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Contribution of Fungal Spores to Organic Carbon Aerosol in Indoor and Outdoor Environments in the Greater Cincinnati Area

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ABSTRACT

Airborne fungi may contribute to the organic carbon (OC) content of particulate matter, which make them relevant to air pollution and climate change issues. This study aimed at assessing the contribution of fungal spores to the inhalable OC in indoor and outdoor environments in the Cincinnati metropolitan area. The contribution was calculated assuming that carbon content per fungal spore was 13 pg (derived from a report from Austria). Air samples were collected from 18 homes during summer. At each site, two air samples were simultaneously taken using Button Personal Inhalable Samplers for 24 hours. One sample was subjected to the total fungi enumeration and the other one was analyzed for OC with Thermal-Optical Transmittance technique. A (1-3)-β-d-glucan analysis was also conducted for indoor air samples using *Limulus* Amebocyte Lysate assay. Additionally, a questionnaire survey was performed on the various factors that might affect the indoor aerosol OC level. The total OC concentration ranged from 0.5 to 19.0 μg/m^3 in outdoor air and from 0 to 36.2 μg/m^3 in indoor air. The concentration of OC originating from fungal spores ranged from 3.8 to 958.4 ng/m^3 in outdoor air while the respective range in indoor air was 0.8 to 351.2 ng/m^3. The (1-3)-β-d-glucan was present indoors at levels ranging from 82.1 to 41,910 pg/m^3. In contrast to studies performed in Austria, Australia and Britain, we found that fungal spores contribute rather little to the local outdoor OC. This could be due to different sampling instruments used for fungal spore sampling and regional differences in fungal spore concentrations. Even smaller contribution of fungal spores was found for indoor OC (average of 0.21%). Statistical analysis revealed that cigarette smoking was a significant factor for the indoor organic carbon level. The results indicate that smoking contributes to the indoor OC level more significantly than fungi.

Keywords: Organic carbon aerosol; Fungal spores; (1-3)-β-d-glucan.

INTRODUCTION

Biological aerosol particles are important not only because of their role in spreading human, animal and plant diseases, and in causing allergies, but also because they are believed to significantly influence the organic carbon (OC) component of an atmospheric aerosol.

In recent years, there has been increasing interest in quantifying the contribution of biological content to atmospheric organic aerosol. Bauer *et al.* (2002) described a procedure for determining the OC content of fungal spores frequently observed in the atmosphere. Spores of *Cladosporium* sp., *Aspergillus* sp., *Penicillium* sp., and *Alternaria* sp. – the four predominant and representative airborne fungal genera – were analyzed for their carbon content, which was estimated to be 13 pg per spore on average. Using this value and the spore counts, Bauer *et al.* (2008a) assessed quantitatively the contribution of fungal spores to PM_{10} as well as to OC in PM_{10}; the measurements were performed at suburban and urban sites in Austria. At the suburban site, fungal spores contributed on average 6–14% to the OC-aerosol mass concentrations. At the traffic dominated urban site, fungal spores accounted for 2–8% of OC. Measurements of OC at the suburban site showed that in summer fungal spores were primary contributors to OC of the PM_{10} fraction, and accounted on average for 60% of the OC in the $PM_{2–10}$ fraction. Based on the analysis of samples collected in London, U.K., Battarbee *et al.* (1997) found that biological particles might form a significant fraction of the urban aerosol (> 20% by particle number), especially after rain events. In a study conducted in Brisbane, Australia, Glikson *et al.* (1995) showed that fungal spores dominated the bioaerosol counts in a particle size range of 2–10 μm. At peak seasons, the total bioaerosol counts represented 5–10% of the PM_{10} mass. The above studies all used microscopic techniques for quantifying biological particles.

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Analysis of chemical composition of biological particles has also been used to assess their presence in atmospheric aerosol. Womiloju *et al.* (2003) analyzed phospholipids in different fungal and pollen genera. The concentrations of phospholipids suggested that fungal cells and pollen grains were responsible for 12–22% of the organic carbon fraction or 4–11% of the aerosol mass. Bauer *et al.* (2008b) used arabitol and mannitol, which are common storage substances in fungal spores, for the quantification of fungi in atmospheric PM_{10}. The average content of arabitol per spore was 1.2 pg and the average content of mannitol was 1.7 pg per spore. Ergosterol – a component of fungal cell membranes – was utilized as a biomarker by Cheng *et al.* (2008) and Lau *et al.* (2006) to determine fungal prevalence in ambient air. An empirical conversion factor (for estimating ambient fungal prevalence from filter ergosterol concentration) of 0.191 ± 0.040 pg ergosterol per spore was obtained.

Cheng *et al.* (2009) investigated fungal contribution to OC aerosol in a subtropical city in Hong Kong. The carbon content of fungal spores was calculated using a laboratory-generated weighted-average carbon conversion factor for each fungal genus (equivalent OC content per spore). This factor varied from 3.6 to 201.0 pg carbon per spore, depending on fungal genus. The fungal concentration was found to contribute to the total OC in $PM_{2.5}$, $PM_{2.5–10}$, and PM_{10} at fractions of 0.1%, 1.2%, and 0.2%, respectively. Heald and Spracklen (2009) studied the contribution of primary biological aerosol particles (PBAP) to the global budget of organic aerosol. Concentration of mannitol, a biotracer for fungal spores, was used to constrain the first global model simulation of PBAP from fungi. Fungal spores were found to contribute 23% of total primary emissions of organic aerosol, or 7% of the fine-mode particle source ($PM_{2.5}$).

(1–3)-β-d-glucan is a biologically active polyglucose molecule comprising up to 60% of the cell wall of fungi and some soil bacteria and plants. (1–3)-β-d-glucan levels in samples of airborne or settled dust have been used in several studies as a surrogate measure of mold exposure (Fogelmark *et al.*, 2001; Schram-Bijkerk *et al.*, 2005a, b; Iossifova *et al.*, 2007; 2009).

Inorganic and carbonaceous components in indoor and outdoor particulate matter were investigated by Lazaridis *et al.* (2008) in Norway. Aerosol measurements were performed at two dwellings in the suburbs of the Oslo metropolitan area during summer/fall and winter/spring periods of 2002–2003. The concentration of OC was higher indoors than outdoors in the fine ($PM_{2.5}$) and coarse (PM_{10}) particle fractions, whereas elemental carbon was higher indoors only in the coarse particle fraction. In regards to the carbonaceous species, local traffic and secondary organic aerosol formation were believed to be the main sources outdoors, whereas in indoor environments, combustion activities such as preparation of food, burning of candles, and cigarette smoking were the main sources. Other studies have also reported smoking as a major source for indoor OC (Na *et al.*, 2005).

Daily (24-h average) indoor and outdoor $PM_{2.5}$ samples were collected (Cao *et al.*, 2005) in six residences in Hong Kong in March of 2004 for OC and elemental carbon. Low indoor-outdoor correlations (r) were found for OC (0.55), indicative of different OC sources indoors. A simple model implied that about one-third of carbonaceous particles in indoor air originated from indoor sources.

Although many studies have been conducted to assess the indoor-outdoor relationship of carbonaceous particles and contributing factors of indoor carbonaceous species, very limited information can be found on the contribution of fungal spores on indoor OC aerosol. The aim of this study was to assess the contribution of fungal spores to the outdoor and indoor OC aerosol through an air monitoring campaign in the Greater Cincinnati area. Fungal spores were identified and enumerated using a high-resolution light microscope and an established conversion factor of 13 pg carbon per spore was also used in this study. Additionally, we utilized (1–3)-β-d-glucan as a chemical marker for fungi.

EXPERIMENTAL METHODS

Sampling Sites

Eighteen homes in the Greater Cincinnati area were selected for this investigation from the cohort of a population-based study entitled, "Mold Exposure in Homes and the Development of Children's Atopy and Asthma" described in detail by Reponen *et al.* (2010). In brief, the study included indoor air and dust sampling and a walkthrough survey on home characteristics. Indoor air samples were analyzed for fungal spores and (1–3)-β-d-glucan. For the purpose of the sub-study reported here, homes that had their indoor assessment between June and August of 2008, were subjected to additional assessment of OC in indoor and outdoor air. Summer was selected because of elevated outdoor fungal spore concentrations (Adhikari *et al.*, 2003).

During each home visit, a questionnaire was administered to a parent and included questions about number of people living in the house, the presence of pets, the use of gas stove for cooking, and the frequency of frying food in the house (see Table 1). Moldy odor, signs of moisture damage, visible mold and cigarette smoking were recorded on a checklist by the study staff. Due to the sensitivity of the issue (people often lie about their smoking habits), evidence of smoking was observed as smell of tobacco smoke or presence of ash trays. In addition, affirmative or negative response to smoking indoor was collected from the parents through a questionnaire. The age of the houses ranged from 3 to 127 years, their floor areas ranged from 1183 to 3388 ft^2.

Sampling Procedures

Both indoor and outdoor samples were collected simultaneously for 24 hours in each home using Button Inhalable Aerosol Samplers (SKC, Inc., Eighty Four, PA, USA) operated at a flow rate of 4 L/min. The Button Sampler has a curved porous inlet that provides low dependence of the sampling efficiency on the wind velocity and direction (Aizenberg *et al.*, 1998). The Button Sampler was evaluated for the collection of different bioaerosols side by side with the widely used Rotorod Sampler (Sampling Technologies,

Table 1. Presence of potential indoor sources of organic carbon inside homes*.

Home	Cigarette smoking	Mold at home	Gas stove	Frying food	#of people	Pet at homes	Fungal spore (spores/m^3)	β-d-glucan (pg/m^3)	Organic carbon (μg/m^3)
A	Y	N	N	1/wk	4	cats	760	2791	24.2
B	N	N	N	3/wk	4	No animals	5118	12640	13.6
C	Y	Y	N	1/wk	5	No animals	1138	1484	36.2
D	N	Y	N	0/wk	4	No animals	228	1191	5.7
E	N	N	N	0/wk	5	dog	270	1048	15.8
F	Y	Y	Y	3/wk	6	dog	33510	41910	21.2
G	N	N	N	0	7	No animals	293	1309	10.7
H	N	N	N	0	5	dog	10900	701.9	< LOD
I	N	N	Y	2/wk	3	dog + other furry animal	5348	154.7	17.0
J	N	N	Y	10/wk	4	No animals	96	82.08	12.3
K	N	N	N	0	7	Cat+ other furry animal	107	8466	12.8
L	N	Y	Y	0	5	cat	487	.	10.7
M	N	N	N	1wk	6	dog	974	1220	6.3
N	N	Y	N	7/wk	6	No animals	130	2387	16.5
O	N	Y	N	1/wk	6	No animals	49	3749	12.7
P	N	Y	Y	1/wk	8	No animals	627	1780	14.2
Q	Y	N	N	0	4	Dog + other furry animals	418	15680	12.6
R	N	Y	N	2/wk	9	No animals	199	7049	22.3

* Home L does not have (1-3)-β-d-glucan data since the air sample for (1-3)-β-d-glucan was lost.

St. Louis Park, MN, USA) and was found efficient for personal sampling of outdoor aeroallergens, especially those of relatively small particle sizes (Adhikari *et al.*, 2003).

For outdoor sampling, the two samplers were placed onto a sampling tripod under a rain shield (7.5 cm below) connected by tubes with a rain and noise-insulated enclosure containing sampling pumps (model 224-PCXR4, SKC Inc.). The inlets were oriented vertically. Each outdoor sampling station was set up 1–2 m away from the house outside wall. For indoor sampling, two Button Samplers were placed next to each other with a noise-insulated enclosure containing sampling pumps (model 224-PCXR4, SKC Inc.). Indoor sampling was performed in the residents' primary activity room (a living room in most cases). The residents stayed at home and performed their normal activities during the measurements.

Out of the two Button Sampler collection filters obtained in each sampling station, one filter (3.0 μm polycarbonate, Millipore Inc., Billerica, MA, USA) was subjected to the fungal spore enumeration and (1-3)-β-d-glucan analysis (for indoor samples only), while the other (quartz, SKC Inc.) was analyzed for OC. The quartz filters were pre-baked at a temperature of 550°C for at least for 24 hours before sampling (Schauer *et al.*, 2000) to eliminate traces of OC contaminants.

Determination of the Concentration of Airborne Organic Carbon

The OC analysis of quartz filters was conducted using the Thermal-optical Transmittance (TOT) technique (Chester LabNet Inc., Oregon, USA). The first phase of the analysis was performed in pure helium. All carbon collected from the filter is oxidized to carbon dioxide and then reduced to methane. The methane is measured using a flame ionization detector (FID). During the first phase, a red light laser (670 nm) and photocell are used to monitor transmittance of the filter, which typically darkens as refractory OC chars and then lightens as the char burns off. The second phase takes place in a mixture of 98% helium and 2% oxygen. After a slight cooling, filter is further heated to 900°C. During the second phase, once the light transmission through the filter equals that seen as the beginning of the first phase, the OC/EC split is set. CO_2 measured at the first phase and during the second phase prior to the split is considered as organic carbon. CO_2 measured after the split is considered elemental carbon. The limit of detection (LOD) of OC was 0.91 μg/m^3.

Determination of Fungal Spore Concentration and (1-3)-β-d-glucan Content

After sampling, the collected particles were extracted from the polycarbonate filters with 5 mL extraction solution (sterile filtered water containing 0.05% Tween 80). The extraction was accomplished by agitation for 15 min using an ultrasonic cleaner (FS20, Fisher Scientific, Pittsburgh, PA, USA) followed by vortexing for 2 min. A 2-mL volume of the extracted solution was filtered through mixed cellulose ester filter, which was made transparent using acetone vapor. The fungal spore enumeration was performed on 40 randomly selected microscopic fields at 400× or 1000× using a high-resolution light microscope (Labophot 2, Nikon Corp, Japan).

The air samples were analyzed for (1-3)-β-d-glucan using Limulus Amebocyte Lysate assay as described by Iossifova *et al.* (2007). An aliquot of 0.5 mL of the air sample extract was used for each analysis. The samples were spiked with (1-3)-β-d-glucan standard of 50 pg/mL to assure that there was no inhibition or enhancement between the extract and the reagents.

Contribution of Fungal Spores to Organic Carbon Mass in Aerosol

The contribution of fungal spores to organic carbon mass in the air (%) was calculated as follows:

$$0.0001 \times 13 \times C_{\text{fungal spore}}/C_{OC} \quad (1)$$

The equation reflects a previously established average carbon content of 13 pg per spore. $C_{\text{fungal spore}}$ is expressed in spores/m^3 and C_{OC} is expressed in μg/m^3.

The contribution of (1-3)-β-d-glucan to organic carbon (%) was calculated as follows:

$$0.0001 \times C_{\text{(1-3)-β-d-glucan}}/C_{OC} \quad (2)$$

where $C_{\text{(1-3)-β-d-glucan}}$ is expressed in pg/m^3.

Statistical Analysis

To assess the impact of various factors on the indoor OC aerosol level, data were analyzed using JMP (read "jump", statistical software from SAS Inc. (Cary, NC, USA) that links dynamic data visualization with robust statistics). The dependent variable was the OC aerosol concentration in the house. The independent variables were: cigarette smoking (yes/no), visible mold at home (yes/no), gas stove (yes/no), frying food frequency (number of times per week), number of people at home (numerical parameter), fungal spore concentration (spores/m^3), (1-3)-β-d-glucan concentration (pg/m^3) and the presence of cat or dog (yes/no). The data were analyzed with fit model using standardized least square method. The independent variables with p-value less than 5% were considered to have a significant impact on the dependent variable (OC level). Descriptive statistics including geometric means and 95% confidence intervals were calculated for the independent variables.

RESULTS

Fig. 1 shows inhalable OC concentrations measured in indoor and outdoor air of study homes. The outdoor aerosol OC concentrations ranged from 0.5 to 6.6 μg/m^3 except for home J which had 19.0 μg/m^3. The indoor OC aerosol concentrations ranged from 1.9 to 36.2 μg/m^3 and exceeded the respective outdoor concentration except for home J and home H (the indoor OC concentration for home H was < LOD). The geometric mean of the indoor-outdoor (I/O) ratio calculated for the OC aerosol concentration was 4.32. Homes C, A, R, and F exhibited the highest indoor OC aerosol concentrations. Among the homes of C, A, R, and F, with the exception of home R, all the homes had indoor cigarette smoking sources, and, with the exception of home A, they all had visible mold indoors. This indicates that cigarette smoking and visible mold might be significant sources of organic carbon indoors.

Fig. 2 shows inhalable fungal spore concentrations measured in indoor and outdoor air of study homes. The fungal spore concentrations in indoor environments ranged from 48 to 33,510 spores/m^3 with an arithmetic mean of 3,267 spores/m^3 and a geometric mean of 557 spores/m^3. The fungal spore concentrations in outdoor environments ranged from 12 to 7,373 spores/m^3 with an arithmetic mean of 2,354 spores/m^3 and a geometric mean of 570 spores/m^3. The geometric mean of I/O-ratio for the airborne fungal spores was 0.96.

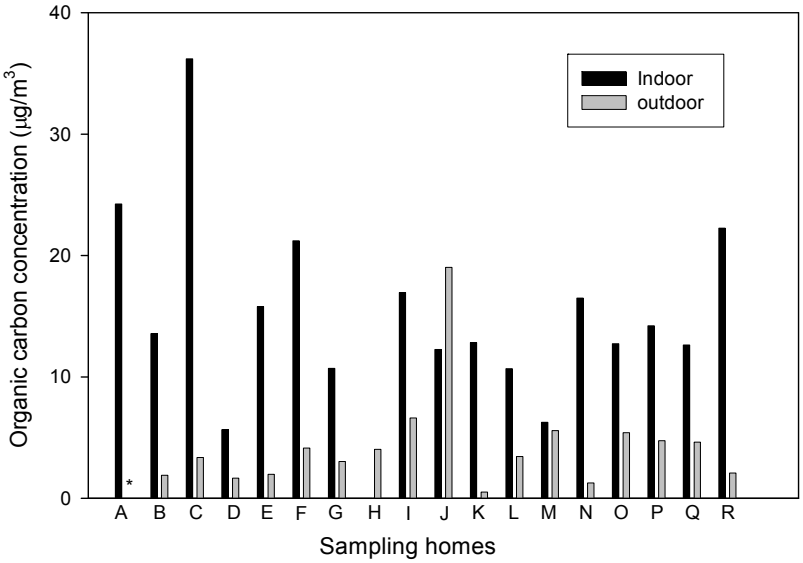

Fig. 1. Organic carbon concentration in the air inside and outside sampling homes. For home H, the indoor organic carbon concentration in the air is 0. * indicates a missing data point for organic carbon concentration (home A did not allow for outdoor sampling).

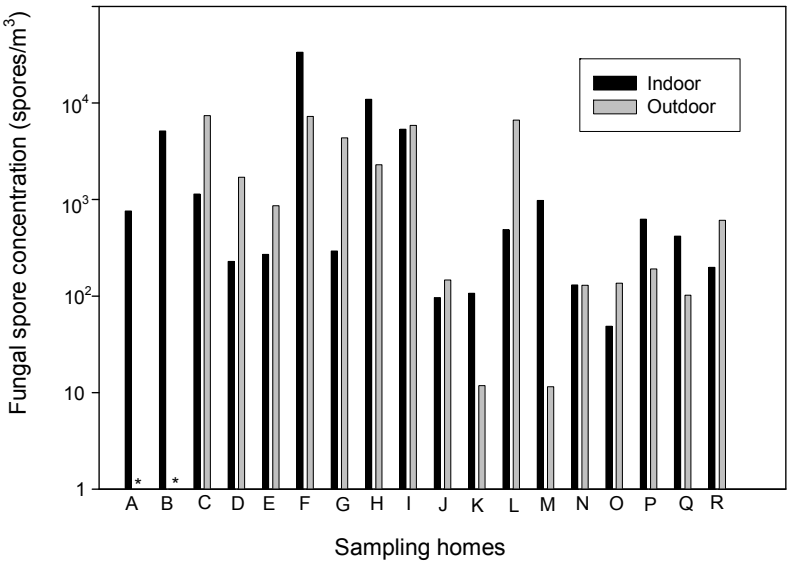

Fig. 2. Fungal spore concentrations in the air inside and outside of sampling homes. * indicate a missing data point for outdoor air concentration (Home A did not allow outdoor air sampling; fungal spore filter from home B was damaged due to rain water).

Table 2 presents the contributions of fungal spores to the inhalable OC mass in indoor and outdoor air environments. The contributions of total fungal spores to the OC-aerosol ranged from 0 to 0.49% in indoors with an average of 0.21% (by mass). The corresponding range for (1-3)-β-d-glucan was 0 to 0.20% with an average of 0.04% (by mass). In the outdoor environment, the contribution of fungal spores to the OC aerosol mass ranged from 0 to 2.85% with an average of 0.87%.

To study the factors affecting the OC-aerosol level inside the homes, the data were analyzed with a fit model using standardized least square method. The independent variable was "indoor aerosol OC". The dependent variables were "cigarette smoking", "mold at home", "gas stove", "frying food", "# of people", "pet at home", "outdoor aerosol OC", "1-3-β-d-glucan", and "indoor fungal spore level". The variables that insignificantly affected the "indoor aerosol OC" (p-value > 0.20) and did not impact the fitness of the model (R-square of the model is larger than 0.5) were removed from the model. The remaining dependent variables were utilized in the model again. The R-square of the new regression line was 0.63. This indicates that the selected fit model could accurately predict the actual OC concentrations in the aerosol in indoor environment. Table 3 shows the statistical data for the regression coefficients (a, b, c, d, e) for the fit model Y = aX1 + bX2 + cX3 + dX4 + eX5. Y represents indoor aerosol OC level. X1, X2, X3, X4, and X5 represent the independent variables: cigarette smoking, frying food, # of people, outdoor OC and (1-3)-β-d-glucan. The second column (coefficient) shows the mean values of the coefficients a, b, c, d, e. The fourth column (95% CI of coefficient) shows the range in which the value of the coefficient belongs with 95% confidence. Among all the independent variables, only cigarette smoking had a significant impact on the indoor OC aerosol concentration (p = 0.002). The indoor OC level in homes with smokers was almost twice higher than that in homes with "no smokers" (mean 23 μg/m^3 vs. 13 μg/m^3). The indoor OC level in homes with "mold at home" was higher than in homes with "no mold at home" for either "smokers" or "non-smokers" homes (data not shown). However, this difference was found statistically insignificant, likely due to the limited sample size of this study. It is worth to point out that "(1-3)-β-d-glucan level" and "indoor fungal spore concentration" had no significant impact on the indoor OC level.

DISCUSSION

We found that the relative contribution of fungal spores to the outdoor OC aerosol mass did not exceed 2.85% with an average of 0.87%. In contrast to the findings of several studies (Glikso *et al.*, 1995; Battarbee *et al.*, 1997; Bauer *et al.*, 2008a), our investigation conducted in the Greater Cincinnati area showed that fungal spores are not a significant component of aerosol organic carbon in outdoor air. Only Cheng *et al.* (2009) has previously reported results in the same range as obtained in the present study. The quantitative discrepancies can be attributed to different sampling methodology as well as regional differences with respect to the proportion of biological vs. non-biological particles in the atmosphere.

According to Bauer *et al.* (2008a), fungal spores contribute significantly (on average 6–14%) to the ambient OC concentration in a suburban Australia site. At a traffic dominated site in Austria, the contributions of fungal spores to OC ranged from 2 to 8%. Bauer *et al.* (2008a) reported a range of 2 to 10 μg/m^3 for aerosol OC concentrations in PM_{10} fraction obtained outdoors in Vienna, which was comparable to those in our study (0.5 to 6.6 μg/m^3 for most homes). However, fungal spore concentrations were higher than in our study: the average

Table 2. Contribution of fungal spores and (1-3)-β-d-glucan to organic carbon concentration in the air.

Home ID	Contribution of fungal spores to OC (%)		Contribution of (1-3)-β-d-glucan to OC (%)
	Outdoor air	Indoor air	Indoor
A	*	0.04	0.01
B	*	0.49	0.09
C	2.85	0.04	0.00
D	1.33	0.05	0.02
E	0.57	0.02	0.01
F	2.28	2.05	0.20
G	1.86	0.04	0.01
H	0.74	**	**
I	1.15	0.41	0.00
J	0.01	0.01	0.00
K	0.03	0.01	0.07
L	2.51	0.06	
M	0.00	0.20	0.02
N	0.13	0.01	0.01
O	0.03	0.00	0.03
P	0.05	0.06	0.01
Q	0.03	0.04	0.12
R	0.38	0.01	0.03
Average ± std	0.87 ± 1.00	0.21 ± 0.50	0.04±0.05

* missing data point (see Figs. 1–2).
** data is unavailable since for OC was below the detection limit for home H.

Table 3. Statistical data about the regression coefficients (a, b, c, d, e) in the fit model Y = aX1 + bX2 + cX3 + dX4 + eX5. Y represents indoor aerosol OC level. X1, X2, X3, X4, and X5 represent the independent variables: cigarette smoking (Yes/No), frying food (number of times/week), # of people (numerical parameter), outdoor OC (μg/m^3) and (1-3)-β-d-glucan (pg/m^3).

variable	coefficient	standard deviation of coefficient	95% CI of coefficient	P-value
Cigarette smoking [No]*	–8.969	2.211	(–13.895, –4.042)	0.002
Frying food	0.774	0.549	(–0.450, 1.997)	0.189
# of people	2.172	1.028	(–0.118, 4.462)	0.061
Outdoor OC	0.620	0.380	(–0.227, 1.466)	0.134
(1-3)-β-d-glucan	0.000	0.000	(–0.001, 0.000)	0.129

* The results of cigarette smoking [No] were based on observation (smell of smoke or presence of ash tray) at first and affirmative or negative response to smoking indoor was later collected from the parents through a questionnaire.

outdoor fungal spore concentrations measured at suburban and urban sites in Vienna were 2.3 ×10^4 and 1.8 × 10^4 spores/m^3, i.e., ten-fold higher than ours. The differences in the fungal spore concentrations might be partially attributed to the different bioaerosol sampler used by Bauer *et al.* (who used a glass impinger). However, the investigators did not identify the manufacturer of the impinger and therefore, the inlet and collection efficiencies are unknown. They reported that about one third (73 of 250 mL) of the collection fluid remained in the impinger at the end of the 24-hour sampling period; this might have affected the collection efficiency (Grinshpun *et al.*, 1997), which helps explain the differences. The rest can be attributed to climatic differences. Vienna lies within a transition of oceanic climate and humid continental climate. The city has warm summer with moderate precipitation. The city of Cincinnati has a subtropical humid weather in summer.

Our results on fungal spore contribution to the OC aerosol are not in a perfect agreement with the data of Battarbee *et al.* (1997), who collected aerosol with the Burkard seven-day volumetric impactor (Burkard Manufacturing, Rickmansworth, Hertfordshire, UK) and enumerated biological and non-biological particles using microscopic counting. Battarbee *et al.* reported that biological particles comprise over 20% of the urban aerosol after rain events. Compared to the ButtonSampler, the Burkard impactor collects a different size fraction as its cutoff size is 5.2 μm (Willeke and Macher, 1999). Therefore, the results by Batterbee *et al.* are not fully comparable with the current study.

Glikson *et al.* (1995) collected air samples on Teflon filters, which were analyzed for particle mass by weighing and for fungal spores by microscopic counting. The investigators found that total bioaerosol counts contributed up to 10% of the PM_{10} mass. The comparison of the results of our study to that of Glikson *et al.* are not meaningful though because they did not identify the type of particle sampler nor did they explain the conversion of fungal

spore count to mass concentrations.

The aerosol OC and the fungal spore concentrations obtained in our study are consistent with those published by Cheng *et al.* (2009) based on their measurements in the subtropical city of Hong Kong. Air samples were collected by a high volume sampler (Model 1200, Graseby, Smyrna, GA, USA) with a PM_{10} size-selective inlet and an impactor with a cut-off aerodynamic diameter at 2.5 μm (Model 231-F, Graseby). OC varied from 2.4 to 9.6 μg/m^3 with an average of 5.1 μg/m^3, and the fungal spore concentrations ranged from 264 to 4,244 spores/m^3 with an average of 1,615 spores/m^3 and a geometric mean of 1,230 spores/m^3.

One potential reason for the differences between our study and earlier studies is that we used an inhalable sampler, whereas $PM_{2.5}$ or PM_{10} samplers have been commonly used by other investigators for the measurement of OC. Martuzevicius *et al.* (2008) collected $PM_{2.5}$ aerosol samples during spring in indoor and outdoor environments of six homes located in the Greater Cincinnati area. The outdoor aerosol in these homes had OC levels of 2 to 7 μg/m^3 whereas indoor OC aerosol concentration varied from 5 to 31 μg/m^3 and exceeded the respective outdoor concentrations. The latter might have occurred due to indoor sources of OC such as cigarette smoking, mold at home, and gas stove; however, these indoor sources were not identified or quantified in the quoted study. Bauer *et al.* (2008a) reported a range of 2 to 10 μg/m^3 for aerosol OC concentrations in PM_{10} fraction obtained outdoors in Vienna. Yu *et al.* (2004) studied the temporal and spatial distributions of the OC-aerosol in the $PM_{2.5}$ size range measured over the continental US during the summer of 1999. The mean values from the eight sampling sites of the Southeastern Aerosol Research and Characterization (SEARCH) networks were between 2.51 to 5.15 μg/m^3. To summarize, the indoor and outdoor OC levels obtained in our study are mostly comparable to those reported in the above quoted three investigations. This suggests a relatively low influence of larger particles (that accounts for the difference between $PM_{2.5}$/PM_{10} and an inhalable aerosol fraction). This also indicates a limited role of the geographical differences in the OC aerosol background.

For indoor environments, the contribution of fungal spores to OC aerosol was even lower than outdoors with the maximum of 0.49% and the average of 0.21%. While various factors affect the indoor aerosol OC, it is interesting to notice that none of the biological variables such as "indoor fungal spore concentration", "(1-3)-β-d-glucan level", or "mold at home" are among those exhibiting significant contribution. Only "cigarette smoking" made a significant impact on the indoor OC level with the levels in homes with smokers exceeding the ones in homes with no smokers almost 2-fold. Previous studies also suggested that cigarette smoking is a major source for indoor OC (Na *et al.*, 2005; Lazaridis *et al.*, 2008).

CONCLUSION

We found that fungal spores contribute rather little to the local outdoor OC compared to studies conducted in Europe and Australia. This could be due to different sampling instruments used for fungal spore sampling and regional differences in fungal spore concentrations. For majority of sampled homes, the indoor OC exceeded the respective outdoor concentration. Among the various factors affecting the indoor OC level, only "cigarette smoking" had significant impact on the indoor OC level. None of the biological variables such as "indoor fungal spore concentration", **"**(1-3)-β-d-glucan level", or "mold at home" contributed significantly to the indoor OC level.

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