Biomarkers as indicators of fungal biomass in the atmosphere of São Paulo, Brazil

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Abstract

The biogenic aerosol contribution to atmospheric particulate matter (PM) mass concentration is usually neglected due to the difficulty in identifying its components, although it can be significant. In the Metropolitan Area of São Paulo (MASP)-Brazil, several studies have been performed to identify sources for PM, revealing vehicular emissions and soil re-suspension as the main identified sources. The organic fraction has been related primarily to biomass burning (BB) and fuel combustion, although there is significant presence of green areas in the city which render biogenic emissions as an additional source of organic carbon (OC). The objectives of this work are to (i) estimate the relative mass contribution of fungal spores to PM concentrations with sizes smaller than 10μm (PM_{10}) in MASP, (ii) assess the main sources of PM_{10}, and (iii) characterise the composition of the PM_{10}. To achieve these objectives, we measured markers of biogenic sources and BB, during the fall-winter transition, which along with other constituents, such as ions, organic/elemental carbon, elemental composition and fungal spore concentrations, help assess the PM_{10} sources. We used receptor models to identify distinct source-related PM_{10} fractions and conversion factors to convert biomarker concentrations to fungal mass. Our results show the mean contributions of fungal aerosol to PM_{10} and OC mass were 2% and 8%, respectively, indicating the importance of fungal spores to the aerosol burden in the urban atmosphere. Using specific rotation factor analysis, we identified the following factors contributing to PM: soil re-suspension, biogenic aerosol, secondary inorganic aerosol, vehicular emissions and BB/isoprene-related secondary organic aerosol (I-SOA) markers. BB/I-SOA markers are the main source representing 28% of the PM_{10} mass, while biogenic aerosol explained a significant (11%) fraction of the PM_{10} mass as well. Our findings suggest that primary biogenic aerosol is an important fraction of PM_{10} mass, yet not considered in most studies.

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1. Introduction

Atmospheric particulate matter (PM) consists of particles in various size ranges, including those with aerodynamic diameters ≤10 µm (PM_{10}) and ≤2.5 µm ($PM_{2.5}$; also known as fine particles). These pollutants affect the most the air quality in the Metropolitan Area of São Paulo (MASP), as described by numerous studies (Pacheco et al., 2017; Kumar et al., 2016; Vara-Vela et al., 2016; Perez et al., 2015; Andrade et al., 2010; Andrade et al., 2017). MASP has the largest vehicular fleet in Brazil, making it a dominant source of PM, especially $PM_{2.5}$ (CETESB, 2015; Kumar et al., 2016; Andrade et al., 2010; Vara-Vela et al., 2016; Andrade et al., 2017). Organic compounds explain the majority (25-40%) of the $PM_{2.5}$ mass concentration (Castanho and Artaxo, 2001; Souza et al., 2014) and a significant fraction (13-19%) of PM_{10} (Caumo et al., 2016). Due to the lack of systematic studies, the role of the biological components in the composition of the organic PM portion is largely unknown around the world. A few studies have estimated the contribution of bioaerosol to PM_{10} mass (Bauer et al., 2008b; Zhang et al., 2010). However, bioaerosol contributions in most parts of the world are still unknown, bringing an uncertainty about their real role in the atmosphere. In MASP, there have been a few studies (Gonçalves et al., 2010; Degobbi et al., 2011; Emygdio et al., 2017) that attempted to characterize the fungal spore number concentration, but not their mass concentration.

Fungal spores are among the most common type of bioaerosol in the atmosphere (Glikson et al., 1995; Ataygul et al., 2007; Després et al., 2012), and the main fungal species in the atmosphere belong to the Ascomycota and Basidiomycota phyla (Fröhlich-Nowoisky et al., 2012; Elbert et al., 2007; Zoppas et al., 2006; Butinar et al., 2007; Emygdio et al., 2017) and the mitosporic fungal group. The main genus is *Cladosporium* sp. and *Penicillium* sp (Elbert et al., 2007; Emygdio et al., 2017). Bioaerosols can be responsible for adverse health effects such as asthma attacks, rhinitis and allergies (Inal et al., 2010; Tariq et al., 1996; Ataygul et al., 2007; Degobbi et al., 2011), and can interfere with atmospheric processes, including rain and hail formation (Morris et al., 2008; 2011; 2012; Gonçalves et al., 2012).

Some studies have used biomarkers to elaborate conversion factors for determining the mass concentration of certain bioaerosols (Bauer et al., 2008a; Burshtein et al., 2011; Lee et al., 2004; Lee et al., 2007). Using biomarkers, it is possible to understand the presence of microorganism and other biological components in PM, estimate their total biomass, and identify and quantify the contribution of the microorganism to the aerosol mass. This methodology simplifies the sampling procedure (e.g., compared to the traditional culturing approach), uses less time to analyse, decreases the uncertainty in the counting and identification, and with only one filter sample it is possible to estimate the concentrations of

several bioaerosols in the atmosphere (Bauer et al., 2008a). The main biomarkers for fungal biomass are arabitol, mannitol and ergosterol (Bauer et al., 2008a; Burshtein et al., 2011; Lau et al., 2006). Bauer et al. (2008b) used their conversion factors (Bauer et al. 2008a and 2002) to estimate a contribution of 4.3% in Vienna (Austria) from fungal spores to OC_{10} and 2.3% to PM_{10}. Later, Zhang et al. (2010) used similar factors to estimate an average spore concentration in the atmosphere of Jianfengling (China); they reported a contribution of 7.9% and 12.1% for PM_{10} and OC, respectively. From the Amazon rainforest, about 50 tons of fungal spores are dispersed into the surrounding atmosphere, which means an average of a thousand fungal spores per m^2 on the earth surface (Elbert et al., 2007). This shows the significance of these sources to the PM_{10} burden, contributing to a substantial percentage of the ambient PM_{10} mass.

As summarized in Table 1, there are several studies around the world that have measured the atmospheric concentrations of mannitol and arabitol and reported their relationship with fungal spore numbers. Table 2 shows studies worldwide that have measured the fungal spore number concentration and mass contribution considering these biomarkers and the conversion factors given by Bauer et al. (2008a). Most of these studies only mention the mannitol and arabitol fungal origin, while they focus otherwise on biomass burning (BB) or characterisation of the organic aerosol in the atmosphere in general (Caumo et al., 2016; Urban et al., 2014; Zangrando et al., 2016; Graham et al., 2003). Other studies (Table 1) considered arabitol and mannitol as important fungal biomarkers but do not estimate the fungal biomass in the atmosphere (Burshtein et al., 2011; Rathnayake et al., 2016; Liang et al., 2016). To the best of our knowledge, just a few studies worldwide have been performed using these carbohydrates, mannitol and arabitol, as biomarkers for estimating the fungal biomass in the atmosphere and their contribution to the PM_{10} mass, as presented in Table 2 (Bauer et al.; 2008a, Bauer et al.; 2008b; Zhang et al., 2010; Gosselin et al.; 2016), and none of such studies have been conducted in São Paulo. In addition, Liang et al. (2013) found a significant positive correlation between fungal spores and the two biomarkers (mannitol and arabitol), indicating their potential as tracers, and estimated the content of mannitol per spore. Using this conversion factor Liang et al. (2017) proposed that fungal spores contributed 3.5% of the OC mass in Beijing (China).

Table 1. Review of relevant studies, which include arabitol and mannitol concentrations, and mention their relationship with fungal spores.

Location (Country)	Season	Size	Site type	Arabitol (ng/m^3)	Mannitol (ng/m^3)	Reference
São Paulo (Brazil - 2012)	Winter	PM_{10}	Urban area	9.3	11.3	Caumo et al. (2016)
São Paulo (Brazil - 2013)	Winter	PM_{10}	Urban area	11.6	14.8	Caumo et al. (2016)
Balbina (Brazil)	Winter	PM_{10}	Rainforest	41.7	53.3	Graham et al. (2003)
Rehovot (Israel)	Winter	PM_{10}	Urban area	8.4	21.9	Burshtein et al. (2011)

Vienna (Austria)	Summer	PM_{10}	Suburban	22	34	Bauer et al. (2008b)
Vienna (Austria)	Summer	PM_{10}	Urban area	28	42	Bauer et al. (2008b)
Colorado (USA)	Summer	TSP	Semi-arid Forest - Dry	10.6	11.9	Gosselin et al. (2016)
Colorado (USA)	Summer	TSP	Semi-arid Forest - Rainy	35.2	44.9	Gosselin et al. (2016)
Jianfengling (China)	Spring	PM_{10}	Tropical forest	44	71	Zhang et al. (2010)
Belgrade (Serbia)	Fall-Winter	TSP	Urban	62.5	35.9	Zangrando et al. (2016)
Iowa (USA)	All	PM_{10}	Urban area	19.7	31.8	Rathnayake et al. (2016)
Beijing (China)	Autumn	PM_{10}	Urban area	21.1	31.9	Liang et al. (2016)
São Paulo (Brazil - 2015)	Fall-Winter	PM_{10}	Urban area	11.7	23.3	This study

Table 2. Review of relevant studies, which include fungal spore number concentrations (FSNC), and fungal spore mass contributions (FSMC) to PM and OC mass, considering the conversion factors reported by Bauer et al. (2008a) for mannitol and arabitol.

Location (Country)	Season	Site type	FSNC (spores/m³)	FSMC to OC[3]	FSMC to PM[3]	Reference
Vienna (Austria)	Summer	Suburban	29000[1]	10%	4.8%	Bauer et al. (2008a, b)
Vienna (Austria)	Summer	Urban area	26000[1]	4.3%	2.3%	Bauer et al. (2008a, b)
Jianfengling (China)	Spring	Tropical forest	41940[2]	12.1%	7.92%	Zhang et al. (2010)
Colorado (USA)	Summer	Semi-arid - Dry	6900[2]	NA	3.7%	Gosselin et al. (2016)
Colorado (USA)	Summer	Semi-arid - Rainy	26400[2]	NA	20.7%	Gosselin et al. (2016)
São Paulo (Brazil)	Fall-Winter	Urban area	5724[1] /13694[2]	7.6%	2.1%	This study

NA – not available

[1]Total spores counted manually

[2]Total spore concentration obtained using the conversion factor from Bauer et al. (2008a)

[3]The estimation of the contribution of fungal spores to PM and OC mass was performed here with the mannitol conversion factor, in order to facilitate the comparison between other studies.

In urban areas in Brazil, only a few studies have included mannitol and arabitol in their organic analysis (Caumo et al. 2016; Urban et al., 2016), and in those cases, only their relationship with fungal spores was pointed out, without consideration of these compounds as potential fungal biomass tracers. It should also be noted that none of the studies conducted in São Paulo utilized these marker compounds to determine the biogenic PM source contributions. Therefore, we carried out ambient measurements in São Paulo to (i) quantify several organic compounds in PM_{10}, (ii) estimate the mass contribution of fungal spores to the PM_{10} mass concentration, and (iii) assess the main sources of PM_{10} considering these organic compounds.

2. Methodology

2.1 Site description

PM_{10} and $PM_{2.5}$ filter samples and fungal spores were collected on the fourth floor (~12 m above ground) of the main building terrace, in the Institute of Astronomy, Geophysics and Atmospheric Sciences (IAG) of the University of São Paulo campus "Cidade Universitária" (Figure 1). The University of São Paulo is located in the western district of São Paulo, Brazil (23°33'33.77" S latitude and 46°44'0.21" W longitude), and currently has a total land area of 3,648,944 m² and built area of 860,628 m² (Universidade de São Paulo, 2013).

Large avenues, with a large circulation of vehicles, surround the sampling site (i.e., av. Engenheiro Billings and Nações Unidas - A, Escola Politécnica - B, Corifeu de Azevedo Marques - C), as presented in Figure 1. The surrounding area is also one of the few areas with vegetation in São Paulo, such as the Forest Reserve 'Armando Salles de Oliveira', which is ~450 m from the collection site and is a protected preservation area (Figure 1).

Founded in 1554, São Paulo city is currently one of the largest metropolises in the world (Demographia, 2016). It has an estimated population of ~12 million inhabitants and the metropolitan area had ~21 million inhabitants in 2015 which equals to ~10% of the total population of Brazil (IBGE, 2015). The MASP includes 39 cities and has a diverse industrial district and one of the largest (7.1 million) vehicle fleets in Brazil (CETESB, 2014; IBGE, 2015; DENATRAN, 2011), leading to a large variety of aerosols emitted into the atmosphere.

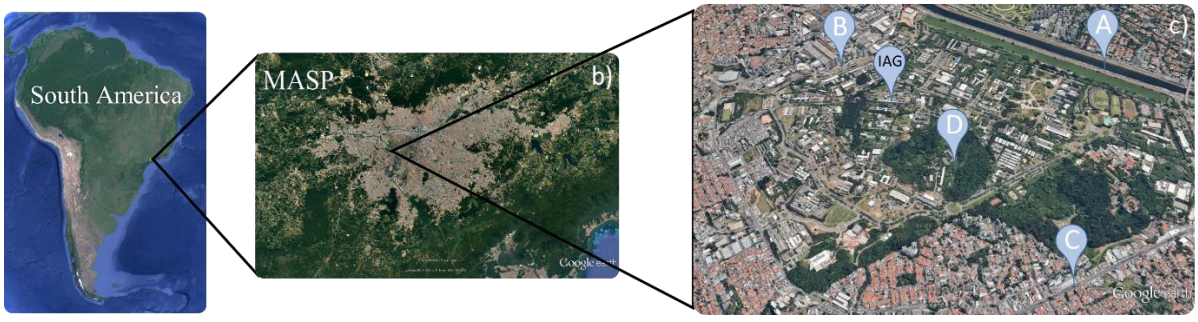

Figure 1. Maps of (a) South America, (b) MASP, (c) Proximities of the sampling site. IAG – Sampling site; A – Marginal Pinheiros; B – Escola Politécnica; C - Corifeu de Azevedo Marques;

D - Forest Reserve "Armando Salles de Oliveira". The pictures were obtained from Google maps (https://maps.google.com/).

2.2 Instrumentation and data collection

In order to achieve the goals described above, PM samples were collected and various chemical analyses were performed. The resulting data include (i) carbohydrate compounds: arabitol, mannitol, threitol, 2-methyl-treitol (2-MT), 2-methyl-erythritol (2-ME), levoglucosan, mannosan, galactosan; (ii) water-soluble organic carbon (WSOC); (iii) organic carbon (OC) and elemental carbon (EC); (iv) cations: Ca^{2+} (calcium), Mg^{2+} (magnesium), K^{+} (potassium), Na^{+} (sodium); (v) anions: Cl^{-} (chloride), F^{-} (fluoride), NO_3^{-} (nitrate), NO_2^{-} (nitrite), $C_2O_4^{2-}$ (oxalate), PO_4^{3-} (phosphate), SO_4^{2-} (sulphate) ; (vi) elemental composition: Al, Si, P, S, K, Ca, Ti, Mn, Fe, Cu, Zn. Furthermore, the number concentration of fungal spores was obtained through microscope slide analyses from samples collected simultaneously with the PM filter samples.

We sampled the PM with "MiniVol" portable air samplers (Airmetrics, Inc.) with a continuous flow of 5 L/min. The sampler is equipped with an inlet that has an impactor stage allowing the collection of PM_{10} or $PM_{2.5}$ samples (Albuquerque et al., 2012). Baldauf et al. (2001) compared the efficiency of the "MiniVol" with a fixed-site sampling technique (Dichotomous Sampler and continuous mass sampling system), and they found a good correlation and a high level of confidence on the "MiniVol" data. Two samplers were used to collect the data simultaneously; one, collecting $PM_{2.5\text{-}10}$ (coarse) samples using a 47 mm polycarbonate filters (pore size of 8.0 µm) and the other collecting PM_{10} samples using 47 mm quartz filters (Whatman, UK). The samplers were placed close to each other and at the same height (~1.5 m above the sampling platform).

The collection of viable spore particles (spores that can form another fungi) and non-viable (spores that cannot form another fungi) was carried out with a Hirst-type suction slit impactor, a Burkard Spore Trap (Burkard Manufacturing Co., Ltd., Rickmansworth, UK) concurrently with "MiniVol" filter sampling, resulting in 24 hour slides. The Burkard impactor has a narrow intake slit (2×14 mm) for pulling the particles through it at a flow rate of 10 L/min. Inside the Burkard trap is a drum that is covered with an optically clear tape (Melinex) and is coated with an adhesive collection substrate (Lubrisol). The sampled particles are impregnated in the drum, which moves in an anti-clockwise manner at a rate of 2 mm per hour, resulting in a continuous 48 mm trace during a 24 hour sampling period (Burge et al., 2006). After sampling, the tape is cut into seven parts with 24 hours sampling in each part. The tape is then put onto a slide using an adhesive mixture (Mowiol, H_2O, glycerol and phenol). After drying, the cover slip is fixated using a few drops of glycerine jelly.

Sampling was conducted from 16 May 2015 until 9 July 2015, therefore, during fall and wintertime, providing a total of 54 samples. All samples were collected during 24 hours daily. The average, standard deviation, maximum and minimum values of meteorological data (temperature, relative humidity, wind speed, precipitation and atmospheric pressure) considering the sampling period were obtained from the "Estação Climatológica do IAG" and can be seen in SI Table S1. PM_{10} mass concentration was obtained from a telemetric automatic station from the Environmental Agency of São Paulo State (CETESB) located 3.36 km from the

collection site. For the determination of OC, EC and fungal spore concentrations, only 24 samples were analysed. The $PM_{10-2.5}$ polycarbonate filters were used to determine the elemental composition, while the quartz PM_{10} filters were used for the other analyses (concentrations are given in SI Table S2). Quartz filters were baked at a temperature of 800 ºC for 12 hours and packaged in pre-baked (at 500 °C for 4 hours) aluminum foil (Chemical Institute and Physics Institute - USP) prior to sampling. After the filters were collected, they were stored at a temperature below –18 °C until their use. All the handling of the filters was carried out with proper sterility, using gloves and clean utensils. Blanks were sampled one day a week (at a total of eight blanks samples) to account for potential contamination or artifacts due to handling or other reasons.

2.3 Chemical analysis

The methodology describes by Zhang et al. (2013, 2015) was adopted for carbohydrate analyses. The protocol used for the analysis of carbonaceous species was based on Lin et al. (2014), and it was done in the Department of Biomedical Engineering and Environmental Sciences at National Tsing Hua University, Hsinchu, Taiwan. For carbohydrate and water-soluble ion analysis, half of each filter (38 cm/19 cm) was extracted with 3 mL deionized ultrapure water (resistivity > 18.2 MΩ-cm) in prebaked 4 mL amber glass vials under ultrasonic agitation for 60 minutes. The extracts were filtered through a syringe filter (PTFE membrane, 25 mm diameter, 0.45 µm pore size, Pall Corporation, NY, USA) to remove insoluble materials. The extract solutions were stored at 4 ºC to prevent degradation of analytes prior to analysis. The Blanks samples were submitted to the same analysis protocols as the ambient samples, and the resulting data were used to correct all measurements by subtracting the blank values from the sample values.

Carbohydrates were analysed by High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD) on a Dionex ICS-3000 system, using a Dionex CarboPac MA1 guard column and Dionex CarboPac MA1 analytical column (4×250 mm). A sodium hydroxide solution (400 mM, 0.4 mL min^{-1}) was used as eluent (Engling et al., 2006; Zhang et al., 2013). The water-soluble ions were analysed on a Dionex ICS-3000 ion chromatography (IC) system to determine the anionic and cationic species. The anions were separated with a Dionex IonPac AS22 (4x250 mm) column with a Dionex IonPac AG22 guard column (4×50 mm) and an eluent consisting of 4.5 mM Na_2CO_3/1.4 mM $NaHCO_3$ solution with a flow rate of 1.2 mL min^{-1}. The cations were separated with an IonPac CS12A (4×250 mm) column with a Dionex IonPac CG12A guard column (4×50 mm) and an eluent of 20 mM Methanesulfonic acid solution with a flow rate of 1.0 mL min^{-1} (Zhang et al., 2013).

The WSOC was determined through the dilution of 1 mL of the extracted sample in 7 mL of deionized ultrapure water and then quantified with a Model 1010 Total Organic Carbon (TOC) analyser (OI Analytical, USA). For OC and EC quantification, a small punch of 0.5 cm^2 was cut from the quartz filter samples and stored at 4 ºC until their use. They were analysed with a DRI Model 2001A OC/EC Carbon Analyser (Atmoslytic Inc., CA, USA), using the IMPROVE-A temperature protocol (Chow et al., 2001) in the Department of Environmental Engineering at National Cheng Kung University, Tainan, Taiwan.

For the number concentration of fungal spores, the fixed slides resulting from the Burkard sampler were examined under an optical microscope at 1000 x magnification, using the methodology where only one longitudinal trace of the slide is analysed (Rogers et al., 2001). The collection of particles, slide assembly, and counting of the spores were carried out following the approach of Rogers et al. (2001). The spore identification was performed according to Haines et al. (2000) as well as Smith (1990) and Burge et al., (2006).

2.4 Statistical analysis

Percentages of the carbohydrates, ions, WSOC and OC/EC were obtained for the PM_{10} mass. The contributions of fungal particles to OC and PM_{10} mass were estimated based on the measured biomarkers, by using conversion factors from the literature (Bauer et al., 2008a, 2002). The data obtained in this work were subject to Pearson correlation analysis (SI Table S3-S6). The source identification was made through absolute principal component analysis (APCA) using a normalized Varimax rotation. The estimations of the contribution of each factor to the PM mass concentration were performed through the regression, as described in Andrade et al. (2010).

To confirm the source identification obtained by APCA, a Positive Matrix Factorization (PMF) was performed using the EPA PMF5.0 software (Paatero and Tapper, 1994; Al-Dabbous and Kumar, 2015). This model uses a source profile, mass quantity and residue (error) to estimate the factor contribution in each sample through the equation $X_{ij} = \sum_{k=1}^{p} g_{ik} f_{kj} + e_{ij}$, where *X* is the data matrix, *g* is the mass quantity, *f* is the source profile, *e* is the model error, *i* is the samples, *j* is the variable, and *p* is the number of factors. The better solution for the X_{ij} will be obtained when a smaller Q (loss function) is found. The Q function solution will determine the difference between the adjusted and measurement, weighted with the uncertainty of the measurements. Variables can be classified as *strong*, *weak* and *bad* depending on the signal-to-noise ratio (*S/N*). All the variables were characterized as 'strong' considering that all signal-to-noise ratios (*S/N*) were bigger than 2 (Lang et al., 2015). The number of runs was 100 using a random start and 4-8 factors were examined. The number of factors analyzed were 4-8, because the Q_R (Q robust value)/Q_T (Q theoretical value) ration became too small after the 7º factor was tested, which means that there might have been an excess number of factors (Brown et al., 2015). Q_R was calculated by the software and Q_T was estimated the same way as in Lang et al. (2015). The Bootstrap method was used and the data were rotated with an "Fpeak" of –0.5 (smaller Q).

3. Results and Discussion

3.1 Carbohydrate concentrations

In order to understand the variation of certain organic compounds and their relationship with each other and with ions, we assessed the concentrations of carbohydrates and others PM constituents (OC/EC, WSOC and ions). Supplementary Information, SI Table S1 shows the meteorological variables for the sampling period, with an average temperature of 17.3 ºC, relative humidity of 81.9%, wind speed of 5.5 km/h, air pressure of 929.4 hPa and precipitation of 122.0 mm. SI Table S2 shows the average concentration, standard deviation, as well as the

maximum and minimum concentration of all carbohydrates, ions, OC, EC, WSOC and PM_{10}. Figure 2 shows a boxplot with the concentrations of sugar alcohols and 2-MT and 2-ME, galactosan, mannosan and levoglucosan, while their average percentage contributions to OC are displayed in Figure 3, together with the contributions of ions, OC, EC, and WSOC to the PM_{10} mass. The total average concentration of all carbohydrates was 363.2±271.3 ng/m³, corresponding to a relative abundance ranging from 0.6-2.0% (average 1.3%) of PM_{10} mass concentrations. As presented in Figure 2, the concentrations of mannitol and arabitol were determined with average concentrations of 23.3±9.9 and 11.7±6.4, respectively. Their individual contribution to the OC mass concentration was 0.4% for mannitol and 0.7% for arabitol. These results demonstrate that these compounds alone do not contribute significantly to the PM_{10} or OC mass. However, these compounds can be indicators of the presence of other organics components of the same or similar sources, as also shown in others studies (Bauer et al., 2008a; Rathnayake et al. 2016; Gosselin et al. 2016).

Arabitol and mannitol concentrations observed in our study are similar to those found by Caumo et al. (2016) for São Paulo in winter of 2012 and 2013, Gosselin et al. (2016) in a semi-arid montane environment and Burshtein et al. (2011) in Israel (Table 1). However, higher concentrations were observed in numerous studies as presented in Table 1 (Bauer et al., 2008b; Graham et al., 2003; Urban et al., 2014; Zhang et al., 2010; Rathnayake et al., 2016; Zangrando et al., 2016; Gosselin et al., 2016 - rainy period). These locations are examples of sampling sites in urban and forest environments, rendering the differences in concentrations mostly due to the difference in land use characteristics and/or sampling period. Higher concentrations are found in places that are less urbanised and with more biogenic sources, such as a tropical forest (Zhang et al., 2010), suburban area (Bauer et al., 2008b), agro-industrial area (Urban et al., 2014), rainforest (Graham et al., 2003), smaller cities (Rathnayake et al., 2016; Zangrando et al., 2016) and in more humid periods (Gosselin et al., 2016). Moreover, bioaerosol concentrations can vary depending on meteorological conditions (Emygdio et al., unpublished results).

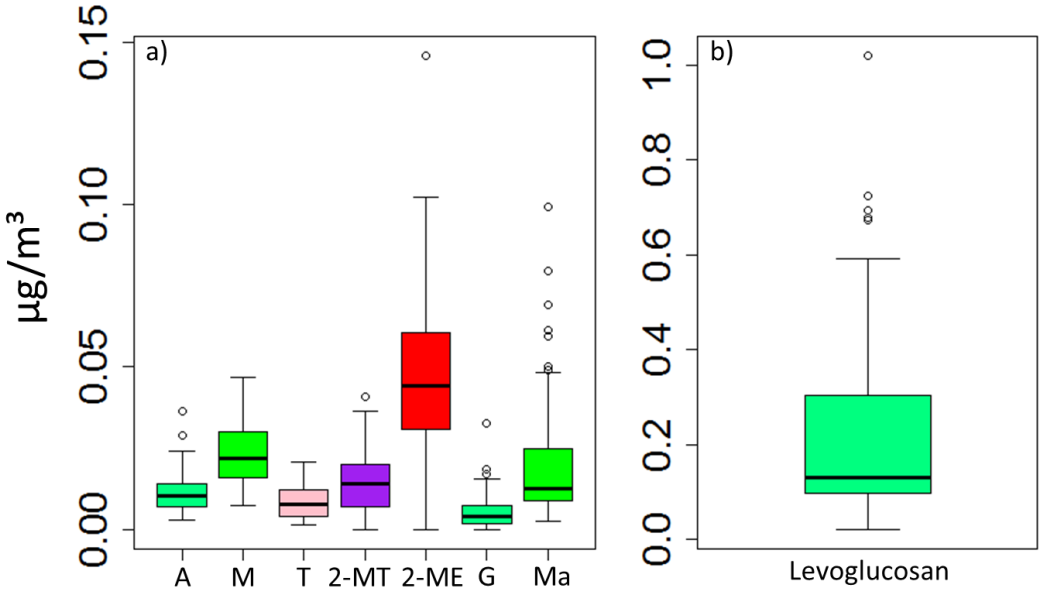

Figure 2. Box-plot with concentrations of (a) A, arabiol; M, mannitol; T, threitol; 2-MT, 2-methyl-threitol; 2-ME, 2-methyl-erythritol; G, galactosan; Ma, mannosan; and (b) levoglucosan.

The concentrations are in µg/m³. The line in the middle of the boxes indicates the median values, the upper part of the box represents the 75th and the lower part of the box represents the 25th percentile of the data. The vertical line above and below the box represents the range of the non-outliers. The circles above the box represent the outliers.

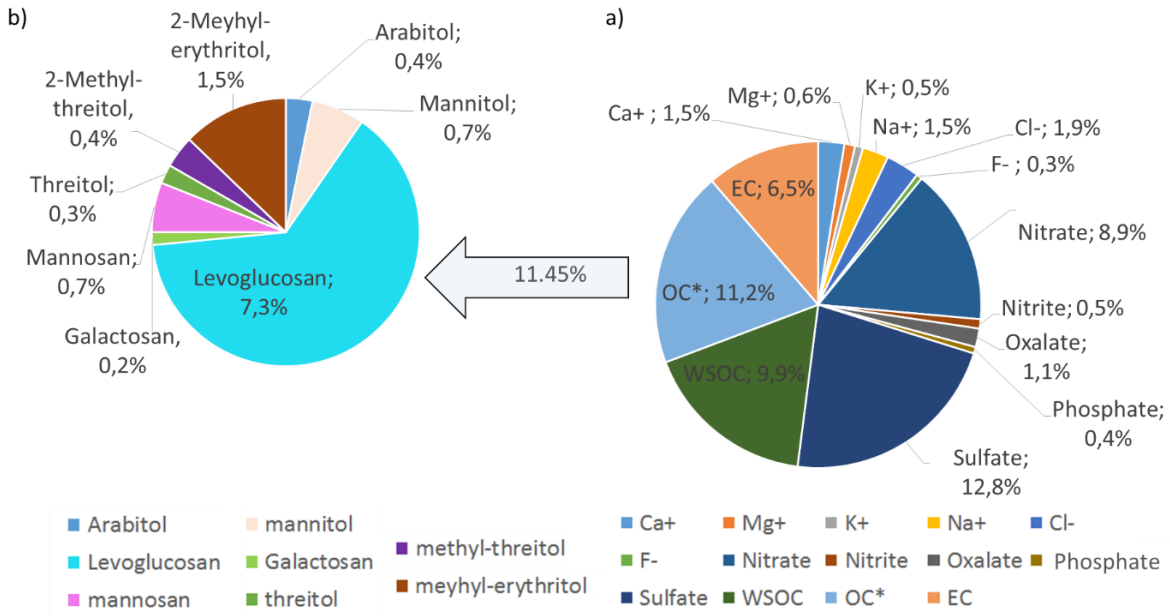

Figure 3. The average percentage of the contribution of each (a) ions, WSOC, OC and EC to the PM_{10}, and (b) the contribution of each carbohydrate to OC, together accounting for 11.5% of the total OC mass.

Considering all carbohydrates, the highest concentration was observed for levoglucosan. This anhydrosugar, together with its isomers galactosan and mannosan, well known tracers for biomass burning emissions (Simoneit et al., 1999; Yang et al., 2012; Graham et al., 2002 and 2003; Sullivan et al., 2011; Zhang et al., 2015), collectively accounting for 0.9% of the PM_{10} mass. The anhydrosugars are observed with their highest amount in smaller size fractions of the ambient particulate matter, since aerosols originating from smoke are typically in the accumulation mode of submicron particles (Graham et al., 2003). Galactosan and mannosan show smaller concentrations (5.5±5.9 ng/m³; 21.8±21.2 ng/m³) compared to levoglucosan (231.9±220.0 ng/m³) due to their origin from different biomass components (pyrolysis products of hemicellulose only, while levoglucosan is a combustion product of both cellulose and hemicellulose), as noted previously (Caumo et al., 2016; Souza et al., 2014; Tsai et al., 2010). It can be noticed from Figure 3 that combined, the anhydrosugars contributed 8.2% of the total OC mass, which demonstrates that OC was highly impacted by biomass burning emissions.

It was suggested that the levoglucosan/galactosan (L/G) ratio is useful for indicating the biofuel that they are derived from (Caumo et al., 2016). For example, galactose (precursor of galactosan) has a high content in hemicellulose from sugarcane residues (Peng et al., 2009),

resulting in a relatively smaller L/G ratio, which is indicative of sugarcane burning emissions (Caumo et al., 2016). The average L/G ratio was around 42 in this work. Caumo et al. (2016) proposed that L/G ratios smaller than approximately 30 may be indicative of regional sugarcane burning. The L/G ratio found in this work was similar to that found by Graham et al. (2002) (44 and 52) and Claeys et al. (2010) (37) in Rondônia, that are mostly impacted by tropical forest fires. The high L/G ratios can be an indication that during our sampling period the concentrations of biomass burning tracers may be influenced by advected air from areas such as Rondônia.

Levoglucosan, mannosan and galactosan concentrations for São Paulo have been previously measured by Caumo et al. (2016) in PM_{10} in winter (330 ng/m³, 27 ng/m³ and 17.5 ng/m³ in 2012 and 510 ng/m³, 40 ng/m³ and 21 ng/m³ in 2013) and by Souza et al. (2014) in $PM_{2.5}$, also in winter (284 ng/m³, 22 ng/m³ and 16 ng/m³ in 2008). Vasconcellos et al. (2010) also obtained levoglucosan concentrations for São Paulo in the wet season (39 ng/m³). In general, the São Paulo anhydrosugar concentrations found in other studies were similar and consistent with this work. However, different concentrations of levoglucosan, galactosan and mannosan were observed in the Amazon rainforest (Brazil). Balbina during winter showed mean concentrations of levoglucosan, galactosan, and mannosan in the coarse size fraction $PM_{2.5\text{-}10}$ of 2.0 ng/m³, 0.1 ng/m³ and 0.1 ng/m³, respectively (Graham et al., 2003), while the marker mean concentrations in Rondônia during spring ("biomass burning season") in PM_{10}, were 2460 ng/m³, 55.4 ng/m³ and 126 ng/m³ on a pasture and 1180 ng/m³, 22.7 ng/m³ and 49.5 ng/m³ in a forest, respectively (Graham et al., 2002). These higher concentrations of anhydrosugars are probably due to their relation with biomass burning that occurs intensively in Rondonia during spring.

Threitol, 2-ME and 2-MT showed average concentrations of 8.4±4.9 ng/m^3, 14.0±10.1 ng/m^3, 46.6±26.2 ng/m^3 respectively, representing 0.3%, 0.4%, 1.5% of the OC mass. Claeys et al. (2004) carried out sampling in the Amazon rainforest and proposed that the 2-methyltetrols (2-ME and 2-MT) are photooxidation products of isoprene, consequently both are considered isoprene-related secondary organic aerosol (SOA) markers. The high concentrations of 2-ME and 2-MT observed here are therefore indicative of an atmosphere with a high abundance of isoprene. Caumo et al. (2016) investigated 2-MT and 2-ME patterns in São Paulo and obtained smaller concentrations compared with this work, with 2.5 ng/m³ and 6.1 ng/m³ for 2012, and 2.3 ng/m³ and 5.5 ng/m³ for 2013, respectively. Based on the chamber experiments by Surrat et al. (2006), these compounds are formed in clean atmosphere conditions (low-NO_x), which indicates that during our sampling period lower concentrations of NO_x were prevalent compared with the observations by Caumo et al. (2016). Threitol is a homologous carbohydrate of 2-ME and 2-MT and to our knowledge, there is no data reported about threitol concentrations in ambient air in São Paulo. Threitol can be derived from the combustion of biofuel species such as leaves, grasses, and branches (Sullivan et al., 2011), and is also present in fungal structures (Frisvad et al., 1998). Graham et al. (2002) found threitol concentrations with an average of 2.3 ng/m³ in a pasture area and 1.7 ng/m³ in an Amazonia forest area, which are higher than present in this work, probably due to the larger forested area.

The above results allow concluding that knowing the organic composition of PM_{10} is important for understanding the PM sources, including several biogenic sources as well as possible anthropogenic sources such as biomass burning.

3.2 Ions, organic and elemental carbon and PM_{10} mass concentrations

To better understand the PM_{10} composition and sources the concentration of some ions were assessed. Figure 4 shows a boxplot with the concentrations of various species including (a) nitrate and sulphate b) oxalate and potassium c) WSOC, OC, OM, EC, TC and TCA), while their average, maximum and minimum concentrations are displayed in SI Table S2, together with other ionic species. Ions contribute highly (30%) to the PM_{10} concentration, with a total concentration of 8.4 µg/m^3. The dominant ion species was sulphate, followed by nitrate (Figure 4). These ions account for 12.8% and 8.9% of the total mass concentration of PM_{10}, respectively. A straightforward explanation for the higher contributions of sulphate is that it is formed through the reaction between sulphur compounds and other reactive substances, and in the São Paulo atmosphere a high concentration of radicals, that favour the oxidation of sulphur dioxide to sulphate, has been reported (Miranda et al., 2002; Albuquerque et al., 2012). Furthermore, both sulphate and nitrate are associated with diesel and gasoline combustion, industrial emissions and secondary aerosol formation (Andrade et al., 2010). Therefore, these compounds represent anthropogenic sources, which are substantial in São Paulo. The high concentration of nitrate can be explained by the fact that it is formed from NO_x precursors, which are abundant in polluted areas like the São Paulo atmosphere. Miranda et al. (2002) show that sulphates are one of the main aerosol particle constituents in the São Paulo atmosphere, and Albuquerque et al. (2012) show that ammonium sulphate is one of the main components of the $PM_{2.5}$ mass in both polluted and unpolluted periods aside from OC/EC.

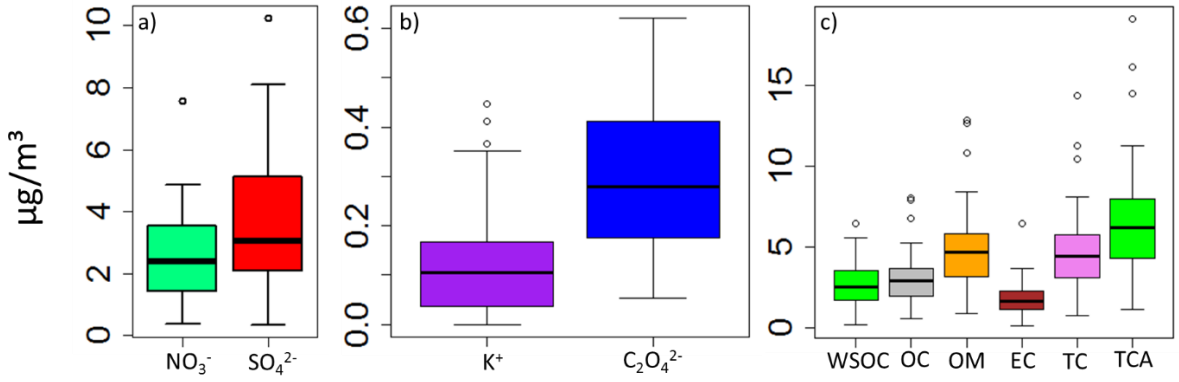

Figure 4. Boxplots showing concentrations of (a) NO_3^- and SO_4^{2-} (b) K^+ and $C_2O_4^{2-}$ (c) WSOC, OC, OM, EC, TC and TCA. The concentrations are given in µg/m³. The number of sampling days for OC, OM, EC, TC and TCA are *n* = 27. The line in the middle of the box indicates the median value, the upper part of the box represents the 75th and the lower part represents the 25th percentile of the data. The vertical line above and below the box represents the range of the non-outliers. The circles above the box represent the outliers. Abbreviations: WSOC, water soluble organic carbon; OC, organic carbon; EC, elemental carbon; TC, total carbon; TCA, total carbonaceous aerosol.

Potassium and oxalate showed average concentrations of 128.9 ng/m³ and 302.0 ng/m³ respectively, representing 0.5% and 1.1% of PM_{10} mass (Figure 4). These contributions are not high, but these species can be important tracers in the atmosphere for biogenic emissions and biomass burning (Graham et al., 2002; Andreae et al., 1988). Vasconcellos et al. (2010), investigating the São Paulo atmosphere, found an average concentration of 357 ng/m³ for potassium in the wet season (March-April) and 579 ng/m³ in the dry season (August). The concentrations observed in both periods were higher than those observed in this work, probably due to the smaller PM_{10} levels present during this study period. Elbert et al. (2007) noted that the average potassium concentration in the Amazon in coarse PM (1-10 µm) observed in different studies, was between 14 ng/m³ and 270 ng/m³. They also estimated that the potassium emissions resulting from the active release of fungal spores (Ascospores) correspond to 60% of the average concentration of potassium found in Balbina and almost 100% in Rondônia. In contrast, Zhang et al. (2015) observed in coarse PM (2.5-10 µm) an average concentration of 30.8 ng/m³ in China, which is lower than the concentrations presented in this work, possibly because of the different particle size range. For oxalate, Vasconcellos et al. (2010) and Souza et al. (2014) obtained a concentration of 285 ng/m³ (PM_{10}) and 243 ng/m³ ($PM_{2.5}$), respectively, during the winter season in São Paulo, which is similar to this work. On the other hand, in Balbina an oxalic acid concentration of 62.8 ng/m³ was observed (Graham et al., 2003) as opposed to 619 ng/m³ and 329 ng/m³ in Rondônia in a pasture area and in a forested area during the "burning season", respectively (Graham et al., 2002). The higher concentrations of oxalate in periods with large biomass burning activities confirms that oxalate is emitted from biomass burning or produced upon oxidation of combustion products downwind of fires (Gao et al., 2003).

The average concentration found for WSOC was 2.8 µg/m³, which corresponds to 9.9% of the total PM_{10} mass concentration (Figure 4). WSOC is related to SOA, and can also be emitted directly during biomass burning processes. Graham et al. (2002) reported higher concentrations of WSOC, with 17.3 µg/m³ for a pasture site in Rondônia, probably because the samples were collected during the biomass burning season (spring), indicating its higher emission from biomass burning events.

The average concentrations of OC and EC were 3.2 µg/m³ and 1.8 µg/m³, respectively. Their contribution to the PM_{10} mass ranged from 5% to 13% (average 11.1%) for OC and 1% to 10% (average 6.5%) for EC, and total carbon (TC) represents ~18% of PM_{10}. The organic matter (OM) concentration was obtained through conversion of the OC mass, by multiplying with a factor of 1.3, as proposed for urban sites (Turpin and Huntzicker, 1995; Rengarajan et al., 2011; Ram et al., 2011). The OM concentration includes elements other than carbon present in the organic aerosol, e.g., hydrogen, oxygen and other elements, and explained 14.5% of the total PM_{10} mass, with an average concentration of 4.1 µg/m³. The sum of EC and OM constitutes the total carbonaceous aerosol (TCA), which represents all carbonaceous aerosol in the atmosphere, and had an average concentration of 6.0 µg/m³ accounting for 21% of the PM_{10} mass in this study.

The PM_{10}, OC and EC concentrations in São Paulo were much lower during this study period than those reported by Caumo et al. (2016), i.e., 89 µg/m³, 10.4 µg/m³, 5.1 µg/m³ for 2013 and 39 µg/m³, 7.0 µg/m³, 2.2 µg/m³ for 2012, respectively; Vasconcellos et al. (2010), also obtained

higher PM_{10} concentrations in São Paulo, with an average of 38 μg/m³. However, the percentage contributions of OC to the PM_{10} mass were similar to those observed in 2013 (13%) in São Paulo (Caumo et al., 2016). This indicates that the lower OC and EC concentrations are the result of lower PM_{10} concentrations during the sampling period of this study, probably due to decreased emissions or weather conditions that favoured the dispersion of airborne particles. Interestingly other studies conducted in São Paulo pointed out higher (40% in winter and 35% in summer) OC contributions to $PM_{2.5}$ mass (Castanho and Artaxo et al., 2001). High contributions of other inorganic sources (e.g., soil re-suspension) to the PM_{10} mass may also decrease the relative abundance of OC.

It is also relevant here to examine the OC/EC ratio, which can be used to obtain insights regarding the influence of different types of emissions and transformations of carbonaceous aerosol (Pachauri et al., 2013). This ratio can be an indicator of the presence of primary versus secondary organic aerosols (Chow et al., 1996; Castro et al., 1999; Pachauri et al., 2013). Values bigger than 2 for the OC/EC ratio indicate that secondary organic aerosol formation influenced the ambient aerosol in addition to the primary organic aerosol contributions to the measured OC concentrations (Chow et al., 1996; Castro et al., 1999; Pachauri et al., 2013). The OC/EC ratio in our sampling period ranged from 1.2 to 4.3, with an average rate of 1.9, indicating primary anthropogenic carbonaceous aerosol with important contributions of SOA and/or primary biogenic OC. The OC/EC ratios in this work were very similar to those measured by Caumo et al. (2016) with a range of 1.9 to 4.8 for 2012 and 1.5 to 4.3 for 2013.

Another approach to exam the relative contributions of primary organic carbon (POC) and secondary organic carbon (SOC) is often performed with the EC tracer method (Castro et al., 1999). The mass of SOC and POC were determined using the equation proposed by Castro et al. (1999), where $SOC = OC_{total} - POC \; and \; POC = EC \times (OC/EC)_{min}$. SOC contributed 27% of the OC, while POC contributed 73% in the sampling period of this study. The OC is therefore mainly constituted of particles emitted directly in the atmosphere, corroborating with the relatively low OC/EC ratios. The SOC concentrations are influenced by weather conditions and the sampling season, e.g., measurements conducted during winter may present lower SOC concentrations due to weaker photochemical activity (low solar-UV insolation) (Castro et al., 1999), which could explain the lower percentage of SOC. While examining $PM_{2.5}$ in India, Pachauri et al. (2013) noticed that in a place with high vehicle traffic SOC contributed only 18% of OC, which may have been the case in this study where primary emissions from traffic were high as well.

3.3 Correlation analyses

In order to verify how the analysed compounds related with each other, and to better understand their variation in the atmosphere, we correlated each compound with each other as seen in SI Tables S3-S6. Figure 5 presents the time series of these compounds (i.e., arabitol (A), mannitol (M), threitol (T), 2-MT, 2-ME, levoglucosan (L), mannosan (Ma), galactosan (G), potassium (K^+), oxalate ($C_2O_4^{2-}$), nitrate (NO_3^-), sulphate (SO_4^{2-}) and WSOC). As indicated by the temporal variations in Figure 5, the correlations are strong (r ~0.90) between arabitol (A) and mannitol (M), and between levoglucosan (L), galactosan (G) and mannosan (Ma), as expected (Zhang et al., 2010; Burshtein, et al., 2011; Zhang et al., 2015; Graham et al., 2003 E 2002;

Nirmalkar et al., 2015; Sullivan et al., 2011; Graham et al., 2003; Gosselin et al., 2016). The high correlation coefficient between the sugar alcohols (r>0.9) suggested that they have the same source, probably a fungal origin. Threitol has good correlation with sugar alcohols (r>0.7) and anhydrosugars (r>0.6), while 2-ME and 2-MT show a correlation of 0.7 between each other. Mannitol, arabitol and threitol are known indicators for the presence of primary organic compounds in PM since they are present in many vegetable and fungal structures (Frisvad et al., 1998; Loescher, et al., 1992; Shen et al., 1997; Burshtein, et al., 2011). Furthermore, 2-ME and 2-MT may also have a biological origin, since they are produced by plants, algae, insects and microorganisms (Nozière et al., 2011). The good correlation between threitol, 2-ME and sugar alcohols (r ~0.7) is a strong indication of the biological origin of these compounds. Furthermore, the good correlation of threitol and the median correlation of 2-ME and 2-MT (r ~0.5) with anhydrosugars are also indicative of and association with biomass burning sources of these compounds, pointing to a dual origin.

The lack of correlation between the sugar alcohols and anhydrosugars indicates that sugar alcohols contribute to the natural background aerosol, i.e., primary biogenic aerosol (Graham et al., 2003). Moreover, many authors point out that the concentrations of the sugar alcohols are higher in PM_{10} than at smaller size fractions (Zhang et al., 2010; Zhang et al., 2015; Elbert et al., 2007; Nirmalkar et al., 2015; Sullivan et al., 2011), which is the opposite from the typical size distribution of anhydrosugars, that are more enriched in fine PM (Graham et al., 2003). Time series of WSOC and the anhydrosugars closely follow each other and thus show a high correlation (around 0.8), indicating that polar organic species originated mainly from biomass burning processes, as suggested by Sullivan et al. (2006). However, WSOC can also be produced by secondary organic aerosol formation and may be an indicator of SOC in polluted environments (Weber et al., 2007). The correlation (around 0.6) of WSOC with 2-ME and 2-MT, which are isoprene-related SOA tracers, indicates moderate biogenic SOC contribution to WSOC, as shown above in conjunction with the observed OC/EC patterns.

It can be seen from Figure 5 that the time series of potassium, oxalate, and anhydrosugar compounds closely follow each other. The correlation coefficients between all these variables range mostly between 0.7 and 1.0, with anhydrosugar correlations being larger than 0.9; only the correlation between oxalate and galactosan concentrations was somewhat lower, yet still around 0.6. Potassium may be present in the fine fraction of PM associated with biomass burning events (Graham et al., 2002), and oxalate can be related to biomass burning as well (Andreae et al., 1988), which explains its high correlation with anhydrosugars (biomass burning tracers). The time series of sugar alcohols, potassium and oxalate also are somewhat similar, with r values mostly around 0.5 and 0.4. The explanation for this moderate correlation is that potassium can also be derived from biogenic sources, particularly from active release of fungal spores and other fungal derivatives (Pöhlker et al., 2012; Elbert et al., 2007; Zhang et al., 2015), while oxalate is present in plants and is produced by the incomplete oxidation of carbohydrates. These correlations indicate that both potassium and oxalate have more than one source, but are predominantly of biological origin. Among all ions analysed (SI Table S2), potassium and oxalate can be considered the most relevant ions for biomass burning and fungal source processes.

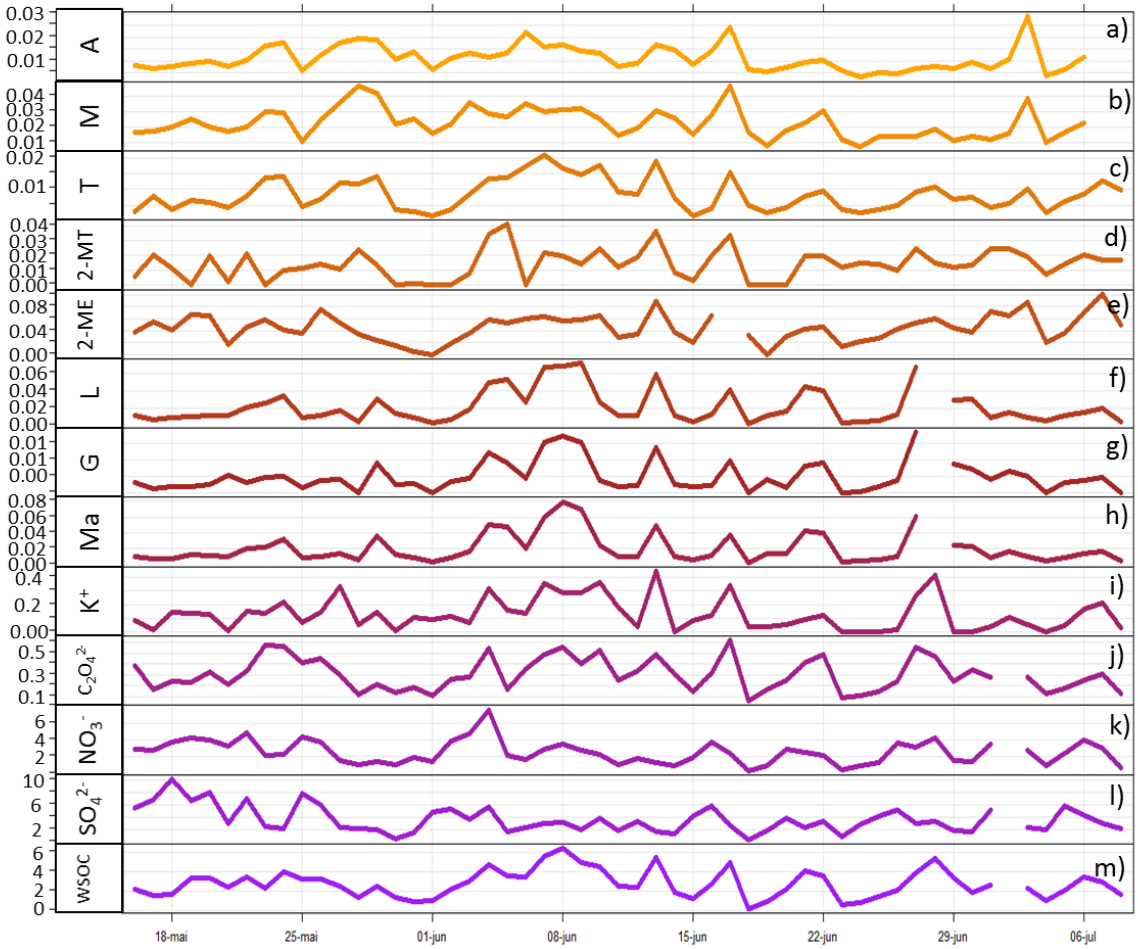

Figure 5. Temporal variations of sugar alcohols (a) arabitol, (b) mannitol, (c) threitol; methyltetrols (d) 2-MT, (e) 2-ME; anhydrosugars (f) levoglucosan, (g) galactosan, (h) mannosan; cations (i) K^+; anions (j) NO_3^- (k) $C_2O_4^{2-}$, (l) SO_4^{2-}; and (m) WSOC. Note the similarity between anhydrosugars and WSOC, and between sugar alcohols. Gaps in the timelines are due to missing data. 2-MT, and 2-MT stand for 2-methyl-threitol and 2-methyl-erythritol, respectively, and WSOC stands for water-soluble organic carbon. More information is provided in SI Tables S2-S6.

Figure 6 shows the correlation between OC and EC, including the regression equation and correlation coefficient. The significant positive correlation observed between OC and EC (r = 0.9) indicates that both are predominantly derived from common sources (Rengarajan et al., 2011), i.e., OC is mostly derived from primary sources or both OC and EC are influenced by the same meteorological factors (Turpin and Huntzicker, 1995). This correlation was also observed in other studies that analysed fine particles (Pachauri et al., 2013). Considering the data analyses discussed in the previous paragraphs (Section 3.2 - OC/EC ratios and POC/SOC estimation), the high correlation between OC and EC suggests that OC is mostly derived from primary sources, such as biomass burning.

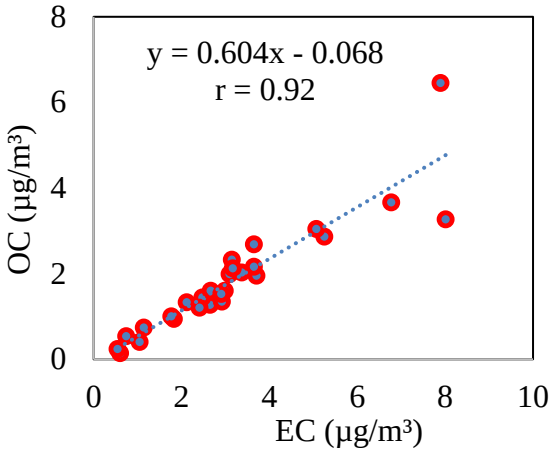

Figure 6. Correlations of OC with EC concentrations. Regression equation and *r* are presented on top of the plot. Abbreviations: OC, organic carbon; EC, elemental carbon.

3.4 Fungal Biomarkers

In order to estimate the fungal mass contribution to the PM_{10} mass, and understand how much of the PM_{10} mass the fungal spores are responsible for, it was necessary to verify the correlation between fungal spores and fungal biomarkers. Therefore, thirty-five fungal types were identified in the ambient samples (SI Table S7), with a total mean concentration of 5724 ±2592 spores/m³. The total spore numbers showed a positive correlation with arabitol (*r*~0.8) and with mannitol (*r*~0.7) (Figure 7), as also observed by Bauer et al. (2008b) and other more recent studies (Rathnayake et al. 2016; Gosselin et al. 2016). These significant correlations suggest that arabitol and mannitol are strong indicators of the presence of fungal spores in the atmosphere. They also correlate with threitol (*r*=0.6) and potassium (*r*=0.5), that are not much discussed in the literature, yet are also related to fungi.

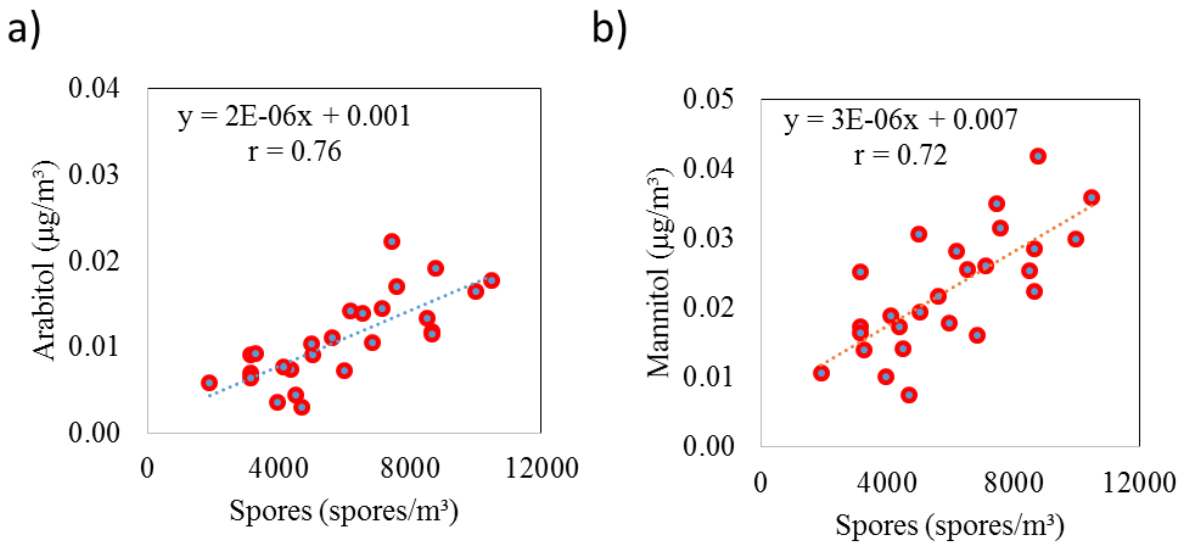

Figure 7. Correlations of (a) arabitol (µg/m³), and (b) manitol (µg/m³) with the total spore number concentration. Regression equation and correlation (*r*) are presented on top of the graphs.

The conversion factor proposed by Bauer et al. (2002, 2008b) allows estimating the relative contribution of fungal spores to the mass concentrations of PM_{10} and OC. The conversion factors utilized were 1.2 pg arabitol/spore, 1.7 pg mannitol/spore (Bauer et al., 2008b), 33 pg fresh mass PM_{10}/spore, and 13 pg C/spore (Bauer et al., 2002). The average fungal spore concentration in the atmosphere, considering the mannitol conversion factor (1.7 pg mannitol/spore) was 13694 spores/m^3, and while considering the arabitol conversion factor (1.2 pg arabitol/spore) it was 9731 spores/m^3. These values are much higher than the fungal spore numbers obtained through the Burkard analyses (5724 spores/m^3). One possible reason is the difference in the counting methodology, which was performed manually using optical microscopy in this work as opposed to Bauer et al. (2008a) who enumerated the spores using an epifluorescence microscopy. The fungal spore concentrations obtained from the Burkard sampler show a significant correlation (*r*) around 0.7 with the fungal spore concentrations estimated with the arabitol and mannitol data.

The fungal spore concentrations obtained with the mannitol conversion factor were then multiplied by the conversion factors for PM_{10} and OC. We found that the contribution of fungal spores to PM_{10} and OC mass to be on average 2% and 8%, with a maximum of 5% and 22%, respectively (Figure 8 and SI Table S8). This means that fungal spores in the MASP atmosphere accounted for 0.5 µg/m^3 of PM_{10} mass and 0.2 µg/m^3 of OC mass. These findings reveal the importance of the fungal spore contribution to the total aerosol burden in the atmosphere. Considering urban areas, these data are consistent with the findings by Bauer et al. (2008b). The contribution of fungal spores to OC was higher in this study, while the contribution to PM_{10} was similar. Compared to various other areas, such as suburban, tropical forest or semi-arid montane locations, the percentages found in this study are smaller, which was expected to some extent, since fungal spores tend to be less abundant in urban areas (Table 2).

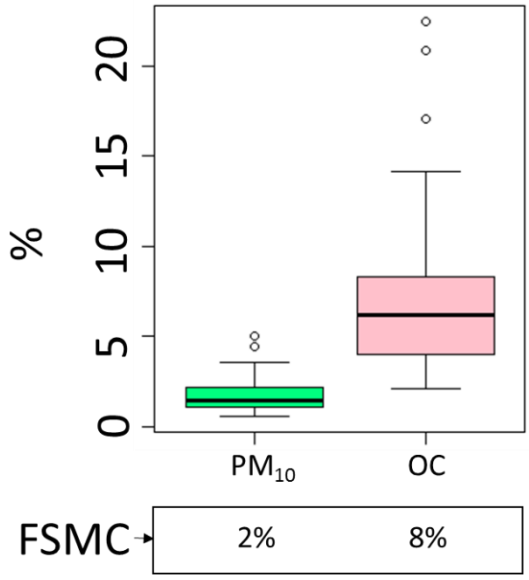

Figure 8. Fungal spore mass contributions (FSMC) to PM_{10} and OC mass. The y-axis indicates the percentage contribution. The line in the middle of the box indicates the median value, the upper part of the box represents the 75th and the lower part represents the 25th percentile of the data. The vertical line above and below the box represents the range of the non-outliers. The circles above the box represent the outliers.

The conversion factors proposed by Bauer et al. (2002 and 2008b) include a number of assumptions and uncertainties, due especially to the difference between the environments. Although the sampling periods were similar, the sampling site of this study was São Paulo city, one of the largest metropolises in the world (Demographia, 2016) with a tropical climate. In contrast, Bauer et al. (2002 and 2008b) collected samples in Vienna (Austria) in a moderate climate zone, with humidity, temperature and chemical composition being significantly different. In addition, fungal species and concentrations in the atmosphere of São Paulo and Vienna are different and can, therefore show differing mannitol and arabitol concentrations. Considering these differences, it is important to note that these estimates are associated with the inherent uncertainties.

3.5 Sources of PM_{10}

To assess the main sources of PM_{10}, including fungal spores and others organic compounds, the concentration variations were analyzed using PCA and PMF. Figure 9 (a) shows the species considered and the varimax normalized rotated PC loading matrix results. Five factors were extracted accounting for 83.3% of the total variance, with eigenvalues greater than unity. The commonalities for all of the species were mostly greater than 60% except for 2-MT (SI Table S9). To confirm this statistical analysis, PMF analysis was performed, providing similar results to the APCA (Figure 10). The uncertainty was set as 0.1%, smaller than the original data, and an additional 25% of uncertainty was added to the model. The five-factor solution was the best fit, with more meaningful results.

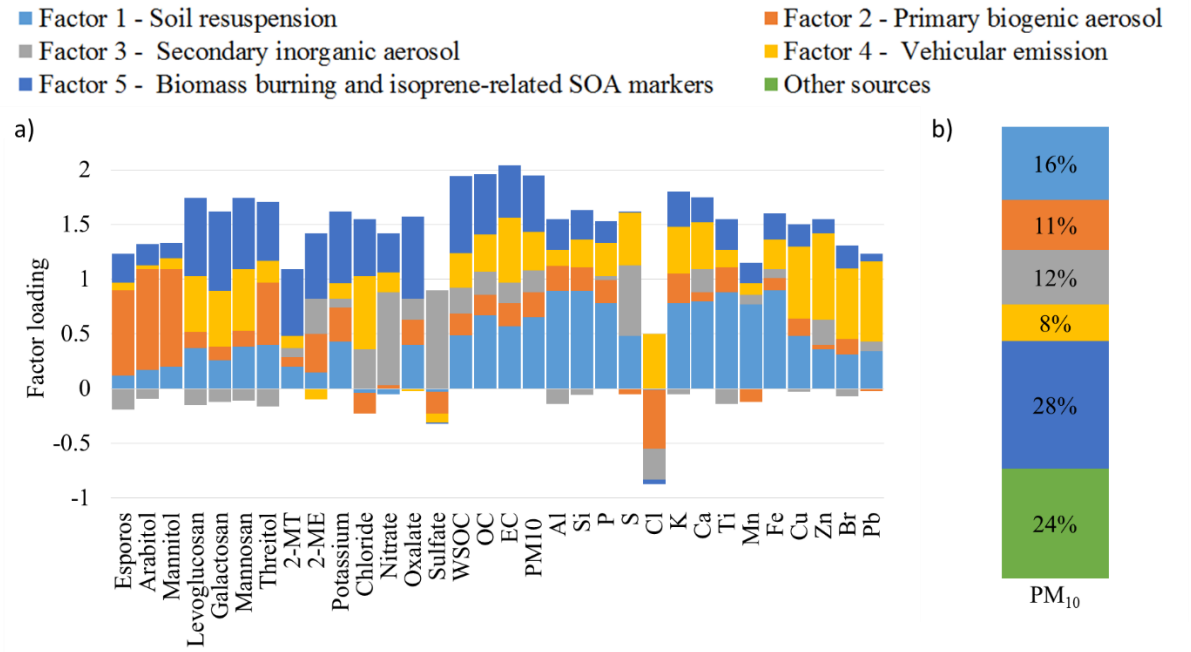

Figure 9. PCA analyses (a) normalized varimax rotated principal component loading matrix. Factor loadings bigger than 0.6 are considered as high loading. Note that for the PM elements, aerosol components measured in the particle size range of 2.5–10 µm served as input in the PCA; (b) mass concentration percentages of each factor to the PM_{10} mass, obtained with regression analysis of the absolute factor scores.

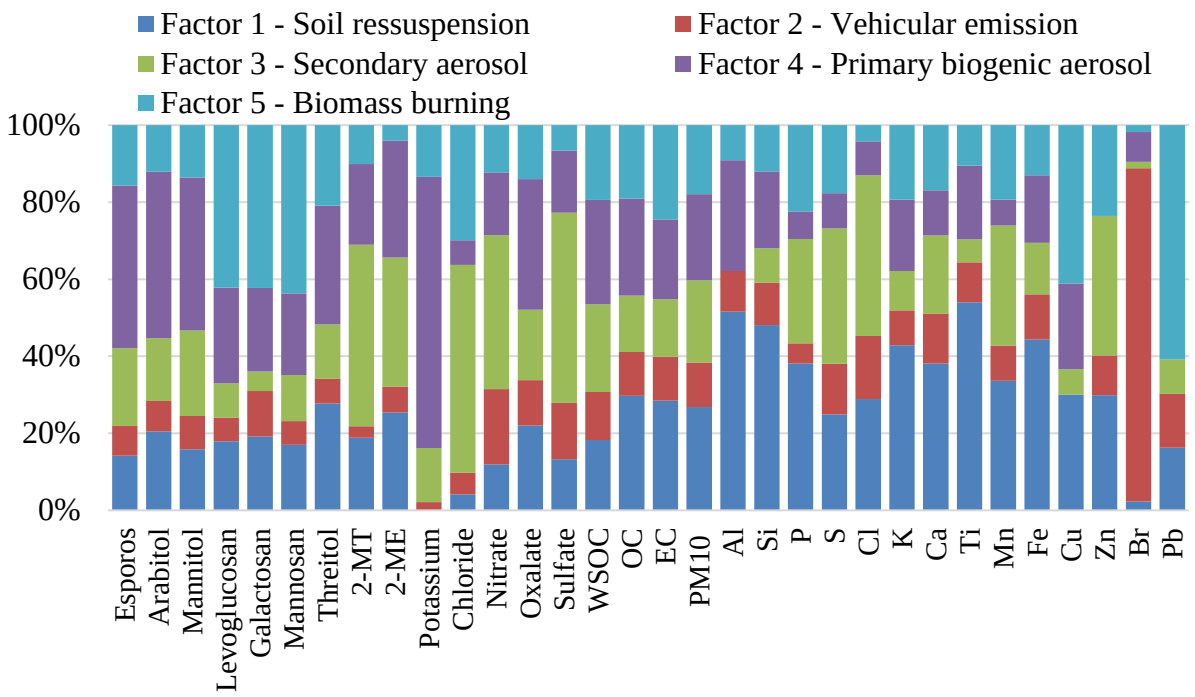

Figure 10. PMF analyses performed with the same APCA data. The five factors identified in the APCA were also confirmed by this analyses.

Factor 1 (APCA and PMF) was characterized by a high loading of soil re-suspension tracers, i.e., Al, Si, P, K, Ca, Ti, Mn, Fe, OC, PM_{10}. This factor also represents vehicular related emissions, due to the high loading of PM_{10}, EC and OC. Factor 2 (APCA) and factor 4 (PMF) present high loadings of fungal spores, with mannitol, arabitol and threitol as fungal tracers. The mannitol and arabitol concentrations vary with different species of fungi; moreover, mannitol can be found in others primary biogenic aerosols such as bacteria, algae and higher plants (Shen et al., 1997). Factor 3 (APCA and PMF) indicated secondary inorganic aerosol (SIA) due to the presence of nitrate, sulphate and S, and may also indicate oil-burning boiler and industrial emissions. Factor 3 for PMF also represents the isoprene-related SOA, due to the high percentage of 2-ME and 2-MT. Factor 4 (APCA) shows high loadings of chloride, Cl, Cu, Zn, Br and Pb, indicating vehicular emissions (VE). Factor 5 (APCA and PMF) represents biomass burning, based on high loadings of levoglucosan, mannosan, galactosan, oxalate, potassium and WSOC. For APCA this factor also represents the isoprene-related SOA, since there is a high loading of 2-ME and 2-MT. Factor 5 (PMF) has contributions of vehicular emissions as well (Pb, Cu). Zhang et al. (2015) conducted PCA analysis using similar compounds and found four factors (fungi related aerosols, sea-salt and secondary nitrate, secondary sulphate and ammonium, biomass burning and dust aerosols), similar to this work.

A regression analysis of the absolute factor scores, using APCA, was performed to quantify the sources that contributed to the PM_{10} mass concentration (Andrade et. al., 2010), and the concentration percentages of each factor to the total PM_{10} mass (Figure 9 b). We follow the methodology proposed by Andrade et al. (1994; 2010). Briefly, daily scores were calculated for the five factors and then a regression analysis was carried out between the normalized absolute factor score and the PM_{10} mass to obtain a mass scale for each factor. Factor 5,

biomass burning and isoprene-related SOA, were the main sources of PM_{10} mass concentration. Probably this factor also includes an intrinsic re-suspension soil emission, considering their large mass contribution. Nonetheless, this strong contribution indicates that biomass burning can strongly influence PM_{10} mass concentrations. The isoprene-related SOA markers were measured a few times in São Paulo (Caumo et al., 2016) and represent secondary organic emissions derived from biogenic sources. Factor 5 represents, therefore, the contribution of biogenic species to the atmospheric aerosol. Factor 4 represents vehicular emissions and does not contribute to the PM_{10} mass concentration as much as others in São Paulo (CETESB, 2015). However, factor 3, factor 1 and factor 5 has also some intrinsic vehicular emissions. Furthermore, the majority of the undetermined contributions (other sources, 24%) are likely from vehicular emissions, but we did not have additional tracers to better characterize this source. Similar challenges arise when considering the industrial sources since such sources are scarce at the sampling site.

The primary biogenic aerosol factor estimate (11%) is one of the major goals of the present work, and indicates not only that the fungal contributions to PM_{10} are significant, as presented in the previous chapter (Section 3.4), but also other primary biological sources, including bacteria, pollen, viruses, plant fragments and even small animals such as insects and other arthropods and protozoa. Until today, this biological source in São Paulo has been neglected in the mass balance or could not be determined due to the lack of tracers to characterize this source (Andrade et al., 2010; Oyama et al., 2016; Brito et al., 2013). The environmental agency of São Paulo (CETESB), one of the most important environmental agencies in Brazil, does not consider this source in the mass balance in the 2015 report on air quality in São Paulo state, stating that 25% is from secondary aerosol, 25% from soil re-suspension, 10% from industrial sources, 1.9% from motorcycles, 31.4% from heavy vehicles, and 6.7% from light vehicles. Likewise, the London Atmospheric Emissions Inventory for 2013 did not include the biogenic emissions as a source of PM_{10}; it only considered the natural VOC emissions from forest vegetation (GLA, 2013).

The difficulty in characterising this biogenic component is that there is still a very big gap in the knowledge of how much bioaerosol is present in the air, their biodiversity, and what are their contributions to atmospheric processes, that include bacteria, pollen, algae and other species asides from fungi. Besides that, it is known that fungal bioaerosol, in general, is allergenic, and can cause several adverse health effects, such as respiratory and infectious diseases, chronic diseases like asthma, bronchitis, rhinitis (Haines et al., 2000; Inal et al., 2010; Tariq et al., 1996; Ataygul et al., 2007; Douwes et al., 2003; Burge and Rogers, 2000), and compounds present in some fungi cell walls can cause an inflammatory response (Douwes et al., 2003). Among the main fungal types associated with asthma attacks are *Alternaria* spp, *Cladosporium* spp (Tariq et al., 1996). Other bioaerosols, as listed above, can also cause problems; for example, the increase in the concentration of atmospheric pollen shows a relationship with a greater number of hospitalizations due to asthma attacks (Knox et al., 1997). It is also known that primary biogenic aerosols, such as certain bacteria and fungi can be important *ice nuclei*, since they can form *nuclei* at temperatures below –10 °C (Morris et al., 2012; Mohler et al., 2007; DeMott et al., 2010), and because of that can interfere with atmospheric and precipitation processes.

4. Summary, Conclusions and Future Work

We measured tracers for primary biogenic aerosol and biomass burning, as well as other organic and inorganic compounds in PM_{10}, and fungal spore number concentrations in MASP. The aims were to estimate the fungal mass contribution to PM_{10}, assess the main sources of the PM_{10} considering biomarkers, and quantify the concentration of several organic compounds, that have not been measured previously. We measured several organic compounds that are not usually measured in São Paulo and we compared and correlated their concentrations with ions and OC/EC concentrations as well as with other studies conducted in São Paulo. In particular, we quantified the fungal spore contribution to PM_{10} using the measured ambient tracer concentrations and conversion factors proposed in the literature. Using APCA and PMF analyses, we proposed the main sources for PM_{10}, including organic compounds never used in a mass balance in São Paulo before.

The following key conclusions are drawn from this study:

- The most abundant carbohydrate species was levoglucosan (231.9 ng/m^3), an important biomass burning tracer.
- Carbohydrates contributed collectively to the PM_{10} and OC mass with 1.3% and 11.5%, respectively; certain carbohydrate compounds such as threitol were measured in São Paulo for the first time.
- Fungal spores contributed 2% and 8% of the total PM_{10} and OC mass, respectively, in MASP. The fungal biomass contribution was not estimated previously for an urban area in Brazil, and were only estimated for the Amazon region, while being an important fraction of the PM and OC burden in urban areas.
- Our results show that PM_{10} had 5 main sources: soil re-suspension, primary biogenic aerosol, secondary inorganic aerosol, vehicular emissions, biomass burning and isoprene-related SOA.
- The biomass burning and isoprene-related SOA sources contributed approximately 1/4 of the PM_{10}, indicating that biomass burning has a strong influence on the PM_{10} concentration.
- Primary biogenic aerosol contributed 11% of PM_{10} mass, which is a significant value given that it was never quantified before.

The findings from this study suggest that it is necessary to consider the primary biogenic aerosol as an important source of urban PM_{10}. This component can influence atmospheric processes in many ways such as immune-active particles can cause respiratory diseases or aggravate them. There is need for detailed chemical analyses of organic tracers, collected from diverse sites during different seasons, to assist in building better understanding of their spatial and seasonal distributions.

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