from each batch. This generated 20 data points for the regression analysis. The slope of the regression line provided the specific OC content of the species or strain studied. The 95% confidence levels were determined at the same time.

2.2. Air sampling

The studied region, Hong Kong (22.12°N, 114.08°E), is located in the subtropics on the south eastern coast of the Asiatic mainland with an annual average temperature of 23.0 °C. Atmospheric aerosol samples were collected on the rooftop of a residential building in the campus of the Hong Kong University of Science and Technology (HKUST) on the east side of Hong Kong during November 2005–April 2006. The samples were collected by a high volume sampler (Graseby, GMWT 2200) equipped with a PM_{10} size-selective inlet (Graseby, Model 1200) and an impactor with a cut-off aerodynamic diameter at 2.5 μm (Graseby, Model 231-F). Six viable samples were collected in parallel with each filter sample. A total of 28 filter samples were collected. Details of sampling were described in Cheng et al. (2008b).

2.3. Quantification of carbon contents in aerosol samples

The filter samples were analyzed for OC and elemental carbon (EC) using DRI 2001 OC/EC Carbon Analyzer following IMPROVE thermal–optical reflectance (TOR) protocol (Chow and Watson, 2002). Punches of samples were heated stepwise at temperatures of 120 °C, 250 °C, 450 °C and 550 °C in pure helium atmosphere, and 550 °C, 700 °C and 800 °C in 2% oxygen and 98% helium atmosphere. The carbon that evolved at each temperature was oxidized to carbon dioxide, and then reduced to methane for quantification with a flame ionization detector. Reflectance of each filter sample was monitored during the process and OC/EC spilt was set when the reflectance returned to its initial value. The carbon evolved prior to the spilt was considered as OC and the carbon evolved after the spilt was considered as EC.

2.4. Comparison of TOC system and TOR protocol

TOR is a very common method employed for determination of the OC concentrations in filter samples as the protocol has a correction for pyrolyzed OC due to the charring phenomenon in the heating process (Cao et al., 2005; Kim and Hopke, 2005; Hwang and Hopke, 2007; Brown et al., 2007). However, it is not the optimal approach for analyzing the OC contents in fungal spores directly. Though fungal spores can be transferred to a filter for TOR analysis, the evenness in spreading the spores on the filter is not guaranteed and there will be inevitable error due to transfer loss. Direct measurement of the organic carbon content is achieved with direct combustion in TOC analyzer. The total OC content of filter samples can also be quantified with the TOC analyzer; however, the organic and elemental portion of the carbon cannot be differentiated for the filter sample. Therefore, the OC in filter samples was analyzed with the TOR protocol while the fungal spores were quantified with the TOC analyzer. As the OC data from the two analytical approaches were cross-compared, the consistency of the two approaches is important. This was tested by subjecting the filter samples to both approaches for total carbon analysis. Filters were cut into pieces and combusted in the TOC analyzer as that of the fungal spores. The results were compared with the total carbon contents (TC which was the sum of EC and OC) measured by the TOR protocol. Paired *t*-tests with $\alpha = 0.05$ were used to evaluate the