


Article

PM₁₀-Bound Sugars: Chemical Composition, Sources and Seasonal Variations

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Abstract: The presence of anhydrosugars and sugar alcohols in airborne particulate matter <10 μm (PM₁₀) samples collected between December 2018 and June 2019 was studied for two urban environments in Coimbra. Anhydrosugars were used to estimate the biomass burning contribution, and sugar alcohols were investigated regarding biological sources. Anhydrosugars contributed more than sugar alcohols to the total sugars, mainly levoglucosan. Higher levoglucosan concentrations were linked with the use of biomass-fueled heating appliances, mainly during cold periods. A significant contribution from biomass burning smoke was registered, accounting for 20% to 23% of the PM₁₀ mass in the colder period. Xylitol presented higher concentrations in the colder period and was well correlated with levoglucosan, indicating a common origin. Mannitol and arabitol were well correlated with each other but did not present any kind of correlation with anhydrosugars or xylitol, suggesting a natural source. A quantitative estimation based on the concentration of ambient tracers (mannitol) was evaluated, and the results reveal that, for the two sites, the fungal spore relative contribution to PM₁₀ (roadside site: 2.7% to 2.8%; urban background: 1.9% to 2.7%) and OC mass (roadside site: 6.2% to 8.1%; urban background: 3.9% to 7.5%) was significant and always higher in the warmer period.

Keywords: sugars; PM₁₀; molecular tracers; urban environments; biomass burning



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1. Introduction

Airborne particulate matter (PM) is composed of primary and secondary compounds arising from anthropogenic and biogenic sources. In Europe, in recent years, there has been a substantial reduction of pollutant emissions from anthropogenic sources, especially from industrial processes and vehicle exhaust [1]. Among other reasons, this decrease can be attributed to increased regulation, better technologies and, in some cases, the relocation of heavily polluting and energy-intensive manufacturing industries outside Europe. Meanwhile, the awareness of the contribution of biogenic derived sources to airborne particulate matter loadings has been increasing [2–4]. Emissions from biologically derived sources include biomass burning, the contribution from the soil, dust and associated biota, and primary biological aerosols (PBAs) such as pollens, spores, yeasts, bacteria, fungi, algae, virus, fragments of vegetative debris, human or animal epithelial cells and parts of insects [5–7]. To better understand the contribution of each primary source and to establish the specific tracers of each source, it is necessary to conduct organic speciation studies. Estimations of particle emissions and burdens for different aerosol sources at the global level have been obtained in several studies. These specific tracers include hopanes, steranes and elemental carbon (EC) from fossil fuel combustion, high molecular weight *n*-alkanes for vascular waxes, anhydrosugars from cellulose and hemicellulose thermal degradation

and *n*-alkanoic and *n*-alkenoic acids for meat charbroiling, among others [8–10]. However, studies on the contribution of specifically PBAs, soil, dust and associated biota are still limited due to the lack of appropriate tracers, particularly on a regional scale. A few compounds have been suggested as PBA tracers: phospholipids, β -1,3-D-glucan, ergosterol, D-mannitol and D(+)-arabitol [9–12]. As major products of photosynthesized carbon in ecosystems, saccharides (carbon-hydrates) and their classes have been proposed as primary tracers for these biologically important organic materials on a global scale and to all particle size ranges. Their contribution is important, considering that approximately 13–26% of the mass of total compounds identified in continental aerosols were saccharides, and this number can reach as high as 63% in oceanic aerosols [2,7]. Atmospheric saccharides derived from different source types are mainly composed of: (a) monosaccharides including D(+)-glucose (dextrose), D(–)-fructose (levulose), D(+)-xylose, etc. and disaccharides such as D(+)-sucrose and D(+)-trehalose (mycose), among others released by microorganisms, plants, animals and soil suspension [5–7,13,14], (b) sugar alcohols or polyhydric alcohols (saccharide derivatives) such as D(+)-arabitol, D-mannitol, D-sorbitol (glucitol) and xylitol produced by fungi, lichens, bacteria and yeasts [5,6,9,11,15,16], and (c) anhydrosugars such as the three stereoisomers levoglucosan, galactosan and mannosan derived from primary thermal degradation of cellulose and hemicellulose (structural polysaccharides) present in biomass [6,17–19]. The chemical composition and seasonal variations of particulate matter depend on factors such as emission/transformation rates, distance from the source, and physical and meteorological conditions (e.g., precipitation, wind speed and prevailing wind direction) [20].

This paper will present for the first time the seasonal variation of concentrations, chemical speciation and sources of airborne particulate matter <10 μm (PM_{10})-bound sugars in the urban background and roadside sites in a Portuguese city. By adding sugar speciation to the chemical composition profile of environmental aerosols, this study seeks to contribute to the assignment of primary sources of biogenic carbon to the atmospheric particulate matter levels. This paper results from a larger research work that includes organic and inorganic characterization of PM_{10} , namely water-soluble ions and elements, which will be presented and discussed in future publications.

2. Experiments

2.1. Studied Area and Sampling Details

Sampling was carried out in the city of Coimbra between 1 December 2018 and 19 June 2019, a time period corresponding to the late autumn, winter and spring. Two sampling sites were selected: the Geophysical Institute (40°12'25" N; 08°25'30" W) located in a residential area with limited traffic and classified as an urban background site, and nearby the University Stadium (40°12'24.44" N; 08°26'13.43" W) located in a traffic-impacted site, classified as roadside. The straight-line distance between the two locations is approximately 2 km. Although the distance is relatively short, the two sampling sites are located at different altitudes and have dissimilar characteristics. The roadside site is located at about 17 m a.s.l. The urban background site, consisting largely of detached houses equipped with small scale combustion appliances, is located in the upper part of the city, at a higher elevation (about 140 m a.s.l.), and is more representative of the regional atmosphere. Sampling took place simultaneously at the two places. Coimbra is the fourth most important district capital in the country, with an estimated resident population of around 134 thousand inhabitants. It plays a chief role in central Portugal due to its large higher education community, technology and health sciences industry, administrative offices, financial services, law firms and specialized medical care. Samples were taken every 2 days on a 24 h basis, starting at 00:01 and ending at 23:59 (local time). In both sampling sites, particulate matter <10 μm (PM_{10}) samples were collected on pre-fired and weighed quartz filters (150 mm diameter, Pall Corporation, Ann Harbor, MI, USA) by high-volume air samplers (MCV CAV-A/mb, Barcelona, Spain), following the specifications of the UNE-EN 12,341 norm. Samples were taken at a flow rate of 30 $\text{m}^3 \cdot \text{h}^{-1}$ and used for gravimetric

mass and carbonaceous compositional determination. In parallel, PM₁₀ was also collected on preweighed Teflon filters (47 mm diameter, Pall Corporation, Ann Harbor, MI, USA) using low-volume air samplers (Echo PM Tecora, Cogliate, Italy). The Teflon filter samples were taken at a flow rate of 2.3 m³·h⁻¹ and used for quantification of the water-soluble fraction (ions and sugars) and element analysis. After collection, quartz filter samples were returned from the field in pre-fired aluminum foil envelopes, while Teflon filters were transported in dedicated Petri slides. After sampling, all the filters were frozen until analysis. This campaign resulted in a total of 200 samples. Field blank filters were collected for each batch of approximately 10 samples, following the sampling procedure, but without the pump on.

Meteorological parameters (temperature, relative humidity, wind direction and speed) were continuously measured at the roadside site nearby the University Stadium using a console Vantage Pro 2 with the program Integrated Sensor Suite (ISS) (Davis Instruments, Hayward, CA, USA) and the WeatherLink software for data processing.

To better understand the influence of atmospheric transport on aerosol properties, backward trajectory analysis was carried out using the hybrid single-particle Lagrangian integrated trajectory (HYSPLIT) model developed by NOAA's Air Resources Laboratory [21] with meteorological data from global data analysis system (GDAS). Five days (120 h) backward trajectories ending at the sampling place were calculated.

2.2. Analytical Determinations

The following standards were used: levoglucosan (Aldrich 316 555 1G, CAS 498-07-7, St. Louis, MO, USA), galactosan (Santa Cruz Biotechnology sc-213536, CAS 644-76-8, Dallas, TX, USA), mannosan (Santa Cruz Biotechnology sc-220563, CAS 14168-65-1, Dallas, TX, USA), L-(−)-arabitol (Sigma-Aldrich A3506, CAS 7643-75-6, St. Louis, MO, USA), D-mannitol (Sigma-Aldrich M4125, CAS 69-65-8, St. Louis, MO, USA) and xylitol (Sigma-Aldrich X3375, CAS 87-99-0, St. Louis, MO, USA). This and other information are described in Table S1 (available on Supplementary Material). For the sake of clarity, in this manuscript, L-(−)-arabitol will be referred to as arabitol and D-mannitol as mannitol.

The ultra-pure water prepared by a Milli-Q system (Millipore, Burlington, MA, USA), with a resistivity of 18.2 MΩ·cm⁻¹, was used to extract the samples and to prepare the standard solutions and eluents. For the mobile phase, sodium hydroxide aqueous solutions were prepared from a 50–52% (*w/w*) NaOH solution (Sigma-Aldrich, CAS 1310-73-2, St. Louis, MO, USA).

PM₁₀ of each collected aerosol sample was determined using a microbalance. The filters were weighed under temperature and humidity-controlled conditions before and after sampling to obtain aerosol mass loadings.

Each half of the 47 mm diameter Teflon filters was placed in a screwed cap vial and extracted with 10 mL of ultra-pure water by sonication (30 min, with a stop of 5 min in the middle). After extraction, the solution was filtrated through a 13 mm PVDF syringe filter with 0.2 μm pore size (Whatman™, Buckinghamshire, United Kingdom) to remove any insoluble species. Standard solutions were prepared in the required concentrations.

The anhydrosugars (galactosan, levoglucosan, and mannosan) and the sugar alcohols (arabitol, mannitol and xylitol) selected in this assay were determined by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD), which allows their quantification in a single run. The determination by HPAE-PAD has the advantage of avoiding the use of organic solvents and derivatizing agents, becoming a more ecological, faster, less costly and less labor-intensive technique. The analyses were carried out by means of a Thermo Fisher Scientific (Waltham, MA, USA) Dionex™ ICS-5000+ equipped with an autosampler at 13 °C with a sample injection valve, and a quaternary pump with a 10 μL sample loop. The Chromeleon software was employed for controlling the system and data analysis. The different anhydrosugars and sugar alcohols were separated using a Carbopac PA-1 guard column (2 × 50 mm²) and a Carbopac PA-1 anion-exchange analytical column (2 × 250 mm²), both at 20 °C. For the mobile phase,

three eluents were used: eluent A—ultra-pure water, eluent B—200 mM NaOH and eluent C—5 mM NaOH. The analysis program is schematically reported in Table 1. Note that to prevent CO₂ absorption and consequent carbonate contamination of the eluent, the eluent tanks were purged by an N₂ flow for 10/15 min.

Table 1. Multistep gradient conditions with A—water, B—200 mM NaOH and C—5 mM NaOH.

Time (Min)	Flow	% A	% B	% C
0	0.25	90	0	10
10	0.25	90	0	10
20	0.25	0	100	0
30	0.25	0	0	10
50	0.25	90	0	10

Each analysis took 10 min and was followed by the regeneration of the analytical column consisting of rinsing the column with a more concentrated eluent (NaOH 200 mM). The column was regenerated for 20 min at the end of each analysis in order to obtain reproducible retention times, to elute compounds retained in the column, and finally to preserve the column from carbonate formation. Moreover, to improve the reproducibility, the column was re-equilibrated for a period of 15 min before starting a new measurement. The liquid chromatography system comprised an amperometric detector (Dionex ED50) equipped with an electrochemical cell. The detector cell had a disposable gold electrode and a pH electrode as reference (both from Dionex) and was operated in the pulsed amperometric detection (PAD) mode. For the detector sensitivity, a pulse pattern was used with a measuring potential of 0.22 V. The waveform used was Gold, Carbo, Quad for carbohydrates. The detection limits for each sugar derivative were 0.0039 µg·m⁻³, 0.0032 µg·m⁻³, 0.0204 µg·m⁻³, 0.0034 µg·m⁻³, 0.0043 µg·m⁻³, 0.0034 µg·m⁻³ for xylitol, arabinol, levoglucosan, mannitol, mannosan and galactosan, respectively.

The carbonaceous content (organic carbon (OC) and elemental carbon (EC)) of particulate matter was analyzed with a thermal-optical technique, where carbonaceous compounds were converted into CO₂ using a homemade thermal-optical transmission system. The system includes a quartz tube with two heating zones, a laser and a nondispersive infrared (NDIR) CO₂ analyzer [22,23]. For this determination, small discs with 9 mm diameter were punched out of the quartz filters. For each filter, two replicate analyses were done. Controlled heating in anoxic conditions was performed to separate all organic carbon (OC) of increasing volatility. The last fraction of OC is identified by transmittance and corresponds to pyrolyzed organic carbon (PC) produced in the previous heating steps. The separation between OC and EC was achieved by initially heating the filter punches under an inert atmosphere to evaporate first the OC fraction. The remaining fraction is sequentially evaporated/burnt under a gas flow containing O₂. This last carbon fraction contains initial EC plus OC that has pyrolyzed during heating under an inert atmosphere. The interference between PC and EC can be controlled by continuous evaluation of the blackening of the filter using a laser beam and a photodetector measuring the filter light transmittance. This analysis follows a temperature program similar to the EUSAAR-2 protocol (CEN/TR 16243, 2011). The analyzer is daily calibrated with standard atmospheres and filters impregnated with known amounts of potassium phthalate. Detection limits (DL) depend on the variability of filter blanks. The detection limits were calculated assuming 3 times the standard deviation of each batch of filters used.

2.3. Statistical Treatment

For the statistical treatment, the SPSS software (IBM Statistics software v. 26) was used. Kolmogorov–Smirnov and Levene tests [24] were used to test the distribution of variables and the population variances, respectively. Afterward, the nonparametric Mann–Whitney *U*-test [25] was applied in order to determine the eventual statistically significant differences. A difference between two means was considered statistically significant

when the p -value of the two-tailed Mann–Whitney U -test was lower than 0.001. The main correlations were determined using Spearman’s rank correlation coefficient ρ (ρ) (p -value < 0.01).

3. Results and Discussion

3.1. Meteorological Conditions

Part of the meteorological data was obtained from the portable meteorological station that was installed nearby the University Stadium, but some gaps and additional details had to be filled with data provided by the Geophysical and Astronomical Observatory of the University of Coimbra. As previously mentioned, the sampling campaign started in the last days of autumn, ran throughout the winter and ended in late spring. Although the sampling campaign did not cover the four seasons, the seasonal analysis was carried out by dividing the sampling campaign into a colder period (last days of autumn and winter: 1 December 2018–20 March 2019) and a warmer period (spring: 22 March 2019–19 June 2019). During the monitoring period, temperatures varied from a minimum value of 4 °C (in winter) to a maximum of 24 °C (in spring). The records indicate that the predominant wind directions were W, NW and S, SE. During the sampling campaign, the average wind speed was similar in both periods, 2.3 and 2.6 m·s^{−1} for the colder and warmer periods, respectively. This and other information are described in Table 2.

Table 2. Coimbra meteorological conditions during the sampling period. The range and the mean values are presented for temperature, relative humidity, total accumulated precipitation and wind speed.

Meteorological Conditions	Colder Period	Warmer Period
Temperature (°C)	11 (4–15)	16 (9–24)
Relative humidity (%)	81 (39–100)	72 (28–99)
Total accumulated precipitation (mm)	11 (0.0–1.7)	10 (0.0–1.3)
Wind speed (m·s ^{−1})	2.3	2.6

3.2. Particulate Matter and Organic Carbon Seasonal Variations

Throughout the sampling campaign for the roadside site, the mean PM₁₀ concentration was 26 µg·m^{−3} with a minimum of 10 µg·m^{−3} and a maximum of 76 µg·m^{−3}. For the urban background site, a mean level of 20 µg·m^{−3} was obtained, ranging from 7 µg·m^{−3} and 56 µg·m^{−3} (Figure 1). The PM₁₀ concentrations were lower in the urban background site compared to the roadside site with significant statistical differences (p -value < 0.001). On four days, the PM₁₀ concentrations exceeded the daily limit value of 50 µg·m^{−3} set by the Air Quality Directive 2008/50/CE, as shown in Figure 1.

PM₁₀ showed a marked seasonal variation, with average concentrations for the roadside site of 32.3 ± 12.3 and 19.4 ± 6.12 µg·m^{−3}, in the colder and warmer period, respectively. The urban background levels were 24.5 ± 10.9 and 15.1 ± 5.56 µg·m^{−3}, for the same periods (Table 3). Those differences were statistically significant (p -value < 0.001).

The study of PM_{2.5} aerosols from urban background sites, conducted by Alves et al. [26] in Coimbra (winter season, 2007), depicted similar values of 19.3 ± 13.3 µg·m^{−3}, representing 70.6 ± 16.1% of the PM₁₀ mass. The PM₁₀ concentrations (present study) are in agreement with the values reported for other cities referenced by the 2019 report of the European Environmental Agency [27]. The PM₁₀ average concentration in Coimbra was lower than those in Asian cities like Beijing [28] in China, and Faisalabad [29] and Islamabad [30] in Pakistan.

During the sampling campaign, long-distance dust transport from North Africa was observed, influencing the PM₁₀ concentrations between 25 and 27 December, 18–22 February and 26–30 March. These episodes were described on the website of the Portuguese Environment Agency [31] and confirmed with the backward trajectory analysis by the HYSPLIT model (Figure S1 available on Supplementary Material). The PM₁₀ concentrations in both sampling sites tend to be close to each other in the periods under the influence

of Saharan dust. As will be seen below, PM₁₀ concentration peaks coincided with the maximum saccharide levels. In the remaining periods, Coimbra was under the influence of continental air masses, most of which originating and staying over Spain, or maritime air masses. The minimum levels were recorded when backward trajectories indicated an Atlantic origin.

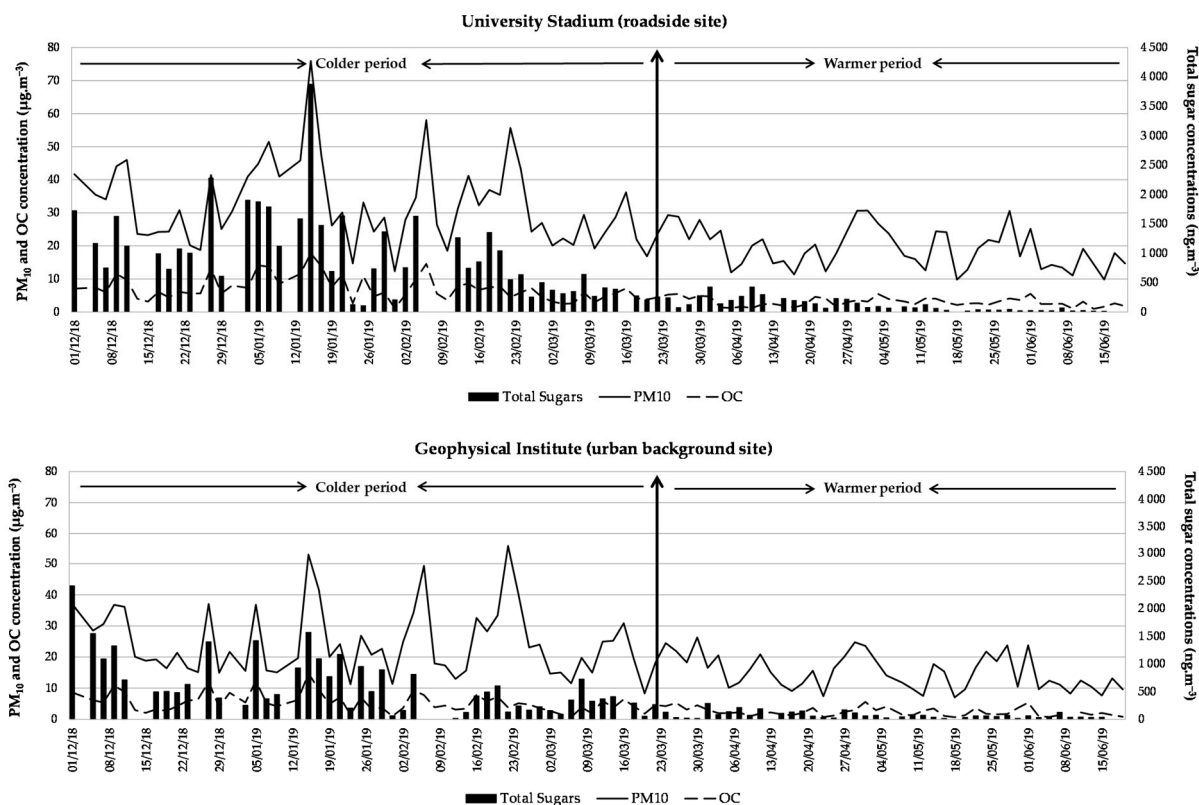


Figure 1. Particulate matter <10 µm (PM₁₀), organic carbon (OC) and total sugar concentrations measured during the sampling campaign for each sampling site.

Table 3. PM₁₀ and OC seasonal mean concentration (µg·m⁻³) for each sampling site.

University Stadium (Roadside Site)						
	Colder Period			Warmer Period		
	Min.	Max.	Mean (SD)	Min.	Max.	Mean (SD)
PM ₁₀	12.3	75.9	32.3 (12.3)	9.80	30.7	19.4 (6.12)
OC	1.14	17.8	7.11 (3.61)	0.97	5.52	3.01 (1.30)
Geophysical Institute (Urban Background Site)						
	Colder Period			Warmer Period		
	Min.	Max.	Mean (SD)	Min.	Max.	Mean (SD)
PM ₁₀	8.28	55.7	24.5 (10.9)	7.02	26.3	15.1 (5.56)
OC	0.66	14.8	5.35 (3.02)	0.65	5.59	2.38 (1.35)

SD—standard deviation.

During the whole sampling campaign for the roadside site, the mean organic carbon (OC) concentration was 5.2 µg·m⁻³ with a minimum of 0.97 µg·m⁻³ and a maximum of 18 µg·m⁻³ (Figure 1). For the urban background site, a mean level of 4.0 µg·m⁻³ was obtained, ranging from 0.65 µg·m⁻³ and 15 µg·m⁻³ (Figure 1). The OC concentrations present significant statistical differences (*p*-value < 0.001) between the two sampling sites.

Although the OC seasonal variation is not so evident, slightly higher values were observed in the colder period (p -value < 0001). The mean concentrations for the roadside site were 7.11 ± 3.61 and $3.01 \pm 1.30 \mu\text{g}\cdot\text{m}^{-3}$, in the colder and warmer period, respectively. The urban background levels were 5.35 ± 3.02 and $2.38 \pm 1.35 \mu\text{g}\cdot\text{m}^{-3}$, for the same periods (Table 3).

3.3. Sugar Concentrations and Seasonal Variations

During the sampling campaign, the total sugar concentrations showed significant fluctuations, ranging from $19.3 \text{ ng}\cdot\text{m}^{-3}$ to $3.88 \times 10^3 \text{ ng}\cdot\text{m}^{-3}$ and averaging $570 \text{ ng}\cdot\text{m}^{-3}$ for the roadside site, which represents 1.8% of the PM_{10} mass. For the urban background site, the mean concentration was $363 \text{ ng}\cdot\text{m}^{-3}$ with a minimum of $19 \text{ ng}\cdot\text{m}^{-3}$ and a maximum of $2.41 \times 10^3 \text{ ng}\cdot\text{m}^{-3}$, accounting for a PM_{10} mass fraction of 1.5%, on average. The total sugar concentrations were higher in the colder period and lower during the warmer period, for both sampling sites (Table 4), with differences statistically significant. This obvious seasonal variation is demonstrated with a strong and negative correlation (roadside site: $\rho = 0.83$; urban background site: $\rho = -0.72$; p -value < 0.01) between the total sugar concentrations and the daily mean temperatures.

Table 4. Seasonal mean concentrations ($\text{ng}\cdot\text{m}^{-3}$) of the selected sugars in PM_{10} samples for each sampling site.

University Stadium (Roadside Site)						
	Colder Period			Warmer Period		
	Min.	Max.	Mean	Min.	Max.	Mean
Levogluconan	70.4	2.89×10^3	692	10.2	291	60.9
Mannosan	8.94	492	115	0.55	46.1	9.07
Galactosan	3.71	218	45.4	1.68	35.7	4.82
Xylitol	1.96	155	41.6	1.96	15.6	5.80
Arabitol	1.59	122	36.4	1.59	70.4	21.4
Mannitol	7.44	100	42.3	1.72	68.4	26.8
Total sugars	113	3.88×10^3	973	19.3	434	129
Geophysical Institute (Urban Background Site)						
	Colder Period			Warmer Period		
	Min.	Max.	Mean	Min.	Max.	Mean
Levogluconan	10.2	1.83×10^3	444	10.2	214	40.2
Mannosan	2.13	329	81.8	1.46	31.9	6.05
Galactosan	1.68	101	28.6	1.68	8.30	2.46
Xylitol	1.96	86.0	19.9	1.96	14.4	2.95
Arabitol	1.59	103	20.9	1.59	48.5	12.9
Mannitol	1.72	80.8	23.4	1.72	43.9	19.1
Total sugars	19.3	2.41×10^3	619	19.3	289	82.9

The total sugar concentrations in Coimbra were in the range of measurements conducted elsewhere (Table 5), including those at the urban background and suburban sites in Oslo, Norway [32], and in Bologna, Italy, but were lower than those observed in the urban atmosphere of Guangzhou (China) [33], the San Joaquin Valley in California (USA) [34] and values documented for cities in Pakistan [29] and India [35]. However, the comparison between results should be made with some caveat since the various studies involve diverse particle sizes, saccharide/sugar compounds and analytical techniques.

Six sugars were determined in PM_{10} samples, including three species of anhydrosugars (levogluconan, mannosan and galactosan) and three species of sugar alcohols (xylitol, arabitol, and mannitol). The anhydrosugar mean concentration was $481 \text{ ng}\cdot\text{m}^{-3}$ and $313 \text{ ng}\cdot\text{m}^{-3}$ for the roadside and urban background sites, respectively. For sugar alcohols, the mean concentration was $88.6 \text{ ng}\cdot\text{m}^{-3}$ and $50.2 \text{ ng}\cdot\text{m}^{-3}$ for the roadside and urban

background sites, respectively. These two groups contributed 84% and 16% (roadside site) and 86% and 14% (urban background site) to the total sugars, respectively.

Table 5. Saccharide/sugar concentrations ($\text{ng}\cdot\text{m}^{-3}$) reported in the literature for other locations.

Location	Sampling Period	PM	Concentration	Reference
MS, Bologna, Italy (urban) San Pietro Capofiume, Italy (rural)	June 2012–May 2013 June 2012–May 2013	PM _{2.5}	210 193	[36]
Curbside site, Norway Urban background site, Norway Suburban site, Norway Rural background site, Norway	September–October 2002 November–December 2001 January–March, May–June 2002 January–December 2002	PM _{2.5}	17–134 10–537 14–1085 1–43	[32]
Sao Paulo State, Brazil	May 2010–February 2012	TSP	2.540 ± 1.760	[37]
Amazonia, Brazil	July 2001	PM _{2.5}	32–115 (61)	[38]
San Augustine, Texas, USA (rural) Clarksville, Texas, USA (rural) Dallas, Texas, USA (urban) Higley, Arizona, USA (agricultural)	November 2005–July 2006 January–July 2006 January–July 2006 January–April 2008	PM _{2.5}	15.4–355.1 (70.4) 7.5–372.2 (128.4) 15.8–196.0 (52.4) 1.1–83.0 (24.1)	[2]
Howland experimental forest, Maine, USA	May–October 2002	PM _{2.5}	10–180	[39]
San Joaquin Valley, CA, USA	December 1995–January 1996	PM _{2.5}	144–3644	[34]
Fudan, Shanghai, China (urban)	March 2013–January 2014	PM _{2.5}	9.4–1652.9 (346.9)	[4]
Tsinghua University, Beijing, China (urban)	November 2010–October 2011	PM _{2.5} PM ₁₀	66.1–3389.1 (600.0) 116.5–4302.3 (792.3)	[28]
Pingtung, Taiwan, China (coastal)	February–April 2013	PM _{2.5}	589.5	[40]
Shanghai, China (urban)	Spring 2012 Summer Autumn Winter 2013	PM _{0.056} –PM ₁₈	120.3 104 292.9 206	[41]
Guangzhou, China (urban)	Spring Summer Autumn Winter	TPS	399.7 443.1 725.6 503.6	[33]
Hong Kong University, China (urban)	Spring Summer Fall Winter	PM _{2.5}	38–129 (84) 83–175 (133) 70–1316 (375) 88–683 (292)	[42]
Chichi-Jima Island, Japan (marine)	1990–1993 2006–2009	TSP	1.34–67.5 (14.8) 3.64–247 (38.7)	[43]
Faisalabad, Pakistan (urban)	2015–2016	PM ₁₀	282–10,995	[29]
Rajim, India (rural)	October–November 2011	PM _{2.5}	4781.1–17,979 (10,166)	[35]

3.3.1. Anhydrosugars

From the results present in Table 4, it is observed that the levoglucosan was the most abundant anhydrosugar in both sampling sites and in the two considered seasons, followed by mannosan and galactosan. Levoglucosan reached a mean value of $692 \text{ ng}\cdot\text{m}^{-3}$ (colder period) and $60.9 \text{ ng}\cdot\text{m}^{-3}$ (warmer period), contributing with 71% and 47% to the total sugars in the roadside site. In the urban background site, this anhydrosugar reached $444 \text{ ng}\cdot\text{m}^{-3}$ (colder period) and $40.2 \text{ ng}\cdot\text{m}^{-3}$ (warmer period), accounting for 72% and 49% of the total sugars. The other anhydrosugars, mannosan and galactosan, presented lower concentrations values when compared to levoglucosan, and galactosan was substantially the less abundant anhydrosugar throughout the sampling campaign, as shown in Table 4. The three stereoisomers were positively and strongly correlated with each other ($\rho > 0.94$; p -value < 0.01), indicating a common origin. Furthermore, their concentrations were significantly higher in the colder period (Figure 2), showing a strong and negative correlation (roadside site: $\rho > -0.72$; urban background site: $\rho > -0.43$; p -value < 0.01) between the three anhydrosugars and the daily mean temperatures (Table 6). No correlation was found with relative humidity and precipitation. The three anhydrosugars were also positively and strongly correlated with OC during the colder period (roadside site: $\rho > 0.76$; urban background site: $\rho > 0.72$; p -value < 0.01) (Table 6). As observed in the present study, higher anhydrosugar concentrations at roadside sites compared to the urban background have

been reported for other European cities, showing the regional nature of the biomass burning aerosol [44]. Differences in concentrations are probably associated with the specificities of the topography and dispersion conditions of the various sites.

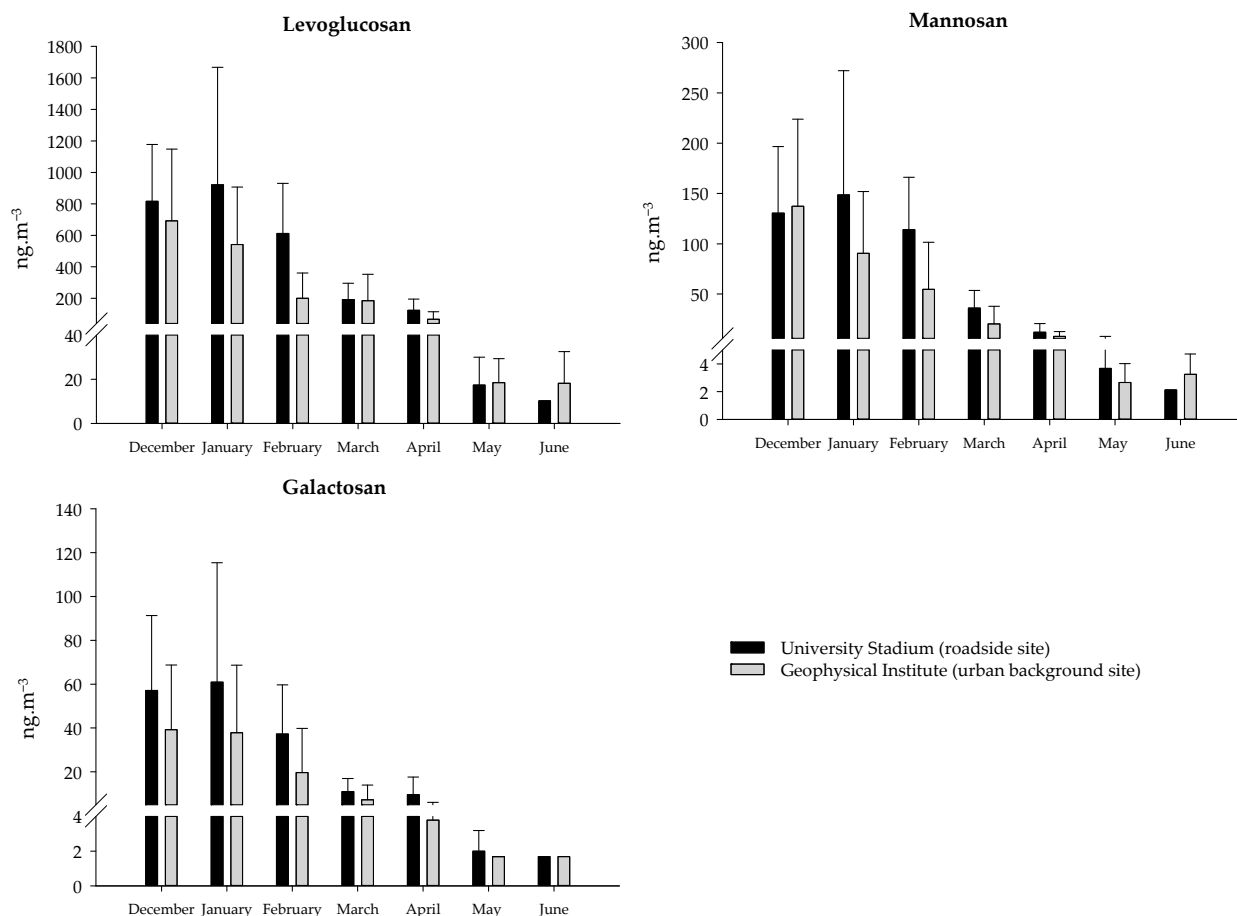


Figure 2. Monthly variation of concentrations of anhydrosugars during the sampling campaign in Coimbra.

Table 6. Spearman correlations between sugar compounds, organic carbon and meteorological parameters.

	University Stadium (Roadside Site)											
	Colder Period						Warmer Period					
	Xylitol	Arabitol	Levoglucosan	Mannitol	Mannosan	Galactosan	Xylitol	Arabitol	Levoglucosan	Mannitol	Mannosan	Galactosan
OC	0.765 **		0.779 **		0.785 **	0.764 **					−0.468 **	
T	−0.748 **		−0.756 **		−0.754 **	−0.724 **	−0.674 **		−0.649 **		−0.468 **	−0.724 **
RH		0.698 **						0.494 **	0.355 *			0.475 **
TP		0.302 *					0.320 *	0.575 **	0.370 *			0.419 **
	Geophysical Institute (Urban Background Site)											
	Colder Period						Warmer Period					
	Xylitol	Arabitol	Levoglucosan	Mannitol	Mannosan	Galactosan	Xylitol	Arabitol	Levoglucosan	Mannitol	Mannosan	Galactosan
OC	0.797 **	0.489 **	0.723 **	0.484 **	0.786 **	0.728 **					−0.572 **	−0.597 **
T	−0.473 **		−0.430 **		−0.494 **	−0.478 **	−0.325 *		−0.415 **		−0.572 **	−0.597 **
RH		0.576 **	0.419 **	0.366 *	0.365 *	0.320 *		0.555 **	0.398 **	0.336 *	0.330 *	0.331 *
TP								0.446 **	0.341 *			

OC—organic carbon ($\mu\text{g}\cdot\text{m}^{-3}$); T—temperature ($^{\circ}\text{C}$); RH—relative humidity (%); TP—total accumulated precipitation (mm) * The correlation is significant at the 0.05 level. ** The correlation is significant at the 0.01 level.

Furthermore, strong and positive correlations were observed between concentrations of the two sampling sites, suggesting that biomass combustion affects air quality not only locally but in a larger area. This marked seasonal pattern is most likely associated with the use of biomass-fueled heating appliances due to low-temperature values in the colder period. Pollution roses, conditioned by temperature, were plotted for the University Stadium (Figure 3a) and the Geophysical Institute (Figure 3b) sites, using the Openair R package [45], and are shown in Figure 3.

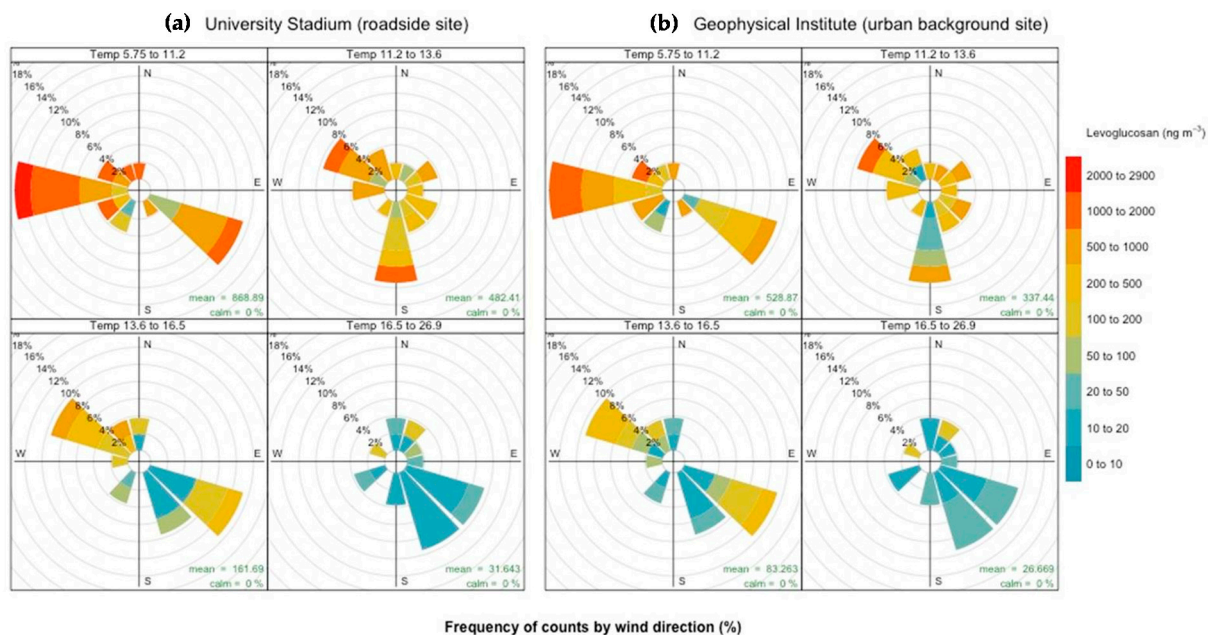


Figure 3. Pollution roses for levoglucosan concentrations conditioned by temperature, for each sampling site: University Stadium (roadside site) (a) and Geophysical Institute (urban background site) (b).

In both sites, the plots indicate that higher levoglucosan concentrations (over $1000 \text{ ng}\cdot\text{m}^{-3}$) were associated with the lowest temperatures and W, SE and NW directions. Colder days, e.g., days with the lowest 25% of observed temperature data, were associated with mean levoglucosan concentrations of $869 \text{ ng}\cdot\text{m}^{-3}$ and $529 \text{ ng}\cdot\text{m}^{-3}$ at the roadside and urban background sites, respectively. On days with temperatures between the first and second quartiles, mean levoglucosan concentrations were still high (roadside- $482 \text{ ng}\cdot\text{m}^{-3}$ and urban background- $337 \text{ ng}\cdot\text{m}^{-3}$). In this group of days, although the prevailing wind was from the S, higher levoglucosan concentrations were registered for air masses blowing from the S and NW directions. As expected, days with higher temperatures showed much lower levoglucosan mean concentrations. To better understand the wind speed role, pollution roses conditioned by wind speed and temperature were plotted (Figure S2 available on Supplementary Material). The plots showed much higher levoglucosan concentrations (roadside— $1295 \text{ ng}\cdot\text{m}^{-3}$ and urban background— $730 \text{ ng}\cdot\text{m}^{-3}$) under low-temperature and low wind speed conditions. Larger concentrations for the NW direction at the traffic site generally point towards more significant rural and suburban sources in that direction (in comparison to less densely populated areas in the SE direction) and a probable dilution effect over the river when compared to the urban background location.

The anhydrosugar mean concentrations are in agreement with the values reported for the same urban background site in Coimbra (PM_{2.5}; winter season, 2007) (levoglucosan: $138 \text{ ng}\cdot\text{m}^{-3}$; mannosan: $31.9 \text{ ng}\cdot\text{m}^{-3}$ and galactosan: $10.5 \text{ ng}\cdot\text{m}^{-3}$) and for an urban background station in the Oporto metropolitan area (levoglucosan: $456 \text{ ng}\cdot\text{m}^{-3}$; mannosan: $43.7 \text{ ng}\cdot\text{m}^{-3}$ and galactosan: $22 \text{ ng}\cdot\text{m}^{-3}$) [22]. The study carried out by Caseiro et al. [44] documented winter mean concentrations of levoglucosan (urban background: $122 \text{ ng}\cdot\text{m}^{-3}$; roadside site: $165 \text{ ng}\cdot\text{m}^{-3}$) and mannosan (urban background: $29.1 \text{ ng}\cdot\text{m}^{-3}$; roadside

site: $34.2 \text{ ng}\cdot\text{m}^{-3}$) in Oporto roughly in the same range of values obtained in the present study. Galactosan mean concentrations (urban background: $0.83 \text{ ng}\cdot\text{m}^{-3}$; roadside site: $0.22 \text{ ng}\cdot\text{m}^{-3}$) were significantly lower than those observed in this study. The values measured in this study are also in the range of data from other European cities like Oslo (PM₁₀; fall season) (urban background/curbside: levoglucosan- $193 \text{ ng}\cdot\text{m}^{-3}/45 \text{ ng}\cdot\text{m}^{-3}$; mannosan: $52 \text{ ng}\cdot\text{m}^{-3}/8.5 \text{ ng}\cdot\text{m}^{-3}$ and galactosan: $1.7 \text{ ng}\cdot\text{m}^{-3}/3.5 \text{ ng}\cdot\text{m}^{-3}$) [32], Florence (PM_{2.5}; winter season) (urban background: levoglucosan— $371 \text{ ng}\cdot\text{m}^{-3}$; urban traffic: levoglucosan— $355 \text{ ng}\cdot\text{m}^{-3}$) [46] and Austrian cities, as described in Caseiro et al. [47]. When compared with the results of countries like China and the USA, the anhydrosugar mean concentrations of the present study are lower.

Several source assignment studies have determined the contribution of biomass burning to the PM₁₀ mass based on levoglucosan to PM ratios. In the present study, a conversion factor derived from source tests involving typical Portuguese wood species [18] was used.

$$\text{Biomass smoke PM} = \text{levoglucosan} \times 11.3 \quad (1)$$

Based on Equation (1), it was estimated that biomass burning represented, on average, 23% and 3.5% of the PM₁₀ mass in the colder and warmer period, respectively, in the roadside site. The contribution in the urban background was estimated to be 20% and 3.1% for the same periods. These results are comparable with a source apportionment study carried out at a traffic impacted site in Oporto indicated that biomass burning accounted for 13% of the particulate mass concentrations on an annual basis, but this contribution was 26–36% on days when the daily PM₁₀ standard was exceeded [48]. Several authors use levoglucosan-to-mannosan ratios (L/M) to distinguish between hard- and softwood smoke contributions. Hardwood comes from angiosperm—or flowering plants—such as Portuguese oak, eucalypt and olive. Softwood comes from gymnosperm trees, usually evergreen conifers, like maritime pine or spruce. In this study, the L/M ratio averaged 8 in the roadside site and 7 in the urban background site, indicating a predominant use of softwood. The L/M ratios are in agreement with the value of 8 obtained by Alves et al. [26] for Coimbra (PM_{2.5}; winter season of 2007). Fine et al. [49] reported ratios around 19–26 and 5.4 for smoke particles from residential combustion of hard- and softwood tree species, respectively. Gonçalves et al. [18] documented ratios in the range 10–35 for hardwoods, while a value of 3 was found for softwood. Schmidl et al. [50] obtained ratios of 4 and 15, respectively, for soft- and hardwoods.

3.3.2. Sugar Alcohols

Regarding the sugar alcohols evaluated in this assay, some fluctuations were observed. Mannitol was the most abundant sugar alcohol measured in both periods and in both campaign sites, followed by arabitol and xylitol (Table 4). However, for the colder period, at the roadside site, this tendency was not observed. In this case, xylitol presented a higher concentration ($41.6 \text{ ng}\cdot\text{m}^{-3}$) than arabitol ($36.4 \text{ ng}\cdot\text{m}^{-3}$).

The mean concentrations of mannitol were $42.3 \text{ ng}\cdot\text{m}^{-3}$ (colder period) and $26.8 \text{ ng}\cdot\text{m}^{-3}$ (warmer period), contributing 4.3% and 20.9% to the total sugars in the roadside site. In the urban background site, mean levels of $23.4 \text{ ng}\cdot\text{m}^{-3}$ (colder period) and $19.1 \text{ ng}\cdot\text{m}^{-3}$ (warmer period) were registered, accounting for 3.8% and 23% of the total sugars (Table 4). Lower concentrations of arabitol were observed throughout the sampling campaign. Xylitol mean levels of $41.6 \text{ ng}\cdot\text{m}^{-3}$ (colder period) and $5.8 \text{ ng}\cdot\text{m}^{-3}$ (warmer period) were recorded in the roadside site. In the urban background site, the corresponding values were $19.9 \text{ ng}\cdot\text{m}^{-3}$ and $2.95 \text{ ng}\cdot\text{m}^{-3}$. The mannitol and arabitol mean concentrations observed in Table 4 are in line with the values reported for the urban area of Vienna (PM₁₀; summer) (mannitol: $42 \text{ ng}\cdot\text{m}^{-3}$ and arabitol: $28 \text{ ng}\cdot\text{m}^{-3}$) but are significantly higher when compared with the values obtained for Oslo (PM₁₀; fall season) (urban background: mannitol- $8.1 \text{ ng}\cdot\text{m}^{-3}$ and arabitol- $5.3 \text{ ng}\cdot\text{m}^{-3}$; urban traffic: mannitol- $20 \text{ ng}\cdot\text{m}^{-3}$ and arabitol- $18 \text{ ng}\cdot\text{m}^{-3}$) [32] or with data from an urban site in Dallas (mannitol $< 6 \text{ ng}\cdot\text{m}^{-3}$; arabitol $< 3 \text{ ng}\cdot\text{m}^{-3}$) [2].

Xylitol closely follows the anhydrosugar patterns, showing higher concentrations in the colder period. Levels of this polyol were positively and strongly correlated with those of levoglucosan ($\rho = 0.97$ and $\rho = 0.82$; p -value < 0.01 for the roadside and urban background sites, respectively), indicating a common origin. Xylitol has been associated with fungal spores and soil biota but was also reported as a constituent of biomass smoke [2,5,7,27,31,51]. It was positively and strongly correlated with OC during the colder period (roadside site: $\rho = 0.77$; urban background site: $\rho = 0.80$; p -value < 0.01) (Table 6). This sugar alcohol did not present correlations with meteorological parameters, except for the temperature, with which a strong negative correlation was observed (Table 6).

Mannitol and arabitol originate from either PBAPs or other biologically derived carbohydrate sources such as resuspended soil and associated biota [5,7,9,39]. It has been described that these sugar alcohols, in temperate climate areas, usually increase from negligible concentrations in winter aerosols to a maximum in late spring–summer [7]. In this study, this trend was not observed (Figure 4). Mannitol and arabitol were well correlated with each other ($\rho = 0.65$ and $\rho = 0.74$; p -value < 0.01 for the roadside and urban background sites, respectively), indicating a common origin and did not present any kind of correlation with anhydrosugars. The lack of correlation between the sugar alcohols and anhydrosugars indicates that sugar alcohols contribute to the natural background aerosol, i.e., PBAPs. In the colder period, arabitol and mannitol were positively correlated with relative humidity. In the warmer period, only arabitol was positively correlated with relative humidity and total accumulated precipitation. Higher humidity and temperature conditions favor the release of fungal spores and the swelling and rupture of the pollen grains and/or bacteria walls, which leads to the increase of the aforementioned sugars [52,53].

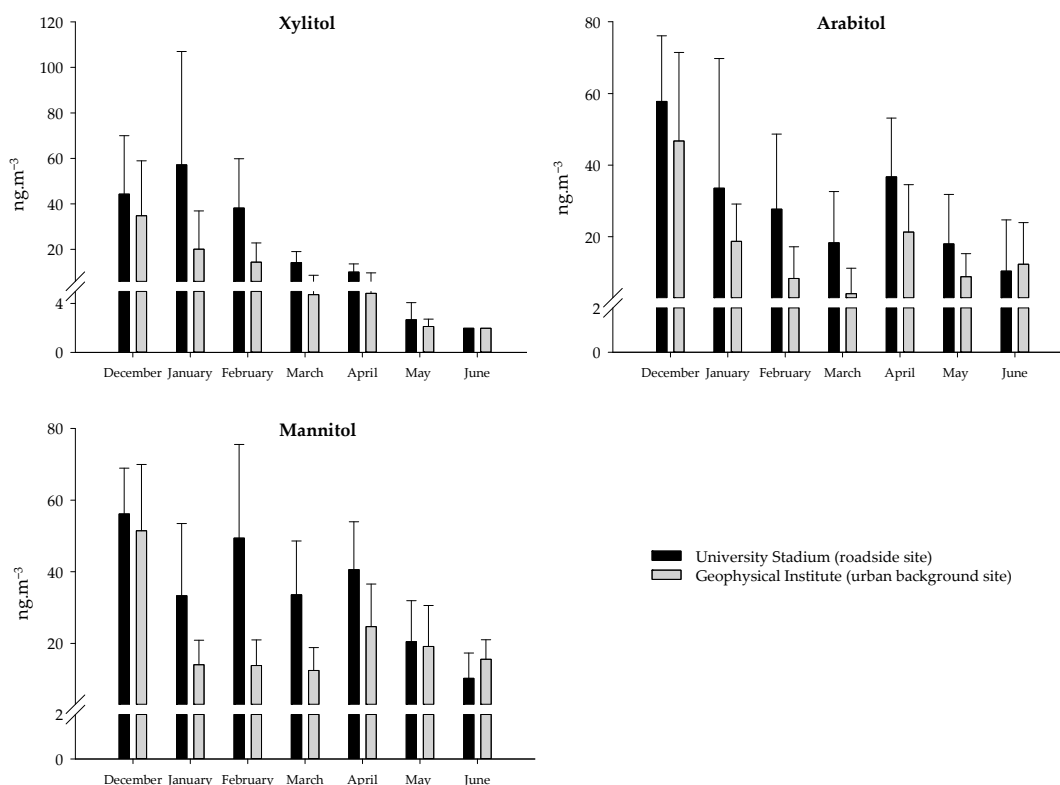


Figure 4. Monthly variation of sugar alcohols during the sampling campaign in Coimbra.

Several authors have tried to make quantitative estimates of the contributions of fungal spores to the PM mass and to OC, based on ambient tracer concentrations. One of the possible methods is to use the conversion factors suggested by Bauer et al. [9,54,55]. In the present study, rough quantitative estimates were obtained by using three conversion factors

(1) arabitol and mannitol concentration to spore number concentration (1.2 pg arabitol spore⁻¹; 1.7 pg mannitol spore⁻¹); (2) spore number concentration to spore fresh mass concentration (33 pg fresh mass spore⁻¹) and (3) spore number concentration to organic carbon concentration of spores (13 pg C spore⁻¹). Using the published conversion factor for mannitol (1.7 pg mannitol spore⁻¹), the average numbers of fungal spores in PM₁₀ during this study for the roadside site were estimated to be 24,982 spores m⁻³ and 15,790 spores m⁻³ in the colder and warmer periods, respectively. For the urban background, levels were estimated to be 13,344 spores m⁻³ and 11,238 spores m⁻³ for the same periods. These values are comparable to the fungal spore counts when using the arabitol conversion factor (1.2 pg arabitol spore⁻¹), as can be seen in Table S2 (available on Supplementary Material). For better comparison with results from other studies, the conversion factors (33 pg fresh mass spore⁻¹ and 13 pg C spore⁻¹) were used to estimate the contribution of fungal spores to ambient PM and OC mass in Coimbra. On average, the relative contributions of fungal spores to the PM₁₀ mass for the roadside site were 2.7% and 2.8% in the colder and warmer period, respectively. For the urban background site, the average relative contributions were 1.9% and 2.7% for the same periods. The mean relative contributions of fungal spores to OC for the roadside site were estimated to be 6.2% and 8.1% in the colder and warmer period, respectively. The corresponding urban background values were 3.9% and 7.5% for the same periods. These results reveal the importance of the fungal spore contribution to the total aerosol burden in the atmosphere. For the two sites, the fungal spore relative contribution to PM₁₀ and OC mass was significant and always higher in the warmer period. These data are comparable with the findings by Bauer et al. [55] (2.3% and 4.3%) and by Emygdio et al. [56] (2% and 8%) in other urban areas. In other areas, such as suburban sites (4.8% and 10%) [55] and tropical forest (7.9% and 12.1%) [57], the percentages are higher than those of this study, which was expected to some extent since fungal spores tend to be less abundant in urban areas.

4. Conclusions

The evolution of six sugars compounds in PM₁₀ samples, collected between 1 December 2018 and 19 June 2019 (time period corresponding to the late autumn, winter and spring), was analyzed for two sampling sites (roadside and urban background) in Coimbra city. It is important to understand and to determine the chemical composition, the natural and/or anthropogenic sources, and the factors that may influence the variation of sugar compounds, such as seasons and respective meteorological parameters. This type of analysis is only possible when a long-term campaign is conducted, as in the present study.

Total sugar mean concentrations of 570 ng·m⁻³ were obtained for the roadside site, which represents 1.8% of the PM₁₀ mass, while a value of 363 ng·m⁻³ was registered for the urban background site, accounting for a PM₁₀ mass fraction of 1.5%. The evolution of the total sugar concentrations showed an obvious seasonal variation. The seasonality was demonstrated with a strong negative correlation between the total sugar concentrations and the daily mean temperatures.

The anhydrosugars and the sugar alcohols contributed 84% and 16% (roadside site) and 86% and 14% (urban background site) to the total sugars, respectively. The three stereoisomers arising from the thermal degradation of cellulose and hemicellulose (levoglucosan, mannosan and galactosan) presented strong and positive correlations with each other between the two sampling sites and the two periods studied, which reinforces the idea that they have in common the same source. The three anhydrosugar concentrations were significantly higher in the colder period, showing a strong and negative correlation with the daily mean temperatures. Events with higher levoglucosan concentrations were linked with the use of biomass-fueled heating appliances. It was estimated that wood-burning represented, on average, 22% and 3.6% of the PM₁₀ mass concentrations in the colder and warmer periods, respectively, at the roadside site. The contribution in the urban background was 19% and 3.1%, for the same periods. Similar contributions from biomass burning in both locations are indicative of the widespread use of wood fuels and that

this emission source should be faced as a regional problem, requiring action, not at the neighborhood level, but by regional, or even national, authorities. Xylitol closely followed the anhydrosugar patterns, showing higher concentrations in the colder period. Levels of this polyol were positively and strongly correlated with those of levoglucosan, indicating a common origin. Mannitol and arabitol were well correlated with each other, suggesting similar sources, and did not present any kind of correlation with anhydrosugars, pointing out to a natural source. In order to better understand their contribution to the PM₁₀ and OC mass, a quantitative estimation based on ambient tracer concentrations was made. The results revealed that, for the two sites, the fungal spore relative contribution to PM₁₀ (roadside site: 2.7% to 2.8%; urban background: 1.9% to 2.7%) and OC (roadside site: 6.2% to 8.1%; urban background: 3.9% to 7.5%) was significant and always higher in the warmer period. Similar contributions of the fungal spores to the PM₁₀ of the two sites are indicative, as observed for biomass burning, of a regional phenomenon and not of local specific sources.

This study has contributed to the assignment of primary sources of biogenic carbon to the PM₁₀ level by adding the speciation of sugars, in urban environments, to the chemical composition profile of environmental aerosols.

Supplementary Materials: The following are available online at <https://www.mdpi.com/2073-4433/12/2/194/s1>, Figure S1: Backward trajectories (120 h) arriving at Coimbra on 26 December, 22 February and 30 March obtained through the NOAA HYSPLIT Lagrangian model; Figure S2: Levoglucosan concentrations pollution roses conditioned by temperature and wind speed, for each sampling site: University Stadium (roadside site) (a) and Geophysical Institute (urban background site) (b). Frequency represented in different ranges; Table S1: Characteristics of sugar standards analyzed; Table S2: The relative contribution of fungal spores to PM₁₀ mass and OC.

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