



Research article

Air pollution and its impact on the concentration of airborne fungi in the megacity of São Paulo, Brazil



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ABSTRACT

In the context of megacities in an urban environment, air quality is an important issue, due to the direct correlation to population's health. The biomonitoring of pollutants can indicate subtle environmental alterations, for that, anemophilous fungi can be monitored for changes in atmospheric conditions related to pollution. In the present study, the concentration of fungi and bacteria in the atmosphere was measured during a specific vehicle fleet reduction in the city of São Paulo, Brazil, from May 24 to 30, 2018, using impactor air samplers. The number of isolated developed colonies was related to atmospheric conditions and the concentration of other air pollutants constantly monitored. *Aspergillus*, *Curvularia*, *Penicillium*, *Neurospora*, *Rhizopus* and *Trichoderma* were identified. The number of colony-forming units increased by approximately 80% during the sampling period in response to environmental changes favored by the fleet reduction. This result implies the relation between fuel emissions, concentration of atmospheric pollutants, and the presence of viable fungal spores in the urban environment, which highlights the importance of combined public policies for air quality in large cities.

1. Introduction

Megacities around the world might present severe air quality issues, as a result of the rapid urban development (Romieu et al., 2012; Slovic and Ribeiro, 2018). The current air pollution levels may compromise the health of the population in a large scale due to the exposure to hazardous compounds (César et al., 2016; Oliveira et al., 2018; Rojas et al., 2019; Górnay, 2020).

Both, light and heavy vehicle fleet, that combust petroleum fuels play a major role in the atmospheric composition. Emissions from vehicles release several toxic air pollutants, such as carbon monoxide and fine particulate matter, which influence the environmental quality as a whole (de Toledo and Nardocci, 2011; Borillo et al., 2018; [Pérez-Martínez et al., 2015]). As the largest megacity in South America, the Metropolitan Area of São Paulo (MASP), Brazil, have a vehicle fleet estimated in 15 million vehicles; with a large amount of those having outdated emission control technology, and inefficient maintenance. As a result, the main

urban pollutants as ozone (O₃), carbon monoxide (CO), hydrocarbons (HC), nitrogen oxides (NO_x), sulfur oxides (SO_x) and inhalable particulate matter (PM_{2.5} and PM₁₀), are frequently monitored due to its greatest health concern and correlation with chronic diseases (Carvalho et al., 2015; Andrade et al., 2017; Habermann et al., 2014; André et al., 2000; Pérez-Martínez et al., 2015).

Along the year, those pollutants reach higher values than the ones preconized by the national and the WHO air quality standards (Siciliano et al., 2020; Chiquetto et al., 2019; Abe and Miraglia, 2016). Some local policies were established willing to reduce those values and minimize the health impact, such as traffic restrictions of heavy vehicles and rotation for light vehicles in the city (Carvalho et al., 2015; Andrade et al., 2017).

The organic fraction of particles suspended in the air can reach up to 25% of all aerosols (Huffman et al., 2013). The bioaerosols include pollen grains, bacteria, viruses, and fungal spores, in addition to their fragments (Heald and Spracklen, 2009). In that context, the use of bioindicators for environmental monitoring is a valuable tool. It can detect changes in the

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Table 1. Sampling information for all the sites.

Date Mmm/dd	Ibiúna street level (rural site)		ICESP Ground floor (City site)		ICESP 23rd floor (City site)	
	Fungi	Bacterial	Fungi	Bacterial	Fungi	Bacterial
May/24	3 samples	3 samples	3 samples	3 samples	3 samples	3 samples
May/25	3 samples	3 samples	3 samples	3 samples	3 samples	3 samples
May/28	-	-	3 samples	3 samples	3 samples	3 samples
May/29	-	-	3 samples	3 samples	3 samples	3 samples
May/30	-	-	3 samples	3 samples	3 samples	3 samples

Each sample was collected during one hour: from 11:00 to 12:00, from 12:00 to 13:00, from 13:00 to 14:00 (local time, UTC-3).

environment due to the presence of specific pollutants, that in turn, affect the biodiversity and also particular species of bacteria, fungi or lichens suspended in the air (Parmar et al., 2016). Those are highly sensitive to environmental disturbances, as they present external characteristic signs according to the pollutant concentration (Pescott et al., 2015). Anemophilous fungi, as a fraction of bioaerosols, can also be considered as bioindicator of air pollution, plant diseases, and allergic reactions that have a wide spectrum of clinical forms. It presents a wide concentration range in urban areas according to the interaction between biological and environmental factors (Emygdio et al., 2018a,b).

The ecological niche of an anemophilous fungus can be anywhere it grows, which often allows for the greater proliferation of one particular species over another. However, many fungi are able to withstand extreme conditions and still reproduce to maintain their life cycle (Sousa et al., 2015; Hoog et al., 2014). Several factors can explain the different concentrations of spores throughout the year in different environments. However, it must be noticed that their viability is influenced by changes in the weather and air quality, among other factors (Emygdio et al., 2018a,b; Ross et al., 2004). Therefore, this study monitored anemophilous fungi and bacteria, and evaluated their correlation with meteorological conditions and atmospheric pollutants at the MASP, during specific traffic periods to evaluate the biomonitoring in a megacity.

2. Materials and methods

2.1. Study area

This study was carried out in the MASP, São Paulo state, Brazil. In the center of São Paulo city, samplings took place at two points, located at the same address, but at different altitudes. Three daily samples were collected at street level (Dr. Arnaldo avenue, Cerqueira César neighborhood), and three samples were collected at the 23rd floor of the ICESP (Cancer Institute of the State of São Paulo) building, 112-meter-high, (23°55'60" S 46°66'81" W). For the control, two days of sampling were carried out in the city of Ibiúna, rural area, in the countryside of the state of São Paulo, 60 km away of the capital (23°39'23" S 47°13'21" W), for a baseline (Table 1).

The Köppen Climate Classification for the area is Humid Subtropical Climate, with subtype "Cfa". Summer period (Dec, Jan, Feb) presents the highest temperatures, while winter months (Jun, Jul, Aug) are usually drier, with low precipitation. The sampling period occurred from 24th to 30th May 2018 (Autumn) at the transition to the winter season. During this period, truck drivers went on a strike across the country, thus changing the habits in the MASP, with reduced circulation of large vehicles. Small vehicles stopped circulating shortly afterwards due to fuel shortage. This strike caused structural changes, affecting the air composition and therefore atmospheric pollution in the city of São Paulo.

2.2. Microbiological monitoring

Different sampling methods for bioaerosols can be found in literature. Nowadays, the impactation method is the most used and the one selected

for the present study (Flores et al., 2014; Moura et al., 2015; Löbs et al., 2020; Emygdio et al., 2018a,b). With a large air volume forced through an impactor equipment, the air flow is uniformized along filter pores and the suspended material fixed on a plate surface (Khan and Karuppaiyl, 2012; Haas et al., 2017; Gniadek et al., 2017). Atmospheric sampling with the exposure of selective and rich media (as substrate) in a petri dish allows the growth of viable organisms, and the calculation of its estimated concentration in the air (Moura et al., 2015; Haas et al., 2017).

Sampling was executed using the M air T sampler (Millipore®, Merck KGaA, Germany) and Mas sampler (model 100, Merck®, Merck KGaA, Germany) (Moura et al., 2015). The samples for fungi and bacteria were collected in clean, sterile plastic petri dishes with 20 mL of two different media: modified Dichloran Rose-Bengal Chloramphenicol Agar Medium (DRBCm) for fungal isolation, and Trypticase Soy Agar (TSB) for bacteria cultivation (Castro e Silva et al., 2015), placed in each sampler for one hour (from 11 am, 12 pm and 1 pm for both devices), with a flow rate of 100 L min⁻¹, reaching a total air volume of 250 L. After sampling, the plates with DRBCm were incubated at 30 ± 2 °C for up to seven days for isolation and phenotypic identification (Hoog et al., 2014). Each viable organism developed a colony, and after identification the number of colony-forming units (CFU) are expressed as concentration (CFU m⁻³).

2.3. Meteorological and air pollutant data

Local atmospheric data (temperature (T), air relative humidity (RH), were obtained from the database of the National Institute of Meteorology (INMET), which provides meteorological information from predefined locations. The database of the stations used in this research are available at: <www.inmet.gov.br/projetos/rede/pesquisa/inicio.php>. Atmospheric pollutant data were obtained from the Environmental Company of the State of São Paulo (CETESB), available online: <<https://cetesb.sp.gov.br/ar/>>.

2.4. Statistical analysis

A database was created with the data collected and processed through descriptive statistics. Kolmogorov-Smirnov test was used to verify the data distribution. The means comparison was performed with t-tests and Wilcoxon non-parametric test. For correlation analysis, Spearman's rho (ρ) and chi-square test were applied. All analyzes were performed using SPSS (version 21.0), and the p-value of p < 0.05 was defined as the level of significance.

3. Results and discussion

The values of temperature, humidity, and cloud cover showed typical variations for this period in the city of São Paulo. The overall meteorological data for May, 2018, were as follows: 18.2 °C of monthly temperature average, approximately 0.6 °C above the climatological average, during the seven days of sampling, the minimum temperature ranged from 10.8 °C to 13.6 °C and the maximum temperature ranged from 21.8 °C to 24.7 °C, average relative humidity ranged from 34% to 57%, total

Table 2. Meteorological data (air relative humidity: RH and temperature: T), air pollutants (particulate matter: PM10, sulfur dioxide: SO2, nitrogen dioxide: NO2, carbon monoxide: CO, nitrogen monoxide: NO, ozone: O3) and CFU concentrations (fungi: FUNG and bacteria: BAC). Data presented as average \pm standard deviation.

Season	Parameter	Urban site (avg \pm sd)	Rural site (avg \pm sd)
Spring	RH (%)	24.6 \pm 18.8	30.3 \pm 18.6
	T ($^{\circ}$ C)	16.3 \pm 6.3	11.1 \pm 6.2
	PM10 (μ g m $^{-3}$)	12.4 \pm 16.2	6.4 \pm 16.5
	SO2 (μ g m $^{-3}$)	0.7 \pm 6.9	3.8 \pm 7.9
	NO2 (μ g m $^{-3}$)	28.5 \pm 20.4	4.6 \pm 19.5
	CO (ppm)	0.4 \pm 9.4	0.6 \pm 9.9
	NO (μ g m $^{-3}$)	16.1 \pm 13.3	2.1 \pm 13.4
	O3 (μ g m $^{-3}$)	46.7 \pm 26.5	68.0 \pm 26.3
	FUNG (CFU m $^{-3}$)	23.1 \pm 44.9	48.9 \pm 49.2
	BAC (CFU m $^{-3}$)	29.2 \pm 75.2	36.3 \pm 76.0
Summer	RH (%)	54.5 \pm 14.2	75.8 \pm 15.4
	T ($^{\circ}$ C)	27.4 \pm 4.1	24.5 \pm 4.7
	PM10 (μ g m $^{-3}$)	23.0 \pm 11.6	11.1 \pm 12.6
	SO2 (μ g m $^{-3}$)	1.7 \pm 1.5	7.0 \pm 25.2
	NO2 (μ g m $^{-3}$)	42.6 \pm 21.5	8.9 \pm 29.4
	CO (ppm)	4.9 \pm 10.6	3.0 \pm 19.0
	NO (μ g m $^{-3}$)	25.7 \pm 18.6	2.3 \pm 16.1
	O3 (μ g m $^{-3}$)	63.9 \pm 29.9	58.0 \pm 30.0
	FUNG (CFU m $^{-3}$)	43.2 \pm 73.3	132.0 \pm 76.4
	BAC (CFU m $^{-3}$)	44.0 \pm 47.4	78.2 \pm 50.8
Autumn	RH (%)	47.2 \pm 14.9	41.7 \pm 15.0
	T ($^{\circ}$ C)	26.2 \pm 5.4	14.8 \pm 5.5
	PM10 (μ g m $^{-3}$)	1.6 \pm 4.9	11.0 \pm 5.4
	SO2 (μ g m $^{-3}$)	31.6 \pm 26.9	6.3 \pm 27.3
	NO2 (μ g m $^{-3}$)	52.4 \pm 32.5	1.7 \pm 33.6
	CO (ppm)	23.6 \pm 20.4	9.0 \pm 20.9
	NO (μ g m $^{-3}$)	27.2 \pm 11.8	0.8 \pm 11.7
	O3 (μ g m $^{-3}$)	6.0 \pm 21.6	57.2 \pm 31.2
	FUNG (CFU m $^{-3}$)	57.7 \pm 78.7	117.8 \pm 78.6
	BAC (CFU m $^{-3}$)	28.1 \pm 51.3	53.5 \pm 51.9
Winter	RH (%)	49.3 \pm 16.2	30.6 \pm 16.6
	T ($^{\circ}$ C)	16.5 \pm 5.3	9.6 \pm 5.2
	PM10 (μ g m $^{-3}$)	25.1 \pm 17.2	16.5 \pm 14.7
	SO2 (μ g m $^{-3}$)	39.1 \pm 30.1	5.1 \pm 30.7
	NO2 (μ g m $^{-3}$)	73.9 \pm 31.1	9.8 \pm 31.7
	CO (ppm)	40.9 \pm 30.9	6.0 \pm 31.3
	NO (μ g m $^{-3}$)	49.7 \pm 19.9	4.5 \pm 18.3
	O3 (μ g m $^{-3}$)	6.6 \pm 33.6	29.4 \pm 33.6
	FUNG (CFU m $^{-3}$)	91.3 \pm 87.5	155.4 \pm 88.8
	BAC (CFU m $^{-3}$)	55.3 \pm 58.1	62.7 \pm 57.6

rainfall was 16 mm, with rain occurring five days before the sampling period.

The occurrence of intense rainfall promotes the cleaning of the atmosphere, that will present lower pollutant concentrations. The description above shows a very stable synoptic condition, which is

characterized by a post-polar high changing to subtropical. These data, and the ones presented in Table 2 with small local variations, represent well the meteorological situation of the entire city of São Paulo.

From Tables 2 and 3, temperature presented is negatively correlated with fungal CFU ($\rho = -0.385$, $p \leq 0.001$) and positively with RH ($\rho =$

Table 3. Spearman's correlation (ρ) of meteorological parameters and air pollutant variables with CFU concentrations.

Parameter	Correlation (ρ)	Significance (p value)
RH	0.273	≤ 0.001
T	-0.385	≤ 0.001
PM10	0.112	0.036
SO2	0.064	0.259
NO2	-0.262	≤ 0.001
CO	0.063	0.263
NO	-0.132	0.009
O3	0.099	0.073

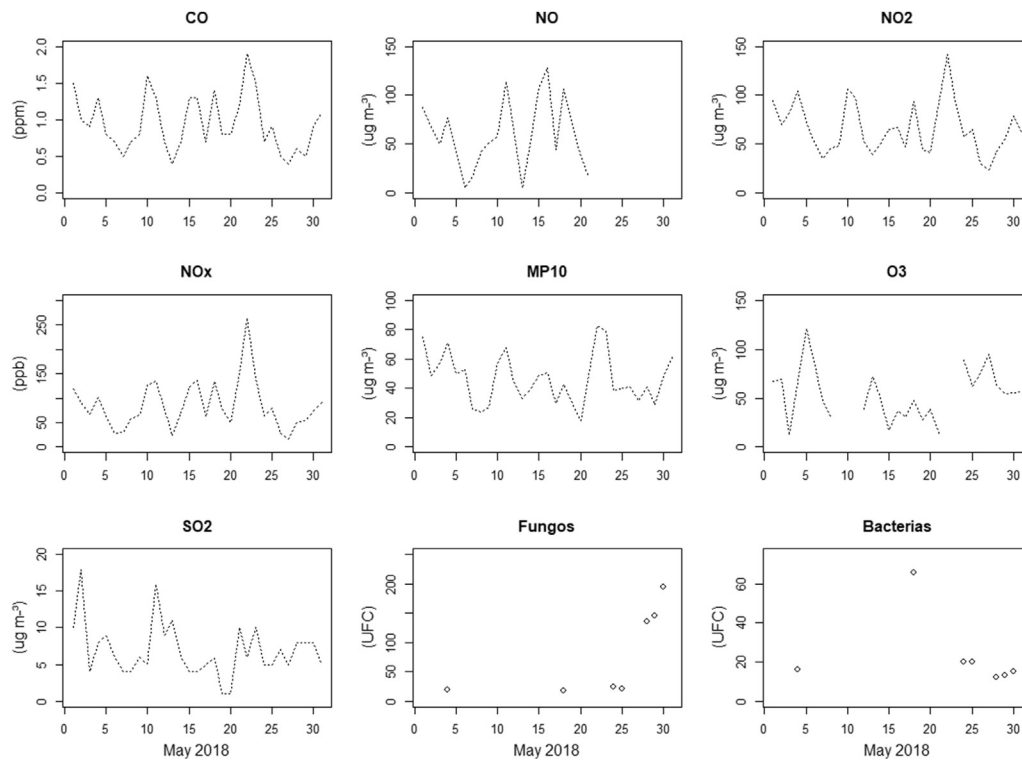


Figure 1. Maximum daily concentration of air pollutants, and average concentration of fungal and bacteria (CFU), during May 2018. Vertical scale in concentration unit, horizontal scale is day of the month for May 2018.

Table 4. Average concentration (in micrograms per cubic meter) of the main pollutants from three monitoring stations nearby the sampling points.

Parameter	Period 1 20-23/MAY	Period 2 24-30/MAY	Reduction
NO _x	200	125	37.7%
PM10	43.4	27	37.8%
SO ₂	2.25	2	11.11%
CO	1.3	1.1	15.4%
O ₃	65	42	35.4%

Period 1: before the sampling period.

Period 2: during the sampling period.

0.273, $p \leq 0.001$), in accordance with literature (Frankel et al., 2012; Grinn-Gofroń and Bosiacka, 2015).

There was no rainfall during the studied period, immediately before or after the strike period. The Figure 1 shows the atmospheric pollutant concentrations along the sampled month. Fungal CFU is negatively correlated to NO₂ ($\rho = -0.262$, $p \leq 0.001$), but does not present a clear correlation or significant pattern with the other air pollution variables (Table 3). During the strike period, the concentration of all pollutants

decreased from 11% (SO₂) to 38% (NO_x and PM10), compared to the previous period, as shown in Table 4.

During the period of study, the concentration of bacteria did not change significantly ($p > 0.05$) compared to overall monthly concentrations. Nevertheless, fungal concentrations seemed to be significantly influenced by the strike (Figure 1). Spearman's correlation was used to assess environmental variables and the concentration of fungi and bacteria, with 95% confidence, as shown in Table 3. Among the environmental variables analyzed, only nitrogen monoxide and bacterial

Table 5. Rural and Urban fungi and bacteria concentrations (CFU m⁻³) at the sampling sites. Total presents the concentration (CFU m⁻³) and the relative composition (%) for the entire month of May 2018. Before shows the values before the sampling period, and after is after the sampling period.

	SITE	TOTAL	BEFORE	AFTER	p-value
Fungi	Rural (Ibiuna)	297 (54.4%)	138 (77.5%)	159 (43.2%)	
	Urban (São Paulo - ground floor)	122 (22.3%)	22 (12.4%)	100 (27.2%)	
	Urban (São Paulo - 23rd floor)	127 (23.3%)	18 (10.1%)	109 (29.6%)	≤ 0.001
Bacteria	Rural (Ibiuna)	43 (49.4%)	13 (48.2%)	30 (50.0%)	
	Urban (São Paulo - ground floor)	26 (29.9%)	6 (22.2%)	20 (33.3%)	
	Urban (São Paulo - 23rd floor)	18 (20.7%)	8 (29.6%)	10 (16.7%)	0.318

Table 6. Number of isolated fungal colonies, by genus, for the three sites, on the 24th and 25th May 2018.

Genus	Rural site (Ibiúna)	Urban site (Ground Floor)	Urban site (23rd Floor)
<i>Aspergillus</i>	0	5	3
<i>Curvularia</i>	2	7	6
<i>Penicillium</i>	2	5	1
<i>Neurospora</i>	0	6	6
<i>Rhizopus</i>	3	5	2
<i>Trichoderma</i>	0	0	2
Total:	7	28	20

concentrations showed statistically significant correlations with fungi ($\rho = -0.80$, $p = 0.03$ for nitrogen monoxide and $\rho = -0.86$, $p = 0.01$ for bacteria) during the strike period. As it is a short and atypical period, where the concentrations of pollutants were influenced by this external event, such values can only be considered as indicative, and a longer period of data should be analyzed for a more robust assessment.

The average concentration of fungi was 80 CFU m^{-3} during a period of 1 year, before the study. Then, by May 2018, during the strike, the average increased to 123 CFU m^{-3} , an increment of more than 50%.

At the beginning of the sampling period, on the 24th and 25th of May, there was $12\text{--}28 \text{ CFU m}^{-3}$ on average from both city sites (ground level and 23rd floor, respectively). However, on the 28th, 29th, and 30th May the average increased to 202 CFU m^{-3} at ground level and 123 at 23rd floor, which shows that the anemophilous fungi reacted to the environmental changes promoted by the reduction of vehicle fleet in the area, increasing the number of isolates by approximately 10 times, while bacteria CFU did not present any clear increase.

The average values of microbial concentrations for the entire study period, presented in Table 1, is between 100 and 150 CFU m^{-3} for anemophilous fungi and below 25 CFU m^{-3} for bacteria. Considering the data from the beginning of May with data from the end of the month, during the strike, an increase of 80% in the number of atmospheric fungi is observed, which shows a significant difference ($p < 0.05$) between the two periods.

The Table 5 shows that the rural site did not present any statistical difference before or after the strike period, only a slight increase of 15% compared to the increase of more than 5 times at both urban sites, before and after the fleet reduction. That result emphasizes the impact of the fuel combustion on CFU concentrations. Additionally, the concentrations of fungi on the 28th, 29th, and 30th May were close to the data obtained from the city of Ibiúna on the 24th and 25th of May, which present the behavior of a pristine area.

From the collected and isolated fungi, were identified the following genus: *Aspergillus*, *Bipolaris*, *Curvularia*, *Penicillium*, *Neurospora*, *Rhizopus*, and *Trichoderma* (Table 6). Fungal particles are ubiquitous when considering a megalopolis, and several sources, including pathogens, are included in this set. For a rural location (control), away from the city, regular and almost constant concentrations are expected for biological particles because of the vast presence of animal vegetation, even with different concentrations and changes in the diversity of the fungi.

In urban environments, such particles can be grouped with other inorganic ones, and therefore they have a more variable concentration. The bacterial count, low in São Paulo during the strike period, may be due to the viability of these organisms.

Analyzing Table 6, which relates the number of isolated fungal genus, few such as *Aspergillus*, *Penicillium*, *Curvularia*, *Neurospora* and *Rhizopus* were presented in most of the sites. The urban sites have more species than Ibiúna. Rural site, for its turn, presented a less diverse genus numbers with 25%–30% smaller when compared to city ground floor and 23rd floor at the same period. Only *Curvularia*, *Penicillium* and *Rhizopus* were found. After the strike the diversity decreased by half. The high concentration of anemophilous fungi near the 23rd floor may be related

to the direct influence of meteorological parameters at that place, which were not measured and can be a focus of another study.

The identified genus are the most common listed in literature for indoor and outdoor areas in urban environments (Andualet et al., 2020; Pyrrri and Kapsanaki-Gotsi, 2017; Fang et al., 2019). Consequently, the environmental impact caused by the increase of vehicles and industries in the urban area may induce to diversity loss (Newbound et al., 2010; Abrego et al., 2020; Bezerra et al., 2014). This study showed that fungi may be sensitive to variations in emission pollutant.

As the dispersion and concentration of anemophilous fungi undergo changes according to environmental conditions, they can be used as bioindicators of pollution. Therefore, fungi can be used to monitor air pollution levels. However, studies using microorganisms as bioindicators of pollution in urban areas are still scarce. Because of the increase of pollutants in the air, it is necessary to implement control measures assessing not only the pollutants but also the life cycle of the airborne fungal organisms.

4. Conclusions

The current population growth in large cities around the world leads to a series of harmful consequences especially atmospheric pollution. In this study, the quantity and diversity of anemophilous fungi in the atmosphere of the city of São Paulo was measured. It has been shown variations during a period of reduced vehicle fleet, due to the truck drivers' strike, May 2018. The isolated identified genus was the most common, but the number of colony-forming units underwent a significant change, as they increased by approximately five times, even when compared to a rural environment with only 15%. The overall fungi diversity shows also a clear increase during the sampling period, higher in the urban environment than the control rural site. The meteorological conditions did not change significantly during the period. Therefore, the indication is that the pollution levels had a decreased up to 38%, in consequence of the reduced fuel combustion, in relation to the previous days. That result emphasizes how the air quality can impact on the living airborne fungi in the urban areas, and therefore the population health. This might be taken in consideration to guide governmental policies.

Declarations

Author contribution statement

Dulcilena de Matos Castro e Silva: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Cybelli Gonçalves G Barbosa: Analyzed and interpreted the data; Wrote the paper.

Fábio Luiz Teixeira Gonçalves: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Maria Regina Alves Cardoso: Conceived and designed the experiments; Wrote the paper.

Rosa Maria N Marcuso: Analyzed and interpreted the data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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