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Short communication

Arabitol and mannitol as tracers for the quantification of airborne fungal spores

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Abstract

Fungal spores constitute a sizeable fraction of coarse organic carbon (OC) in the atmospheric aerosol. In order to avoid tedious spore count methods, tracers for quantifying the spore-OC in atmospheric aerosol are sought. Arabitol and mannitol have been proposed as such tracers, since no other emission sources for these compounds have been reported. By parallel investigations of spore counts and tracer determinations from PM_{10} filter samples we could derive quantitative relationships between the amounts of tracer compounds and the numbers of spores in the atmosphere for different sites in the area of Vienna. We obtained over all average relationships of 1.2 pg arabitol $spore^{-1}$, with a range of 0.8–1.8, and 1.7 pg mannitol $spore^{-1}$, with a range of 1.2–2.4, with a clear site dependence. Thus, using these conversion factors from spore counts to spore-OC and spore-mass, along with analytical data for arabitol or mannitol in filter samples, the contribution of fungal spores to the OC and to the mass balance of atmospheric aerosol particles can be estimated.

Keywords: Fungal spores; Organic carbon; Biogenic aerosol; PM_{10}; Sugar alcohols

1. Introduction

In recent years there has been increasing awareness of the contribution of primary biological aerosol particles (PBAPs) to atmospheric organic particles. Jaenicke (2005) reported that the contribution of PBAPs amounts to between 5% and 50% of the atmospheric aerosol, expressed in number concentrations of particles with radius $>0.2\,\mu m$. Glikson et al. (1995) considered the most dominant fraction of bioaerosols in the size range 2–10 μm to be fungal spores. Battarbee et al. (1997) observed that fungal spores contributed 1–4% and after a rainfall even as much as 23–27% to the total particles. They used a Burkard spore trap and light microscopy to determine the number concentrations and the nature of the airborne particulates, thus stressing the importance of this contribution. Concentrations of total fungal spores have been reported by Bauer et al. (2002a, 2005) and Lee et al. (2006) using microscopic techniques for quantification. However, the microscopic techniques cannot be applied to fibrous filter media (e.g. quartz or glass fiber filters) which are commonly used for

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investigations of the chemical composition of atmospheric particulate matter. Therefore, sampling for the collection of fungal spores had to be performed by liquid impingement, or on Teflon or polycarbonate filters.

A different approach to assessing the occurrence of fungal spores in atmospheric particulate matter is the use of biomarkers. Womiloju et al. (2003) analyzed phospholipids in different fungal and pollen genera and reported a semi-quantitative contribution from fungal cells in the presence of pollen of 12–22% to the organic carbon (OC) and of 4–11% to the aerosol mass in $PM_{2.5}$ samples. Average total phospholipid content in fungal spores ranged between 0.15 and 1.75 pg. Foto et al. (2004) determined the β-1,3 D-glucan content of spores of different fungal species and also glucan concentrations in outdoor air samples. The latter were converted into spore concentrations, assuming only *Cladosporium cladiosporioides* to be present (3.1 pg glucan spore^{-1}), which was in good agreement with spore counts from concurrent samples. Lau et al. (2006) used ergosterol as biomarker and determined its concentrations in fine ($PM_{2.5}$) and coarse ($PM_{2.5-10}$) aerosol as well as average ergosterol contents of spores of three common airborne fungal species, which ranged between 0.68 and 1.89 pg spore^{-1}.

The goal of this study was to introduce fungal spores-OC into the mass balance of atmospheric PM_{10}-OC. To this end we investigated the potential use of arabitol and mannitol, which are common storage substances in fungal spores, for the quantification of fungal spores in atmospheric PM_{10}. The use of a unique biomarker for spores would simplify sampling, avoiding the need for parallel aerosol collection with impingers and filters, and allowing one to determine simultaneously different major constituents of atmospheric PM_{10}, with the exception of siliceous minerals, all from one filter.

2. Experimental

2.1. Sampling sites

Sampling was performed in Vienna at two suburban sites: "Lobau" (UFL) [16°31′36″E, 48°09′45″N] in September, October and December 2004, and "Schafberg" (UFS) [16°18′10″E, 48°14′09″N] in June and July 2005. Parallel to the urban fringe sampling at the UFS, spores collection was carried out at an urban site "Rinnböckstrasse" (UTR) [16°24′28″E, 48°11′05″N], situated near a busy city highway. The three sampling sites belong to the air quality network of the city of Vienna.

2.2. Methods

2.2.1. Sampling

Sampling was performed in parallel with a Hi Vol filter sampler (Digitel DH70) and an impinger sampler for viable particles. The filter substrate used in the Hi Vol sampler was quartz fiber (Tissuequartz 2500QAT-UP, 150 mm Ø, Pall, USA). Filters were analyzed for PM_{10} mass, EC, OC, inorganic ions, arabitol, mannitol and other specific organic compounds. The viable particle sampler was an impinger (AGI-4, Aceglass, USA). The impingers were heat sterilized at 340 °C for 30 min, then filled with 25 mL 17.5% (w/w) glycerol/water solution and autoclaved at 121 °C for 15 min. At the sampling sites the impingers were connected to the sampling unit consisting of an inlet manifold, a pump (KF NO150ANE, Neuberger, Germany) and a dry gas meter (BK2.5, Elster, Austria). The sampling flow rate was 4–11 L min^{-1} and the sampled volumes were between 2.5 and 6 m^3; sampling intervals were between 5 and 24 h. Immediately after sampling, 37% (v/v) formaldehyde was added to avoid bacterial reproduction (1 mL to every 20 mL of sampling solution to give a 1.85% formaldehyde solution). The sampling liquid was stored in polyethylene vials at 4 °C. For the collection of the second series of samples (at UFS and UTR) modified impingers were used with a 10-fold higher volume compared to the original AGI-4 impingers, so that sampling could be continued for 24 h periods. The design of the modified impingers corresponds to the AGI-4 impinger, i.e., the distance between the capillary and the bottom of the impinger is still 4 mm. The height and diameter of the impinger are 310 and 50 mm, respectively, and the diameter of the capillary is 0.8 mm. The impingers were filled with 250 mL of sterile water, After 24 h sampling the liquid levels were still 73 mL on average or 3–6.7 cm depth, which should be sufficient to avoid substantial losses of sampling efficiency (Lin et al., 1997). The sampling flow rate was 7 L min^{-1}, and the sampled volume was around 10 m^3 for 24 h; samples were generally changed at midnight.

2.2.2. Staining/filtration/microscopy

An aliquot of 10 mL of the sampling liquid was dyed with 1 μL SYBR® Gold nucleic acid gel stain

(Invitrogen, USA). After a reaction time of 10 min in the dark, the sample was filtered on an Anodisc filter (0.2 μm, 25 mm Ø, Whatman, England) and mounted on a microscopic slide. Then, 11 μL of anti-fading solution (5 mL glycerol, 5 mL sterile water, 0.5 g ascorbic acid) was added. Subsequently the spores were enumerated by epifluorescence microscopy (Orthoplan 054784, Leitz Wetzlar, Germany; excitation wavelength: 450 nm) at a magnification of 1000. Dyed spores appear as bright green or yellowish-green objects in the microscope field of view. At least 60 fields (4900 μm^2 $field^{-1}$) per filter were counted. The averaged spore count obtained for a field is then extrapolated to the active filter area. The spore count per m^3 is derived taking into account the aliquot from the sampled volume and the sampled volume of air.

2.2.3. Determination of arabitol and mannitol

Arabitol and mannitol were determined in quartz fiber filter extracts, after trimethylsilylation, by gas chromatography with flame ionization detection (GC-FID) using an internal standard calibration procedure, similar to that developed in previous work for the determination of levoglucosan and saccharidic compounds by gas chromatography/mass spectrometry (Pashynska et al., 2002). Standards of arabitol (purity >99%) and mannitol (purity >99%) were purchased from Sigma (St. Louis, MO, USA) and Merck (Darmstadt, Germany), respectively. A filter area of 3 cm^2 was placed in a 20 mL Pyrex glass flask with Teflon-lined stopper and spiked with two internal standard recovery standards, i.e., xylitol (Sigma; purity > 99%, 0.26 μg) for quantitation of arabitol, and dulcitol (Sigma; purity > 99%, 0.25 μg) for quantitation of mannitol. The spiked filter part was extracted three times with 20 mL of dichloromethane/methanol (1:1; v/v) under ultrasonic agitation for 30 min. The extracts were combined and reduced in volume with a rotary evaporator (213 hPa, 30 °C) to approximately 1 mL, then the concentrate was filtered through a Teflon syringe filter (0.45 μm pore size) and dried under a stream of pure nitrogen. The dry residue was derivatized by addition of 25 μL of trimethylsilylation reagent (*N,O-bis*-(trimethylsilyl)trifluoroacetamide + 1% trimethylchlorosilane; Pierce, Rockford, IL, USA) and 15 μL pyridine, and heating the reaction mixture for 1 h at 70 °C. The derivatized sample was analyzed immediately by GC-FID on a model 8000 Top gas chromatograph (Carlo Erba, Milan, Italy), equipped with a deactivated silica precolumn (0.25 mm × 2 m) and a CP Sil 8CB capillary column (95% dimethyl, 5% phenyl polysiloxane; 0.25 μm film thickness, 0.25 mm × 30 m; Chrompack, Middelburg, The Netherlands). The temperature program was as follows: isothermal hold at 45 °C for 3 min, increase to 100 °C at a rate of 20 °C min^{-1} and hold for 10 min, increase to 315 °C at a rate of 5 °C min^{-1} and final hold for 20 min; the total analysis time was 80 min.

3. Results and discussion

3.1. Number concentrations of fungal spores

The first series of measurements included 4 samples that were collected in September, October, and December 2004 at the urban fringe site UFL in the Lobau National Park. The concentrations of fungal spores in autumn samples amounted to between 15,000 and 33,000 spores m^{-3} (on average 25,000). In December, when the surrounding areas were frozen or covered with snow, still around 3000 spores m^{-3} were observed. The second set of measurements was performed from June 30 to July 24, 2005 with parallel sampling at the urban fringe site Schafberg (UFS) and the urban traffic site "Rinnböckstrasse" (UTR). The concentration of spores was frequently higher at UFS and ranged between 20,000 and 42,000, compared to between 17,000 and 32,000 at UTR. Average concentrations of fungal spores were 29,000 at UFS and 26,000 m^{-3} at UTR, respectively.

3.2. Concentrations of arabitol and mannitol

In simultaneous samplings of spores by the viable sampler and of PM_{10} on quartz fiber filters, arabitol and mannitol concentrations were determined from extracts of filter aliquots.

In the autumn of 2004 the airborne arabitol and mannitol concentrations at UFL ranged between 7.0 and 63 ng m^{-3} and between 8.9 and 83 ng m^{-3}, respectively. In the summer of 2005, on average higher concentrations of sugar alcohols (28 ng m^{-3} arabitol and 42 ng m^{-3} mannitol) but lower concentrations of spores (26,000 spores m^{-3}) were observed at the urban traffic site. At UFS by contrast, a higher mean concentration of spores (mean 29,000 spores m^{-3}) but lower mean concentrations of arabitol (22 ng m^{-3}) and mannitol (34 ng m^{-3}) were found. This could be due to different compositions of the fungal flora at the different sites. The concentrations of arabitol were highly correlated ($R^2 = 0.87$)

with those of mannitol and in all samples the concentration of mannitol was 1.5 times higher than the concentration of arabitol (std. dev. 26%).

3.3. Determination of conversion factors from arabitol and mannitol concentrations for airborne fungal spores

The goal of our study was to investigate whether arabitol and mannitol can be used as "unique" tracers for the quantification of fungal spores-OC or fungal spores-mass in atmospheric particulate matter. Thus, we determined simultaneously spore counts as well as the mass of arabitol and mannitol from PM_{10} filter samples.

In Figs. 1a and b relationships between spore counts and sugar alcohols at the three investigated sites are shown. The arabitol and mannitol concentrations are highly correlated with the fungal spore counts at all sites, however, clear site

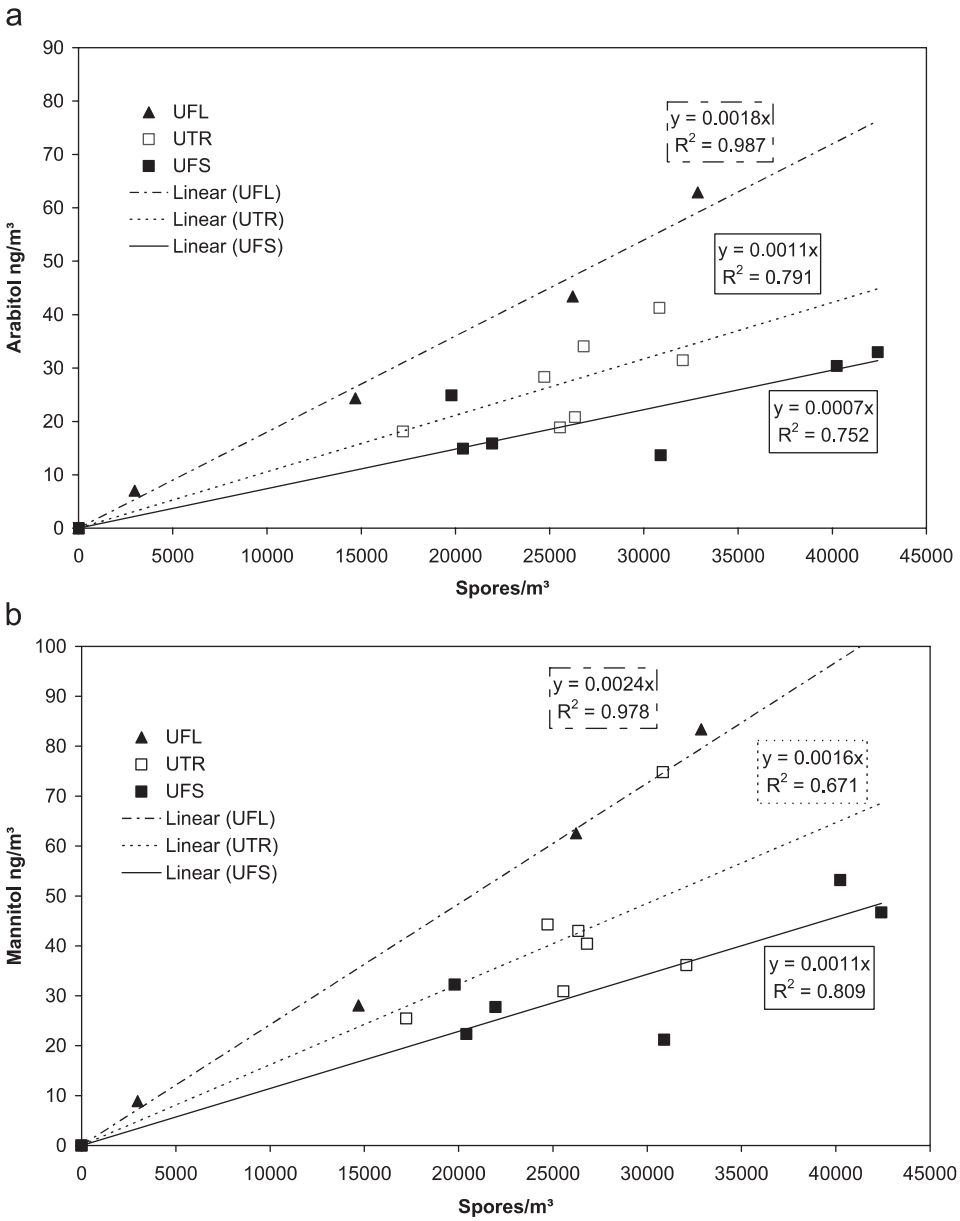

Fig. 1. (a) Correlations between arabitol and fungal spores at the three sites UFL, UFS and UTR. (b) Correlations between mannitol and fungal spores at the three sites UFL, UFS and UTR.

dependences are observed (Fig. 2 or Table 1). Arabitol and mannitol levels per spore were 38% and 33% higher during the summer sampling periods at the UTR (Rinnböckstrasse) compared to the UFS (Schafberg) site. Between the urban fringe sites Lobau and Schafberg, arabitol and mannitol levels per spore were a factor of 2.3 and 2.0 higher, respectively, at Lobau. However at those sites, sampling was performed during different seasons (autumn–winter at Lobau, summer at Schafberg). Thus, the differences of around a factor of 1.4 between the city and urban fringe during the same season and a factor of 2 between seasons might be due to different genera of fungi that may develop different amounts of the storage substances per spore. This issue should be investigated more thoroughly in further studies.

The grand average content of arabitol per spore amounted to 1.2 pg (r.s.d.: 44%), and the average content of mannitol was 1.7 pg spore^{-1} (r.s.d.: 37%). These results can be used as best estimates to convert airborne arabitol and mannitol into concentrations of fungal spores or into OC related to fungal spores. The contents of arabitol and mannitol in fungal spores are found to be in a comparable range to the other fungal biomarkers, such as phospolipids (0.15–1.75 pg spore^{-1}, Womiloju et al., 2003), β-1,3 D-glucan (0.04–3.1 pg spore^{-1}, Foto et al., 2004) and ergosterol (0.68–1.89 pg spore^{-1}, Lau et al., 2006).

Using arabitol or mannitol as conversion factors, the amounts of fungal spores can be tentatively determined from the arabitol or mannitol concentrations obtained for PM_{10} filter samples. The contribution of fungal spores to the organic PM_{10} carbon balance or to the PM_{10} aerosol mass balance can then be determined using further conversion factors, e.g., of 13 pg OC spore^{-1} or 33 pg fresh mass per spore (Bauer et al., 2002b).

A more thorough investigation of tracer/spore relationships at different types of sites and during different seasons of the year should allow one to derive numbers which can be used to infer the contribution of fungal spores to PM_{10}-OC or PM_{10}-mass by measuring the spores' tracers from quartz

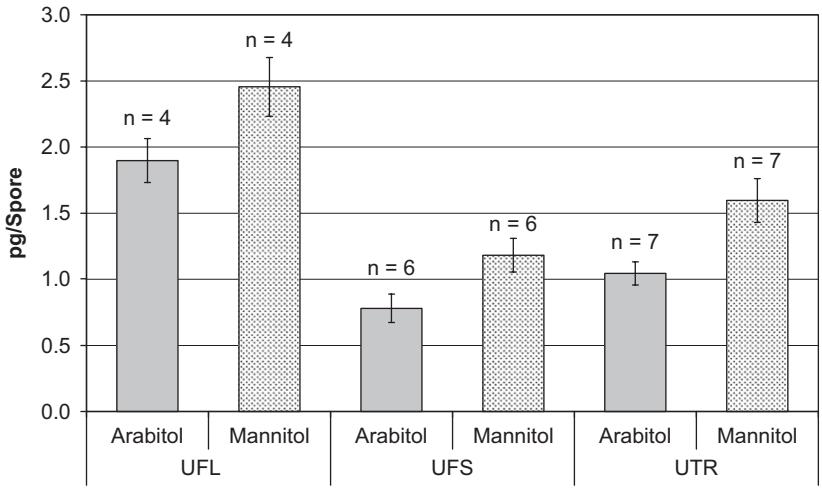

Fig. 2. Average content of arabitol and mannitol per fungal spore at the different suburban (UFL and UFS) and urban (UTR) sites.

Table 1
Site dependence of sugar alcohols in fungal spores

Sugar alcohol	UFL		UFS		UTR	
	pg spore^{-1}	std. dev.	pg spore^{-1}	std. dev.	pg spore^{-1}	std. dev.
Arabitol	1.9	0.33	1.0	0.23	0.8	0.26
Mannitol	2.5	0.44	1.6	0.43	1.2	0.31

fiber filters. Thus a parallel sampling by filter samplers and impingers for viable particles would be avoided.

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