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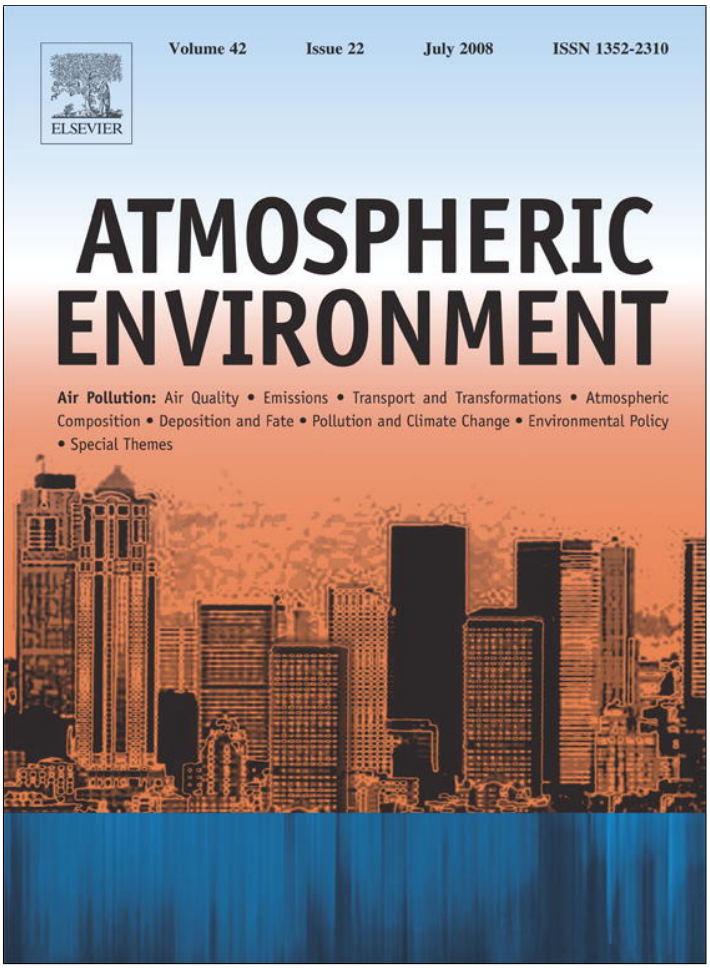

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Significant contributions of fungal spores to the organic carbon and to the aerosol mass balance of the urban atmospheric aerosol

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ABSTRACT

Fungal spores are ubiquitous components of atmospheric aerosols and are therefore also contributors to the organic carbon (OC) component and to the mass of PM_{10} (PM—particulate matter) aerosols. In this study we use spore counts and an experimentally derived factor of 13 pg C and of 33 pg fresh weight per spore for assessing quantitatively the contribution to OC and PM_{10}. The concentrations of airborne fungal spores were determined at a suburban (Schafberg) and a traffic-dominated urban site (Rinnböckstrasse) in Vienna, Austria, during spring and summer. Fungal spores OC ranged from 22 to 677 ng m^{-3} with a summer mean value of around 350 ng m^{-3} at the suburban site and 300 ng m^{-3} at the urban traffic site. At the suburban site fungal spores contributed on average 6% in spring and 14% in summer to aerosol OC mass concentration. At the traffic-dominated site fungal spores accounted for 2% of OC in spring and for 8% in summer. The fungal contribution to PM_{10} was also notable and amounted to 3% and 7% at the suburban and to 1% and 4% at the urban site in spring and summer, respectively. Impactor measurements of OC at the suburban site showed that in summer fungal spores were predominant contributors to the coarse aerosol OC, and accounted on average for 60% of the OC in the PM_{2-10} fraction. Fungal spores thus can be regarded as main components to PM_{10}, total OC and, most importantly, coarse OC even in urban areas.
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1. Introduction

The importance of the contribution of primary biogenic particles to the organic ambient aerosol has become increasingly apparent in recent years. Bioparticles might be allergenic, they might be harmful themselves or carry harmful substances (e. g. endotoxins) (Menetrez et al., 2007), and, because of their size, they might contribute significantly to particulate matter (PM) mass concentrations even if they have low number concentrations compared with non-bioparticles. If PM_{10} is regulated for public health reasons, the relative contribution of non-anthropogenic sources is also of interest for policy-makers.

Quantitative assessment of bioparticles can be achieved by several approaches, including counting individual particles such as bacteria and fungal spores under the microscope (e.g. Glikson et al., 1995; Sattler et al., 2001; Ho et al., 2005). Other biomaterials, such as leaf matter, can be estimated by measuring cellulose which is considered as a macro-tracer (Kunit and Puxbaum, 1996; Puxbaum and Tenze-Kunit, 2003). Alternatively, some minor chemical compounds can be used as micro-tracers once the numerical factors have been established. Polysaccharides (Douwes et al., 1999), phospholipids (Womiloju et al., 2003), sugar alcohols (Carvalho et al., 2003; Graham et al., 2003; Bauer et al., 2008), proteins (Boreson et al., 2004) and ergosterol (Miller and Young, 1997) have

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all been considered in this role. Quite a different approach is to build a chemical profile of each source material and to analyze the total PM by Chemical Mass Balance modelling (e.g. Simoneit and Mazurek, 1982; Simoneit, 2005; Rogge et al., 2006). Other studies (Matthias-Maser et al., 2000; Jaenicke, 2005) have estimated the contribution of bioparticles to the total aerosol in terms of number concentrations. Whichever approach is adopted, the aim is to apportion the total PM to different sources so that they can be ranked in order of their contribution.

Earlier work on atmospheric bioparticles focused on the detection of pathogenic or allergenic microorganisms or pollen. As the spores of many fungal species are known to be potential respiratory allergens (Green et al., 2003; Panaccione and Coyle, 2005; Crameri et al., 2006), most studies concerning fungal spores deal with exposure estimates for airborne fungi. Such studies report concentrations of fungal spores or of spores of different fungal species in the atmosphere (Glikson et al., 1995; Sattler et al., 2001; Ho et al., 2005). The majority of investigations into bioaerosols in general and airborne fungi in particular refer only to the culturable part of the fungal spectrum (e.g. Boreson et al., 2004; Di Giorgio et al., 1996; Lin and Li, 2000; Fang et al., 2005), however, culturable fungi (concentrations expressed as colony-forming units CFU m^{-3}), represent only a limited fraction (usually $<40\%$) of the total airborne fungal spectrum (Lappalainen et al., 1996; Lee et al., 2006). In fact, all bioaerosols—pathogenic or not, allergic or not, dead or alive, are contributors to the aerosol organic carbon (OC), and need to be assessed in terms of their mass contribution to the PM aerosol.

Fungal spores as observed from atmospheric sampling occur predominantly in the size range 2–10 μm (Burge, 2002). Size classified analyses of the sugar alcohols mannitol and arabitol which are chemical tracers for fungal spores, performed in Germany (Carvalho et al., 2003) and in Amazonia (Graham et al., 2003) showed that they mainly occur in the coarse size fraction (aerodynamic diameter, aed $>\sim$2.5 μm). However, at a site in Finland, spore tracers were also found in the fine fraction ($PM_{2.5}$) (Carvalho et al., 2003). In Amazonia both fragments and some entire spores were detected in the fine aerosol (Graham et al., 2003).

The OC associated with bacteria and with fungal spores in background samples collected at an alpine station has already been reported (Bauer et al., 2002a). Elbert et al. (2006) recently reported high contributions to PM—averaging 35% by weight—from fungal spores in Amazonia. However, of greater interest for air quality control authorities in Europe would be the extent of the contribution in densely populated areas. This paper reports spore counts and estimated masses of spores in PM for an urban and a suburban site in Vienna, Austria, and shows that here the contribution especially to the coarse fraction OC is considerable, which helps to account for some of the "unidentified" organic material in airborne PM.

2. Experimental section

2.1. Sampling sites

Samples were taken in parallel at a suburban and an urban site in Vienna from the end of March to July 2005. The suburban site "Schafberg" [16°18′10″E, 48°14′09″N] is

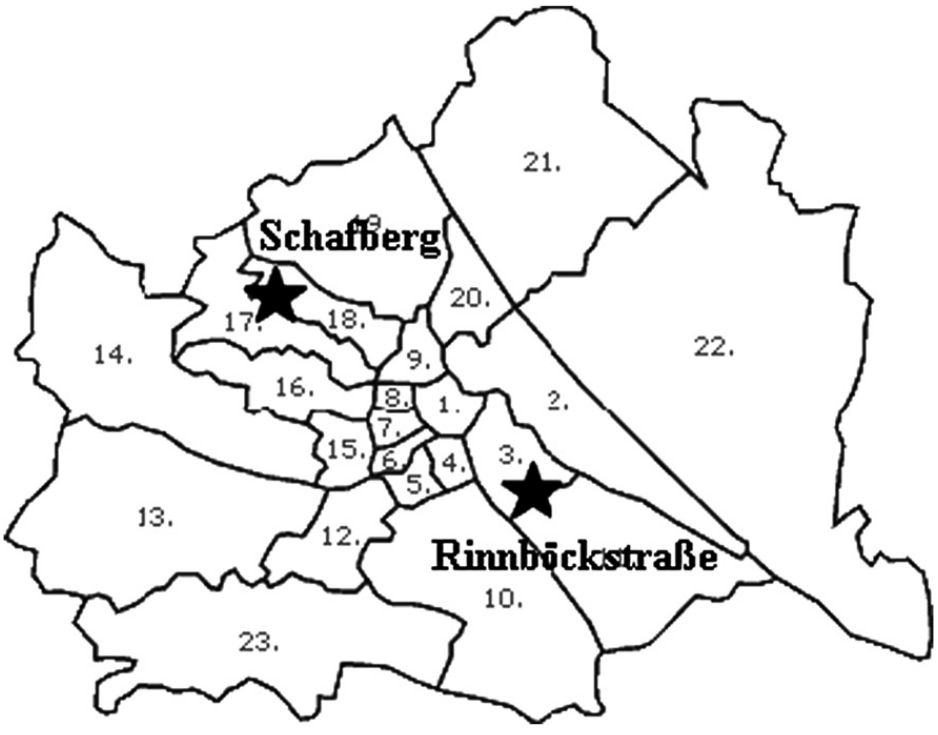

Fig. 1. Map of the city of Vienna, Austria. The urban sampling site (Rinnböckstrasse) is located near the center, the suburban site (Schafberg) is located in the north-west of Vienna.

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situated in a park-like residential area in the northwest of the city next to a park bordered by woodland. The urban site "Rinnböckstrasse" [16°24′28″E, 48°11′05″N] is situated in a mixed residential/industrial area on a grassy strip with trees and bushes between a sidewalk and a street. A major urban freeway (A23) passes within around 200 m. Both sampling sites (Fig. 1) belong to the air quality-monitoring network of the City of Vienna. The sampling height was 4 m above ground.

2.2. Sampling

Samples were collected in parallel with a Hi Vol filter sampler equipped with a PM_{10} inlet (Digitel DA80H, Switzerland) on quartz fiber filters and with an impinger sampler for bioaerosols. The quartz fiber filters (Tissuequartz 2500QAT-UP, 150 mm ∅, Pall, USA) were analyzed for PM_{10} mass, elemental carbon (EC) and OC. The filters were stored frozen (at −20 °C) until analysis. For the collection of the fungal spores modified AGI-4 impingers were used which had a 10-fold higher volume compared to the original AGI-4 impingers (Aceglass, USA), so that sampling could be continued for 24-h periods. We noted that for longer sampling times the AGI-4 loses water, thus reducing the water column in the sampler. For this reason, we constructed a sampler similar to the AGI-4, i.e. the distance between the capillary and the bottom of the impinger is the same as in the AGI-4 (4 mm), but we use a 15 cm water column to have a water reservoir sufficient for 24 h of sampling. At the end of sampling there was still a water column of 3.0–6.7 cm, on average 4.6 cm. The time dependent loss of the sampling liquid was 0.05% per minute and not 1% as reported in Lin et al. (1997). The height and diameter of the modified impinger are 310 and 50 mm, respectively, and the capillary bore is 0.8 mm. Impinger samples and filter samples could therefore be collected during the same time. The impingers were heat sterilized at 340 °C for 30 min and filled with 250 mL of sterile water. After 24 h sampling the liquid levels were still 73 mL on average, which should be sufficient to avoid substantial decreases of sampling efficiency. Atmospheric aerosols also contain surface-active compounds. Facchini et al. (2000) measured a carbon-dependent decline of the surface tension that might reduce the loss of hydrophobic spores. Comparison between pure water and a 17.5% (w/w) glycerol/water solution as sampling fluids showed the same results. Nevertheless, it is possible that a loss of hydrophobic spores may have occurred due to the use of sterile water for sample collection and the airborne spores' concentrations might even be higher. The sampling flow rate was 7 L min^{-1} resulting in sampled volumes of around 10 m^3 for 24 h. Samples were usually changed at midnight. At the suburban site 24 and at the urban site 25 samples were collected in intervals of 2–14 days.

For sampling, the impingers were connected to the sampling unit consisting of an inlet manifold, pump (KF NO150ANE, Neuberger, Germany) and dry gas meter (krom/schroder BK2.5, Elster, Austria). Immediately after sampling, 1 mL formaldehyde 37% (v/v; p.a., Merck) to every 20 mL of sampling solution was added to prevent bacterial reproduction (Kepner and Pratt, 1994, and references therein). The sampling fluid was stored in polyethylene vials at 4 °C. The microscopic analysis was performed within 2 weeks.

At the suburban site, 10 size classified aerosol samples were taken from 27 June to 30 July 2005, in parallel to the impinger samples (24 h of sampling in each case) with 7-stage low-pressure cascade impactors constructed by A. Berner, University of Vienna (model Berner LPI 80/17; Berner, 1984) covering the size range of 0.1–10 μm aerodynamic equivalent diameter with the same sampling schedule as that for spores collection. The impactor had a constant flow rate of 70 Lpm, set by a critical orifice. Sampling substrates were preheated aluminum foils (1 h, 450 °C), which were analyzed for aerosol mass, EC and OC. Particle bounce may have occurred in the size range above 2 μm because the foils had not been coated with vacuum grease as this would have made carbon analysis impossible. Samples were stored at room temperature and analyzed within 4 weeks.

2.3. Determination of spores concentration, carbon content and mass

An aliquot of 10 mL of the sampling liquid of the impinger 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 through an Anodisc filter (0.2 μm, 25 mm ∅, Whatman, England) and mounted on a microscopic slide. Then, 11 μL of anti-fading solution consisting of 5 mL glycerol 87% (p.a., Merck), 5 mL sterile water and 0.5 g ascorbic acid (Normapur, VWR) were added. 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 under the microscope. At least 60 fields (4900 μm^2 $field^{-1}$) per filter were counted, averaged and extrapolated to the whole filter area. Each impinger sample was counted twice. Spore counts per m^3 were calculated using sample aliquot fraction and total sampling volume. The detection limit was derived from counts of field blank impinger solutions. For the field blanks, impingers were filled with sterile water, taken to the field and returned without sampling. Then they were treated like a normal sample. The standard deviation of the counts of 11 blank samples was 1.47 spores mL^{-1}. From this a detection limit of 4.5 spores mL^{-1}, or expressed as air equivalent of 32 spores m^{-3} for a sampling time of 24 h (sampling volume 10 m^3) is obtained.

The carbon content of fungal spores was calculated using a conversion factor of 13 ± 2.3 pg C $spore^{-1}$ obtained earlier as the average carbon content of spores from nine airborne fungal species (Bauer et al., 2002b). For the conversion of fungal carbon to fungal mass a carbon content of 50% of the fungal dry mass (Schlegel, 1992), and a water content of 20 vol% (Sedlbauer and Krus, 2001) were assumed, resulting in an average mass per spore of 33 pg. This is in excellent agreement with the average

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volume of atmospheric spores of 34 μm^3 (spore diameters between 2.5 and 11 μm) determined by Bauer et al. (2002a), who gave the uncertainty of the OC conversion factor as of the order of 20%. For the estimate of the fungal spores mass we assume an uncertainty of around 30%.

2.4. Organic carbon determination

Total carbon (TC) was analyzed from aliquots from quartz fiber filters and from impactor foils using a combustion method originally described by Puxbaum and Rendl (1983), but with a modified detection system. The samples were combusted at 1050 °C in a pure oxygen flow and the resulting CO_2 was detected by a non-dispersive infrared (NDIR) analyzer (MAIHAK Unor 6N). More details are given by Bauer et al. (2002b). EC was determined with a two-step combustion method based on Cachier et al. (1989). During the first step the samples are kept at 340 °C in pure oxygen flow (4.8, Messer; 1.3 L min^{-1}) for 2 h to oxidize organic compounds. In the subsequent combustion step at 1050 °C EC is determined as described above for TC. OC was calculated as the difference between TC and EC. The detection limit of OC is 0.9 $\mu g\, m^{-3}$.

2.5. Determination of mass concentration

Gravimetric analysis of the quartz fiber filters was performed after 48 h of equilibration in a room with 20 ± 1 °C and 50 ± 5% relative humidity (R.H.), according to EN 12341 (CEN, 1998).

The aluminum foils used as collection substrates in the impactors were acclimatized in the laboratory for at least 12 h. The foils were weighed on a Mettler ME 3 microbalance (accuracy: $\pm 0.5\, \mu g$, reproducibility $\pm 3\, \mu g$) equipped with an ion source to reduce electrostatic charging. Equilibration times in the ion atmosphere were 3 min. Laboratory conditions were 25 °C and 16–25% R.H. Lower limit of detection for mass concentration is 0.4 $\mu g\, m^{-3}$ per size class.

3. Results and discussion

3.1. Concentrations of fungal spores

Samples were collected in parallel at the suburban and the traffic-dominated urban site in spring and summer of 2005, so that two variables could be considered: location, and time of year. On average $2.3 \times 10E4$ and $1.8 \times 10E4$ spores m^{-3} were counted at the suburban and the urban site, respectively. The results shown in Figs. 2 and 3 indicate that for both sites there were three distinct periods—April and June/July have higher number concentrations of spores (and levels of PM_{10}) while lower number concentrations were found in May. The test of significance was carried out with ANOVA. However, because of the large variability of the actual counts which suggest an influence of other factors, only the differences (at each site) between May and June/July are statistically significant at the 99% level, not significant difference was observed between the sites, as shown in Table 1. Similar variations are seen at both sites, especially in April and June, which indicate a relatively strong background source affecting both sites.

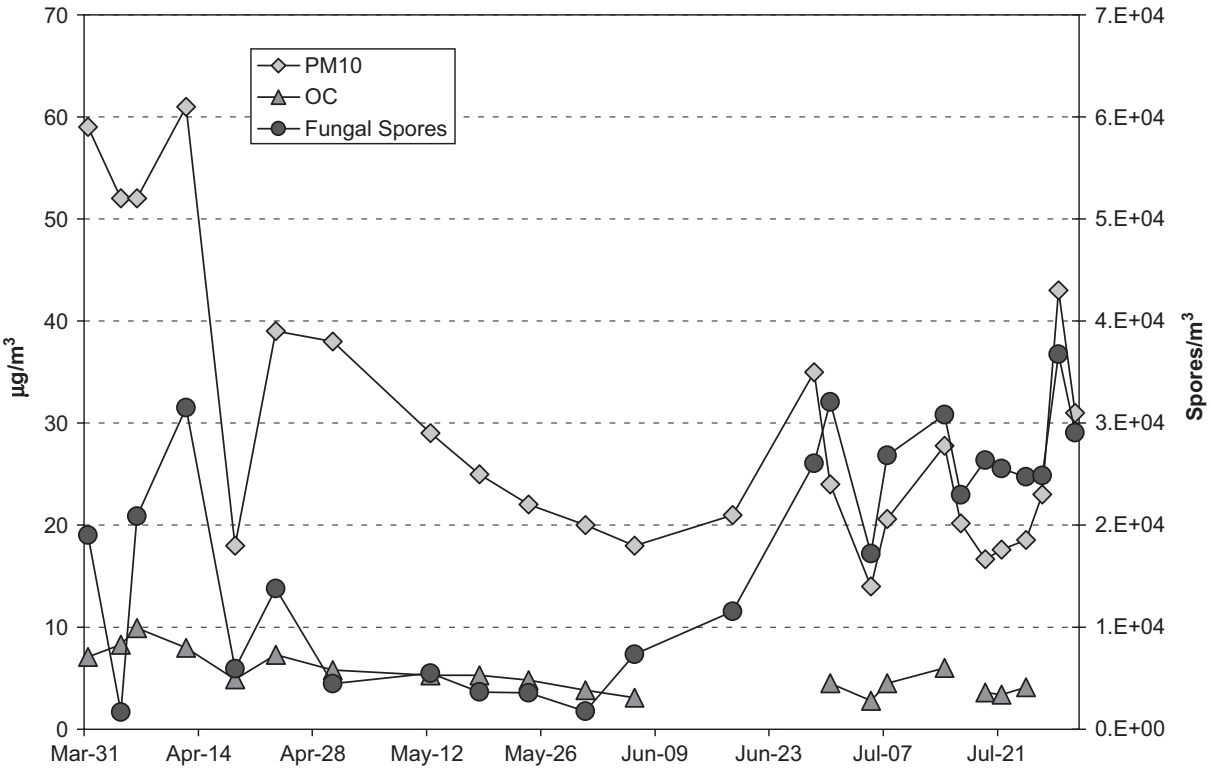

Fig. 2. Time series plot of the PM_{10}, OC_{10} and numbers of fungal spores OC at the urban site in Vienna, Austria.

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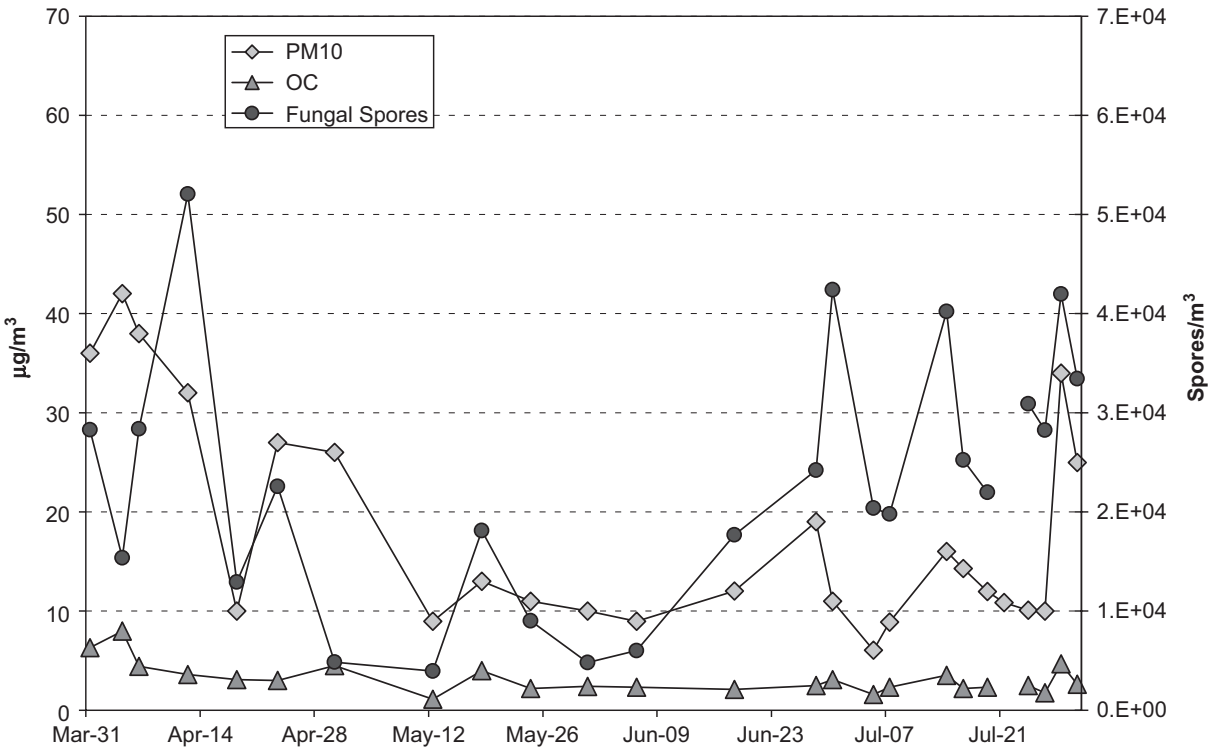

Fig. 3. Time series plot of the PM_{10}, OC_{10} and numbers of fungal spores OC at the suburban site in Vienna, Austria.

Table 1
Numbers of PM_{10} spores per m^3 (arithmetic means of the different periods), at a suburban and an urban site in Vienna, Austria

	No. of samples	Suburban spores (m^{-3})	Urban spores (m^{-3})	Significant difference
April (03.31–04.23)	6	2.7 × 10E4	1.5 × 10E4	No
SD		1.4 × 10E4	1.1 × 10E4	
May (04.30–06.06)	6	7.8 × 10E3	3.6 × 10E3	No
SD		5.4 × 10E3	1.9 × 10E3	
June/July (06.18–07.30)	12/13	2.9 × 10E4	2.6 × 10E4	No
SD		8.9 × 10E3	6.3 × 10E3	
Mean April–July	24/25	2.3 × 10E4	1.8 × 10E4	No
SD		1.3 × 10E4	1.1 × 10E4	

For statistic analysis ANOVA was used. (SD: standard deviation).

3.2. Contribution of spores to atmospheric OC concentrations and PM_{10} mass

When the mass of PM due to the spores is taken as a fraction of the total aerosol mass, or of the OC in the aerosol, a slightly different picture emerges. Using the conversion factors of 13 pg carbon per spore and 33 pg mass per spore (Bauer et al., 2002a), the contribution of spores to aerosol OC and to PM_{10} mass can be assessed quantitatively. Fungal spores OC ranged from 22 to 677 ng m^{-3} with summer mean values of around 350 ng m^{-3} at the suburban site and 320 ng m^{-3} at the urban traffic-dominated sites.

Because the total mass of PM, and also of OC, is generally higher at the urban site than at the suburban site (Figs. 1 and 2) which reflects the urban contribution added to a background component originating outside the urban area, and because the number concentrations of spores are generally higher at the suburban site, the difference in the fraction of OC due to spores is seen more clearly. For two of the three sampling periods this fraction is significantly higher (at the 95% level for the first and at the 99% level for the third period) at the suburban site, by a factor of two or even three (Table 2) with, for example, 8.5% at the suburban and 2.6% at the urban site in April, and 15% at the suburban and 8.4% at urban site over the June/July period. So, it is not so much an increase in the spore count but rather the decreased PM level, which results in the significantly increased contribution of fungal spores to the aerosol OC in the urban background. During the sampling period the mean contribution of fungal spores amounted to 10% and 4.3% to OC_{10} and to 4.8% and 2.3% to PM_{10} mass at the suburban and at the urban site, respectively.

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Table 2
Fraction of OC in PM_{10} due to fungal spores, as % m/m, at a suburban and an urban site in Vienna, Austria

	No. of samples	Suburban spores (m^{-3})	Urban spores (m^{-3})	Significant difference
April (03.31– 04.23)	6	8.5	2.6	Yes, *P* 0.95
SD		5.7	1.6	
May (04.30–06.06)	6	3.9	1.0	No
SD		1.7	0.9	
June/July (06.18–07.30)	12/13	14.7	8.4	Yes, *P* 0.99
SD		3.0	1.1	
Mean April–July	24/25	10.4	4.3	Yes, *P* 0.99
SD		5.8		3.4

(SD: standard deviation).

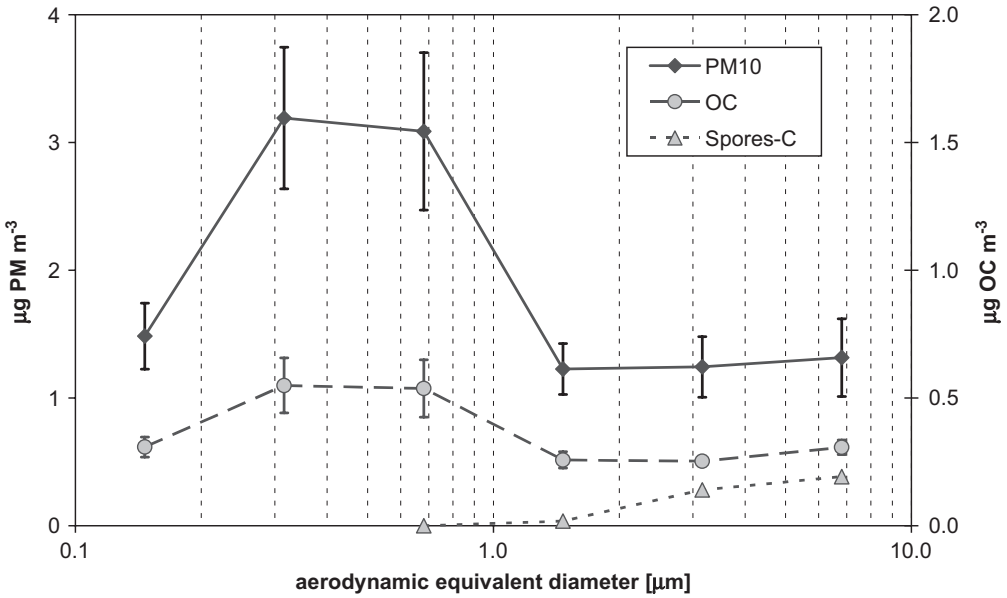

Fig. 4. Mean concentrations of PM_{10} aerosol mass, OC and fungal spores-C determined at the suburban site, as a function of particle size. 10 samples were collected in June/July 2005. The error bars indicate the standard error. Size ranges of stages: 0.1–0.21; 0.21–0.46; 0.46–1; 1–2.1; 2.1–4.6; 4.6–10 μm, indicated are the geometric mean diameters of each stage. The dotted line represents the calculated values for the spores assuming that 10% could be attributed to the fine aerosol in the size range from 0.46 to 2.1 μm, and 90% to the coarse aerosol (2.1–10 μm). The fractions attributed to the two size classes in the coarse aerosol were calculated according the OC concentrations.

3.3. Contribution of spores to coarse aerosol ($PM_{2.1-10}$) OC and total mass ($PM_{2.1-10}$)

At the suburban site l0 size classified samples were collected with low-pressure cascade impactors from June 27 to July 30 in parallel with the impingers. The cut-off sizes of the impactors are given in the caption of Fig. 4. For the size discrimination between fine and coarse aerosol the cut-off of 2.1 μm was used, the fractions between 0.1 and 2.1 μm aed summed to give "fine aerosol" and the fractions between 2.1 and 10 μm aed gave "coarse aerosol", labelled as PM_f and PM_c, respectively. As the spores were collected with a liquid impinger without size discrimination, it was necessary to assume an estimate of 10%/90% for the mass distribution of spores between these two fractions (Womiloju et al., 2003; Carvalho et al., 2003; Graham et al., 2003). The size classified samples showed that the coarse fraction ($PM_{2.1-10}$) contained on average around 28% of the OC and 23% of the aerosol mass. The data and the results are presented in Fig. 4 and Table 3. Fig. 4 shows the size distributions of total mass and OC as averages of 10 sets of data. The dotted line for the OC attributed to the spores in each size fraction is calculated using the 90/10 distribution mentioned above. In the fine aerosol fungal spores were attributed to the size range 0.46–2.1 μm, the distribution of the spores-OC in the coarse aerosol was calculated according the OC concentrations.

The fungal spores do indeed account for a large part of OC in the coarse fraction—a mean of 60 ± 3% of coarse OC (OC_c) and a still considerable part of coarse PM (PM_c) mass, 40 ± 5% ($n = 10$). The surprising uniformity of the experimental results and the large number of sample sets allows one to arrive at these rather small standard errors. On the other hand, the spores account for a smaller part, 18 ± 1% of the total OC in PM_{10}, and 9.3 ± 1%, of the total PM_{10} material, which is normally measured for air quality assessment purposes.

3.4. Comparison with results from other studies

Semiquantitave estimates of the contribution of fungal spores to OC have been reported by Glikson et al. (1995)

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Table 3
Contribution of spores to coarse and fine OC and to coarse and fine PM aerosol at the suburban site from June 27 to July 30 (SEM: standard error of mean)

Measured quantities ($\mu g\,m^{-3}$)					
	PM_{10}	OC_{10}	PM_{2-10}	OC_{2-10}	OC_{spores}
Mean	11.5	2.2	2.6	0.56	0.37
SEM ($n = 10$)	2.1	0.3	0.5	0.04	0.03

Estimated contributions from spores (% m/m)						
	In OC_c	In OC_f	In OC_{10}	In PM_c	In PM_f	In PM_{10}
Mean	60	2.5	18	40	1.2	9.3
SEM ($n = 10$)	3.4	0.2	1.4	5	0.1	0.9

Size classified concentrations of PM and OC were obtained by sampling with a 7-stage low-pressure cascade impactor.
The contribution of spores to fine and coarse fractions of PM and OC (PM_f, PM_c, OC_f, OC_c) were calculated, assuming 10% of the spores to be in PM_f and 90% in PM_c.
PM_f: PM fine; $<2.1\,\mu m$ aed; OC_f: OC fine; $<2.1\,\mu m$ aed. OC_{10}: OC in PM_{10}. aed: aerodynamic equivalent diameter.

and Womiloju et al. (2003). Our results are based on actual spore counts while Womiloju et al. (2003) analyzed phospholipids as tracers for fungal and pollen fragments in the fine aerosol and found 4–11% of PM_c to be due to spores. Glikson likewise reported a contribution in the range 5–10% of PM_c. Our finding of higher fungal spore contributions to OC at the suburban site compared to the urban traffic site is in contrast to the results of Womiloju et al. (2003) who reported higher contributions of fungal cells to OC at an urban/traffic site in Toronto, Canada, with lower ones at a suburban/commercial site and lowest at a rural site around 100 km from Toronto. Elbert et al. (2006) similarly measured a chemical marker, total mannitol, and then estimated the mass of spores in their samples collected from Amazonia. Nevertheless, their end results of around 45% by night and 25% by day for the contribution of spores to the total coarse fraction, PM_c, is in surprisingly close agreement with our result for Vienna, of 40% for a 24-h average.

4. Conclusions

In this study we find that fungal spores are main constituents of coarse organic particulate matter (PM) in the summer season, both in urban and in suburban environments, and contribute an appreciable fraction to total aerosol mass in the coarse size fraction. They account for around 40% m/m of PM_c and 60% of OC_c. Even in the total PM_{10} fraction, they can account for up to 21% of the mass. The occurrence of substantial amounts of biogenic carbon especially in the coarse aerosol is also substantiated by radiocarbon measurements (^{14}C) of size-fractionated urban aerosol (Endo et al., 2004). In our study, fungal spores are shown to be the major constituents of this biogenic carbon, in particular during the warm season. Fungal spores therefore are not only interesting from a health perspective, but also because of their significant contribution to the carbon mass balance in the coarse size range.

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