Research paper

Air pollution monitoring with *Tradescantia* hybrid and optical sensors in Curitiba and Araucária, Brazil

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Abstract. Complex mixtures of substances are in the atmosphere and they can cause diseases in humans and biological communities after acute or chronic exposition. This paper focuses on the physical measurement of particulate matter, a proxy for air pollution, and a biological method for mutation assessment due to plants' exposure to air pollution. The objective of this research was to characterize the air pollution seasonality in municipalities in southern Brazil, and also to understand the relation between air pollution and the biological response of the *Tradescantia* sp. clone 4430. The optical sensor SDS011 was used for measurements of particulate matter (PM) and the Trad-SHM bioassay was chosen to quantify the mutagenic alterations that occurred in stamen hairs during the study period, with PM data being measured every 5 seconds and the flowers being harvested approximately every two weeks for laboratory analysis. The Pearson test was applied to verify the correlation between PM and mutations in stamen hair as a result of which it was observed that there is a positive correlation between these data, with the highest value found being r = 0.61. Also, the period with the highest occurrence of pink cells was between autumn and spring, the same period in which an unusual increase in PM concentrations was also observed, a period that corresponds to a less favorable dispersion of pollutants in the atmosphere. The use of *Tradescantia* sp. clone 4430 showed sensitivity to the environments in which it was exposed. Biomonitoring is an important tool for understanding the effects of pollutants on the ecosystem.

Key words: environmental protection, particulate matter, Trad-SHM bioassay, SDS011 sensor.

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Introduction

Complex mixtures of organic and inorganic substances (such as sulfates, halogenated and nitrated organic compounds, polycyclic aromatic hydrocarbons (PAHs) and strong acids) are apparent in the urban atmosphere due to high levels of pollution, which can be potentially mutagenic and carcinogenic, bringing damage to the environment and human health (Luiz *et al.*, 2005; Gábelová *et al.*, 2004; de Brito *et al.*, 2013). Therefore, the monitoring of these substances becomes important, as it pro-

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vides quantitative information on the compounds present in the atmosphere. The clastogenic or mutagenic effects that such mixtures may have on biota and human health cannot be measured by methods based on pollutant concentration. Thus, biomonitoring becomes necessary to assess the genotoxic potential of the components present in the air.

Biomonitoring uses animal, plant or fungal species continuously exposed in environments to assess the effects that pollutants can have on living beings. The *Tradescantia* plant is an example of a bioindicator of genotoxicity and is sensitive to mutagenic chemical compounds present in the air, therefore it has been widely used to detect such agents and their possible effects (Ma *et al*., 1994; Guimarães *et al*., 2000; De Luccia, 2012). Schairer *et al*. (1978) performed exposures of *Tradescantia* clones 4430 to the air pollutants SO_2, NO_2, and O_3 and to vapors of mutagens such as 1,2-dibromoethane (DBE) and ethyl methanesulfonate (EMS). Their results demonstrated the usefulness of the system as a detector of chemical mutagens. Following exposure to either chemical or physical mutagens, the flowers were analyzed in the laboratory each day as they bloom for approximately three weeks after treatment. Pink mutation rates in *Tradescantia* stamen hairs following a 6-hour exposure to gaseous EMS reach peak values 7–12 days after exposure (Schairer, 1978). In the literature, this value ranged from 10 to 21 days for chronic exposure to radiation. After this period, the highest values of mutations were found in the analyzed flowers matching with values found in the work of Takahashi & Ichikawa (1976), Ichikawa (1981) and Ichikawa *et al*. (1996). In their dose-effect experiments on exposure to different radiation rates, it was observed that mutations were more frequent when exposed to environmental factors about two or three weeks before flowering. Patussi & Bündchen (2013) used specimens of *Tradescantia* sp. clone 4430 to evaluate the genotoxicity of herbicides in a corn crop, where pots with the plant were placed between the plantation during herbicide application and analyzed later to verify if mutations occurred. Their final result is that more alterations were observed mutagenically in the stamen hair of exposed plants compared to the control, thus demonstrating the sensitivity of the biomonitor to harmful agents.

The negative effects that pollutants can cause to plants depend on the concentration and time of exposure and cause alterations in the plants' gas exchange process, leaf necrosis and chlorosis, hamper plant growth and in some cases the suppression of a species in the local flora (Rascio & Navari-Izzo, 2011). Gaseous fractions of air pollution can be absorbed by the stomata of the leaves, particulate matter can be retained on the surface of the plant and can be resuspended by wind or further deposit from the leaves to the ground. The fraction of healthy leaf area, concentration of pollutants and meteorological variables are some of the factors that affect the self-removal of pollutants by plants (Nowak & Dwyer, 2007). The current global trend in urbanization (UN, 2019) increases the pressure on natural and urbanized ecosystems globally.

Long exposure to particulate matter causes adverse effects on human health and can lead to the premature death of millions of people yearly (WHO, 2021). Some diseases caused by this exposure are: cardiovascular diseases, lung cancer, respiratory infections, cerebrovascular accident (CVA) and depression (Vodonos *et al*., 2018; Alexeeff *et al*., 2021). The World Health Organization estimated that air pollution caused the premature death of 4.2 million people worldwide in 2016 and a study by Southerland *et al*. (2022) estimated that 4.14 million people died as a result of air pollution caused by $PM_{2.5}$ particulate matter in 2019. It is noted that mortality attributable to air pollutants remained relatively unchanged between 2000 and 2019 in urban areas globally (Southerland *et al*., 2022).

Particulate Matter (PM) can be considered as a proxy for air pollution and genotoxic effects on plants and negative effects on human health. These particles are subdivided into $PM_{2.5}$, particles with an aerodynamic diameter of ≤ 2.5 µm, and PM_{10}, particles with an aerodynamic diameter of ≤ 10 µm. In the work of Carreras *et al*. (2006) PM was considered as a proxy for the total load of pollutants in the air and through the bioassay Trad-MCN it was found that air pollution caused spontaneous changes in the micronucleus of the pollen mother cells. In the study carried out by Puett *et al*. (2014), a positive relationship was found between exposure to particulate matter and the increased incidence of lung cancer in women, especially among non-smokers. There is strong evidence of causal relationships between $PM_{2.5}$ air pollution exposure and human mortality, as well as acute lower respiratory infections, ischemic heart disease, lung cancer, chronic obstructive pulmonary disease and stroke (Cohen *et al*., 2017; WHO, 2021).

This study aims to understand the air pollution and mutagenic dynamics of the bioindicator *Tradescantia* sp. clone 4430 throughout 2020 and the beginning of 2021 in Curitiba and Araucária, cities located in southern Brazil. Thus, this work seeks to evaluate the relationship between mutations in stamen hair from samples of flowers of *Tradescantia* sp. clone 4430 and PM_{10} and $PM_{2.5}$ measurements made with an optical sensor distributed at eight locations in the study region. We propose a method for estimating the lag time and the exposure window before inflorescences sampling which might help to understand the response of the bioindicator plant to air pollution for the appearance of mutations in the stamen hairs. Few studies were found in the literature to estimate the response time to mutagenic effects in *Tradescantia* sp., therefore, this research brings novelty and contribution to the area of plant ecophysiology and environmental toxicology.

Material and Methods

The study was carried out in two municipalities – Curitiba and Araucária – which are located in the eastern region of the State of Paraná, in Brazil (Figure 1). The estimated population for Curitiba is 1,948,626 and for Araucária 146,214 inhabitants. (IBGE, 2020). The main economic activities of these two municipalities are commerce and industry, in addition to having together a fleet of approximately 1,539,491 vehicles, which contributes to the release of particulate matter and gases into the environment, thus interfering with air quality, public health and the local biome (IPARDES, 2019). According to the Köppen climate classification, the municipalities have a Cfb climate (temperate climate proper), with well-distributed rainfall, mild summer, occurrence of frost (autumn and winter), average temperature below 20°C (except summer) and no defined dry season (Nitsche *et al*., 2019).

Monitoring points

The bioassay with the pots containing *Tradescantia* sp. clone 4430 and the measurement of particulate matter (PM_{10} and $PM_{2.5}$) with the SDS011 sensor were conducted in the urban perimeter of Curitiba and Araucária. The eight sampling points are listed and identified in Figure 1. The negative control point was carried out at the Botanical Station for biological and physical measurements. In each study site the Raspberry Pi computer was assembled with the SDS011 sensor and 5 bioindicator *Tradescantia* sp. clone 4430 were installed in pots.

The negative control of air pollution, located in the same location of sampling point 3, was installed in a place with controlled or low concentration of particulate matter (PM) in the air. At this point, the SDS011 sensor was placed inside an acrylic box closed with filter paper, so that the air was cleaner when entering the box, filtering the particulate matter. The *Tradescantia*

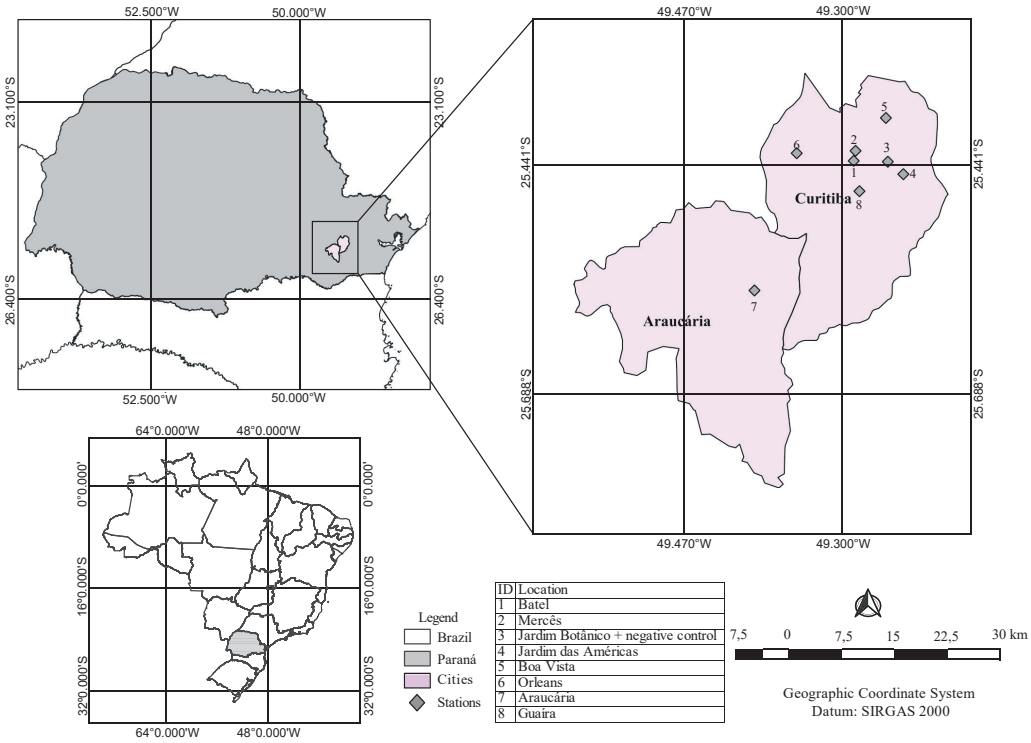


Figure 1. Location of cities and points studied.

pots were placed inside a plastic box and the upper part was also closed with filter paper. A coffee filter was used to isolate the sensor from the external environment. This filter is sterile and has a grammage between 50 and 60g/m^2, it has uniformity in its weaves and is a good retainer of solid particles (Foelkel, 2016).

The SDS011 was installed outdoors, in a place protected from bad weather. The sensor was mounted in the horizontal position, as recommended by the manufacturer. A script was developed in *Python* language for the sensor acquisition of PM_{10} and $PM_{2.5}$ concentration data and storage in Raspberry Pi. This script was programmed to take readings of the measurements every five seconds and save the data in a text file for later processing and analysis. A code was also developed to transmit this data via Wi-Fi to a cloud internet database repository.

The SDS011 sensor is a low-cost device developed in China that reads $PM_{2.5}$ and PM_{10} concentrations. It has a measurement range between 0.0 and 999.9 $\mu g/m^3$ with a response time of 1 second and is characterized by stability over time. The principle of operation of the sensor is through laser scattering, where a fan draws in air through the inlet and the sample passes through a laser beam. The light reflected from the particles is captured and the photoelectric converter processes the signal into particle size and density. Finally, the signal is transmitted to the microcontroller unit where an algorithm processes the data and provides output for particulate concentration in $\mu g/m^3$ (Honeywell, 2021; Shandong Nova, 2021). We have used the sensor manufacturer's calibration.

Plants of *Tradescantia* sp. clone 4430 were installed in polyethylene pots containing commercial substrate. The pots

were initially kept in the laboratory and watered weekly, and then they were placed at the sampling points for a period of 2 months for acclimatization. The field installation was carried out in the eight locations at the end of 2019, with five pots being arranged in each location with the plant, containing five stems with inflorescence, exposed to the natural conditions of the environment. Weekly, 100 ml of water were added to each pot and every fifteen days, 5 g of fertilizer (for every 1 liter of water) consisting of nitrogen, phosphorus and potassium mixed in water was added.

Inflorescence sampling and laboratory analysis

The Trad-SHM assay procedures adopted in this research followed an adaptation of the procedures of Ma *et al*. (1994) and Rodrigues (1999), where the plants followed a chronic exposure, i.e. the pots remained in the study sites throughout the time of the research and not for an acute exposure as suggested by Rodrigues (1999). Figure 2 illustrates the biomonitor used and its main parts. The inflorescences of *Tradescantia* sp. clone 4430 were scheduled to be collected weekly from January 2020 until February 2021 and in each collection all inflorescences were removed by cutting their peduncle. After cutting, the branches were identified according to their sampling point and taken to the laboratory. The flowers were collected in the morning, because during the day they close and wither. If the evaluation was not possible on the same day of collection, two procedures were adopted: the first was to collect the flowers and keep them cooled in pots with lids and moistened filter paper and analyze them up to 24 hours after harvesting, and the second was to collect the flowers and remove the stamens with the help of tweezers and mount the slides, taking care to leave the stamens aligned on the slide; an adhesive tape was used to make the adherence of the lamina on the slide; these finished slides were also kept in refrigeration until the analysis, preferably within 24h (Rodrigues, 1999).

To count the cells, all the flowers collected from each point were used, six stamens were removed with the help of tweezers and arranged on glass slides. A 1:1 solution of 70% alcohol and glycerin was used to fix the biological material. With the help of tweezers and a needle, the hairs were aligned on the slide and observed under a magnifying glass. Then, a coverslip was superimposed and the material was analyzed with the aid of an optical microscope. All the pink cells present in the stamen hair and also the total number of stamen hairs contained in the stamen were counted. If there was no pink cell in the observed stamen hair, only the total number of filaments (stamen hair) was counted. The mutation frequency per flower was then expressed as the number of events/1000 stamen hair.



Figure 2. *Tradescantia* sp. clone 4430 and its main parts, adapted from Small (1922).

Statistical analysis

To analyze the relation between the number of mutations in stamen hair and the concentration of particulate matter, we developed a methodology to estimate the time of exposure of the plant to atmospheric pollution that may result in a certain number of cell mutations. We were interested in discovering how many days of exposure to air pollution the plant needs to experience before its mutagenetic response appears. The Backward Sliding Window (BSW) method was developed for this task, and it is explained in the next subsection. The particulate matter data was resampled to daily average values for applying the BSW method.

Backward Sliding Window (BSW) Method

The Backward Sliding Window (BSW) Method was developed by the authors and applied for detecting two parameters related to the chronic exposure of *Tradescantia* sp. clone 4430 to atmospheric pollution. The parameters are: i) size of the window of exposure, in days; and ii) lag time for mutation appearance after the exposure, counted as days before inflorescence collection, as shown in Figure 3. The method consists of varying the number of days of exposure and the position of the window of exposure to particulate matter, our proxy for air pollution. For different exposure periods the correlation between the mean PM concentration and number of mutations/1000 cells is calculated. The period with the highest Pearson correlation coefficient was chosen for the analysis.

After choosing a reasonable window size and the lag time, a dataset was constructed relating the number of mutations in stamen cells to the mean PM_{10} and $PM_{2.5}$ concentration in the backward exposure window. Only sites with more than 16 inflorescence sampling days were chosen for this analysis, as it was considered that few collection days (≤ 16) would not be statistically significant. The Pearson correlation coefficient between PM and *Tradescantia* mutations were calculated for the sampling points.

Daily profiles with hourly average data of particulate matter were calculated to compare the dynamics throughout the day and night between the sampling points. It is known that in the Araucária sampling point are several industries that contribute to the emission of particles and in Curitiba the main pollution comes from motor vehicles.

Results and Discussion

The collection of flowers of *Tradescantia* sp. clone 4430 for the monitoring points occurred at different dates, quantities and frequency. In the laboratory analysis, all pink cells were counted and some were photographed. Figure 4 shows some examples of pink mutation in stamen hair of *Tradescantia* sp. clone 4430 found during the analysis of the Trad-SHM bioassay, where the stamen hair cells that present a pink coloration can be seen.

Figure 5 shows the boxplot of PM_{10}, $PM_{2.5}$ daily concentrations and mutation frequencies in stamen hair of the sample points, including the control point.

Figure 5(c) shows the frequency of mutations in stamen hair for all points studied, different behaviors were observed between them, which might be associated with the frequency and quantity of flowers collected not being similar between sampling points (the number of sampling days "*n*" are indicated at the top of each boxplot). Figures 5(a) and 5(b) indicate more similar *n* values for particulate matter measurements, but some stations had less data because the sampling started later, like Guaíra and the control point. In addition, these graphs show the outliers as dark diamonds and the averages are indicated as white circles. Unlike the control point, the medians of the other points were closer to the first quartile, thus presenting a positive asymme-

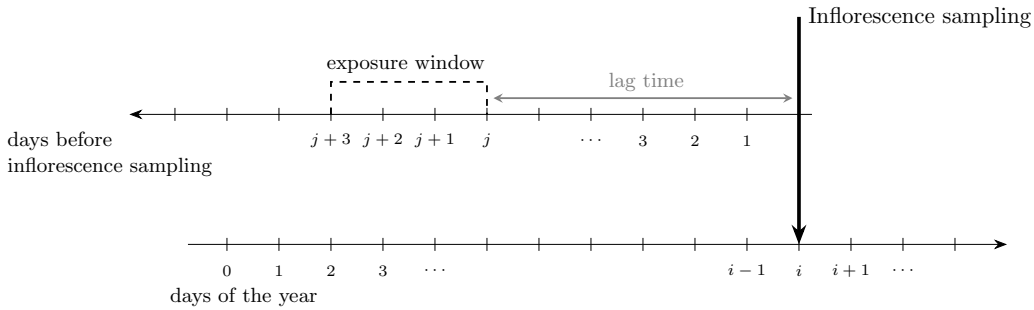


Figure 3. Illustration of the Backward Sliding Window (BSW) Method for detecting possible exposure window size and lag time before inflorescence sampling.

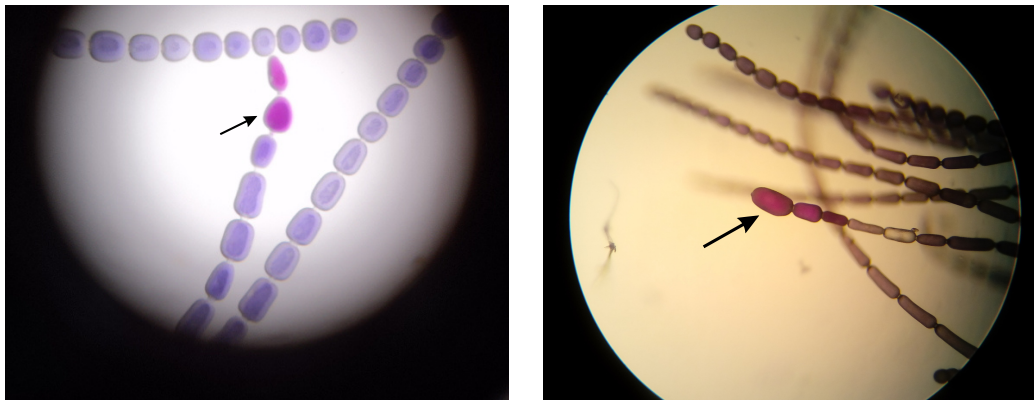
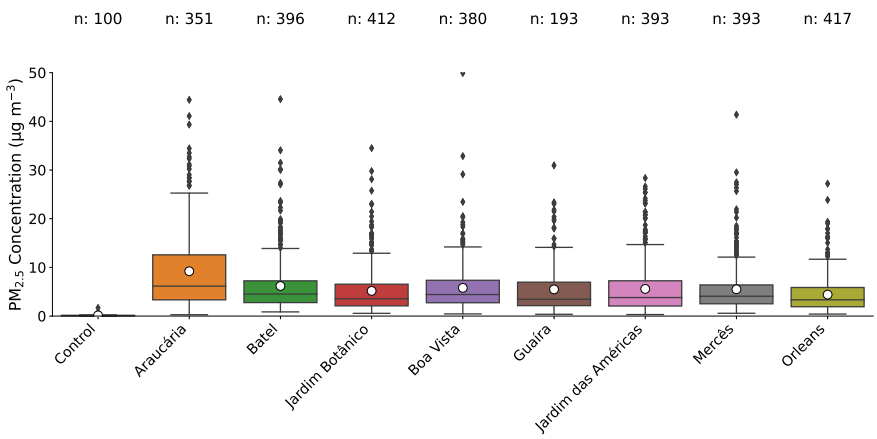


Figure 4. Stamen hair mutations by the Trad-SHM bioassay, indicated by arrows.

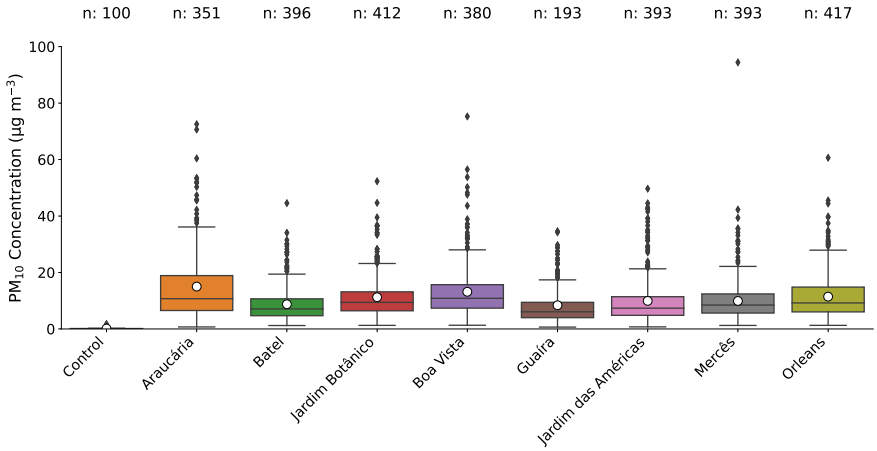
try, since the averages were all above the median, therefore leaving the values more concentrated at the bottom of the boxplot. Also in Figure 5(c), Mercês and Jardim das Américas sampling points presented greater dispersion in relation to the others, due to the difference between the third and the first quartile being greater in these two points, in the Orleans point, it was where the smallest variation of the data occurred.

The particulate matter concentrations were collected by the SDS011 sensor from January 2020 to February 2021. Some stations had few inflorescence sampling days, which would affect the correlation calculation against particulate matter measurements. To circumvent this limitation, a criterion of at least 17 days of inflorescence sampling was adopted. The selected stations for analysis were: Jardim Botânico, Jardim das Américas, Mercês and Orleans. The data was processed, daily averages were calculated for all stations and the data overview given in Figure 6. During 2020 and beginning of 2021 our data shows an increase of mutations in the winter period, between May and November of 2020, coincidently the air quality was also worse between May and October of 2020. It is known that during the coldest months of the year normally there is less dispersion of pollutants in the lower atmosphere. This process is related to the planetary boundary layer height variation, changes in incident solar radiation and meteorological variables, such as rain and wind patterns.

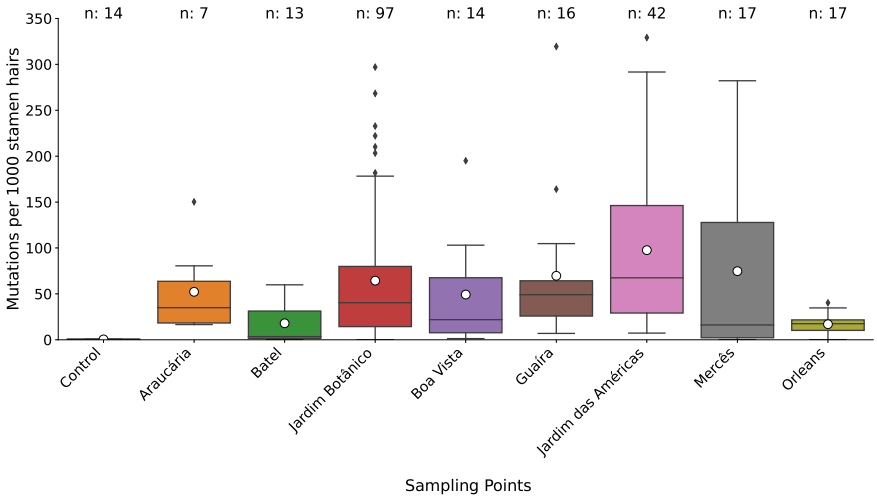
In 2021 the WHO presented guidelines for the guide values for particulate matter and other pollutants that are harmful to health. This update is due to growing ev-



(a)



(b)



(c)

Figure 5. Boxplots of (a) daily mean $PM_{2.5}$ concentration, (b) daily mean PM_{10} concentration and (c) mutation frequencies in stamen hair for the sample points. The number of sampling days (*n*) is indicated for each station, averages are indicated as white circles.

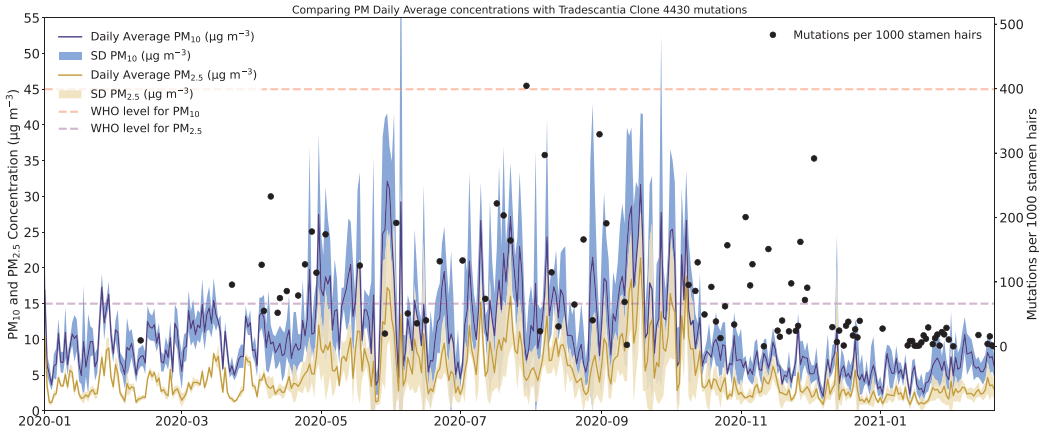


Figure 6. Time series of PM_{10} and $PM_{2.5}$ average daily concentration and *Tradescantia* sp. clone 4430 mutations per 1000 stamen hair according to the sampling day. SD is the standard deviation considering all sampling points, except the control.

idence of the adverse health effects of air pollution, based on advances in the measurement of pollutants and assessment of human exposure to them (PAHO, 2021). The goal of the guideline updates is to provide quantitative recommendations and guidance for minimizing air pollutant levels in order to reduce the damage caused by exposure to pollutants (PAHO, 2021). In comparison with the last report from WHO, the value for $PM_{2.5}$ has become more restrictive because it is a fine particulate that is easily inhaled and can penetrate deeply into the lower airways, reaching the lung alveoli and bronchioles (SISAM, 2019).

The red and purple dashed horizontal lines in Figure 6 refer to the limits imposed by the World Health Organization (WHO, 2021), where the daily average level of PM_{10} and $PM_{2.5}$ must not exceed concentrations of 45 µg/m^3 and 15 µg/m^3, respectively. During the data collection period and considering all the sampling points, 25 days exceeded the limit for PM_{10} and 202 days exceeded the limit for $PM_{2.5}$, not necessarily being the same days. The station that most often exceeded the WHO limits was Araucária (14 days for PM_{10} and 65 days for $PM_{2.5}$), probably due to its location close to the city's industries and also because there are roads with greater vehicle traffic in this region. This fact was observed by Castelhano (2019) who noticed a pattern of PM concentration being higher in Araucária compared to its surroundings. In Curitiba metropolitan area, the sampling points Jardim das Américas, Batel, Jardim Botânico, Mercês exceeded $PM_{2.5}$ levels during 28, 27, 23, 20 days, respectively. The control sampling point did not exceed any of the limits.

Figures 7(a) and 7(b) show the daily dynamics of PM_{10} and $PM_{2.5}$ concentration, respectively, for all sampling points, except the control. Both graphs represent the hourly mean and 5 times the standard error (SE) for the period from January 2020 to February 2021. Araucaria sampling point had the highest PM_{10} and $PM_{2.5}$ concentration for most of the hours of the day, with higher concentrations from 15h to 22h. Jardim das Américas, Batel, Botânico and Guaíra stations had a similar behavior with higher concentrations near the busiest vehicle traffic times, from 6h to 9h and from 18h to 21h. Concentration by time of day can give insight into possible sources of pollution.

Mutation frequency and PM correlation

Figure 8 represents the application of the BSW Method with the correlation results between mutations and mean PM concentration for the selected sampling points.

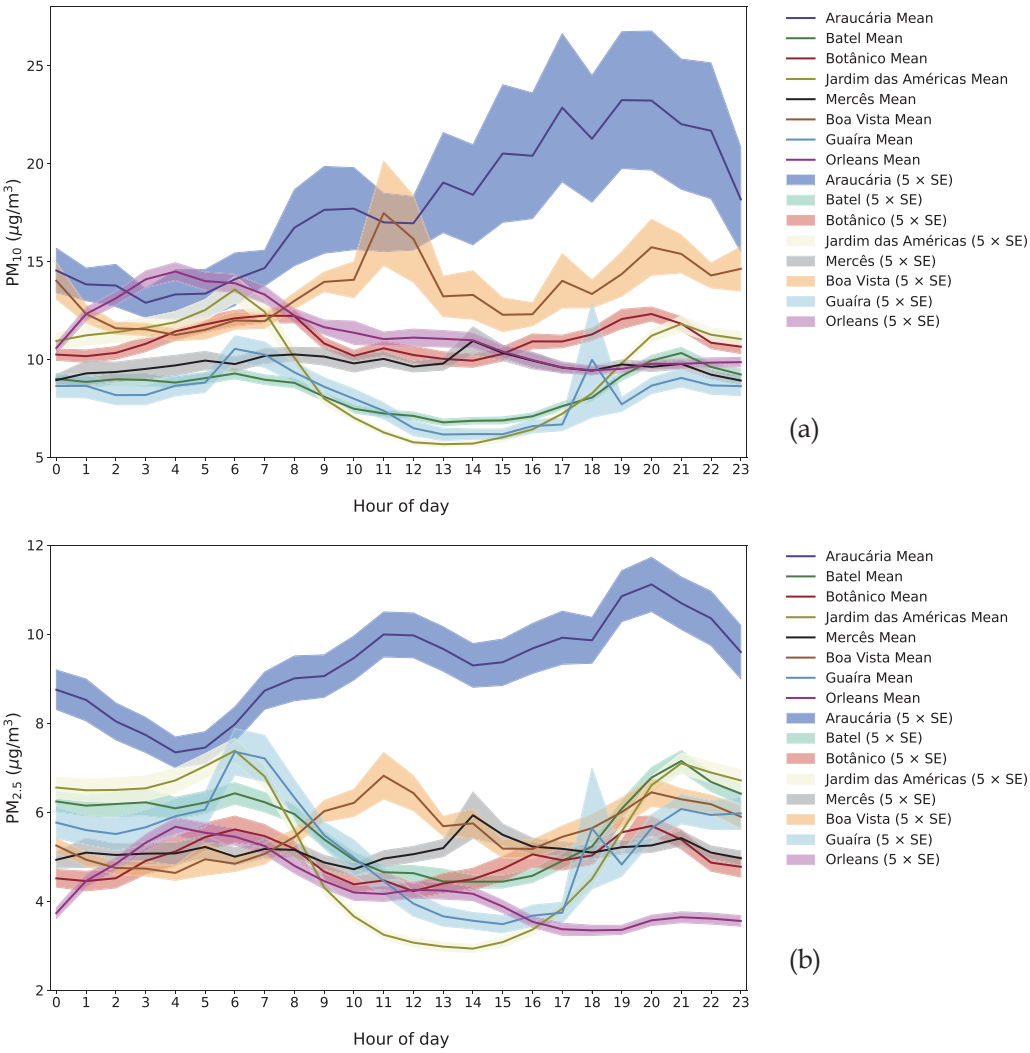


Figure 7. Daily variation of (a) PM_{10} and (b) $PM_{2.5}$ in sampling points, except the control point. Hourly means are depicted with 5 times the standard error (SE) colored shaded area.

We found that a 6-day exposure window beginning 2 days before inflorescence sampling resulted in the highest correlation between PM_{10} and $PM_{2.5}$ mean concentration and cell mutations, considering the 4 selected stations (Jardim Botânico, Jardim das Américas, Mercês and Orleans).

Figure 9 shows the window size (6 days) and lag time (2 days) framework considered for *Tradescantia* sp. clone 4430 analysis of chronic exposure to air pollution.

In order to verify whether air pollution, represented by the particulate matter indicator, is capable of causing changes in the genetic material in plants, specimens of *Tradescantia* sp. clone 4430 were subjected to chronic exposure from January 2020 to February 2021, and the mutation frequency in stamen hair was compared with the concentration of particulate matter PM_{10} and $PM_{2.5}$, collected during the same period.

As already mentioned before, a lag time of 2 days before inflorescence sampling was used to calculate the mean value

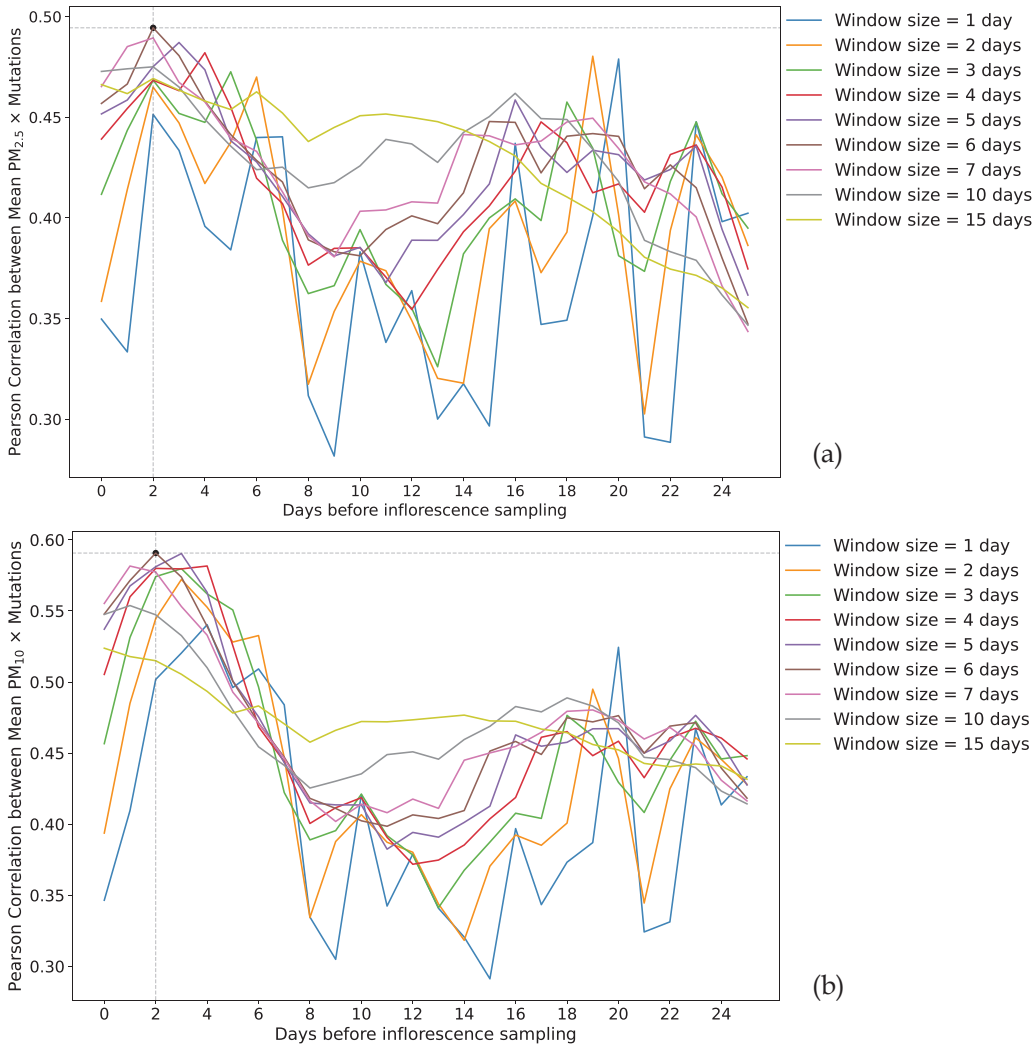


Figure 8. BSW Method correlation analysis between PM and mutations for detecting lag time and window size of exposition (a) $PM_{2.5}$ and (b) PM_{10}.

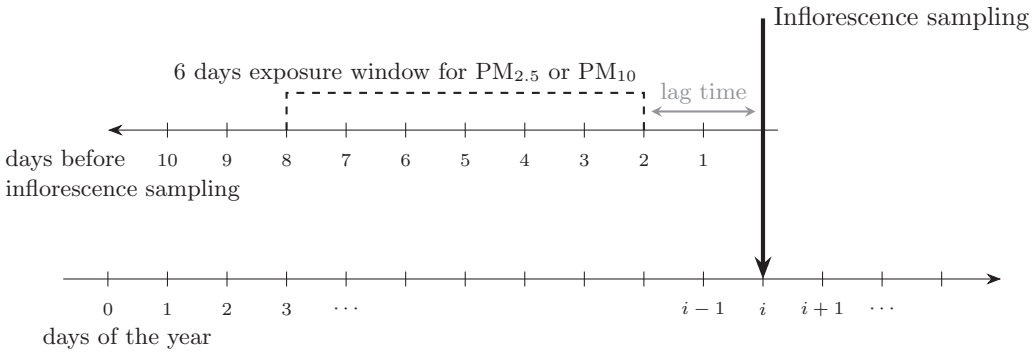


Figure 9. Results of the BSW Method, showing the size of the exposure window and the lag time adopted for $PM_{2.5}$ and PM_{10}.

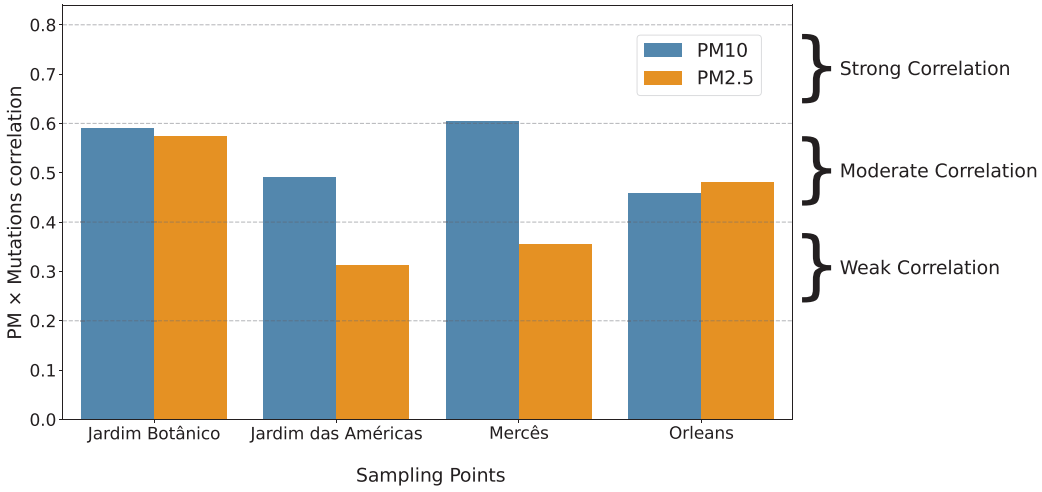


Figure 10. Pearson's correlation between PM_{10} and $PM_{2.5}$ × mutations per 1000 stamen hairs of *Tradescantia* sp. clone 4430 for selected stations.

of particulate matter in the 6-day exposure window, so the statistical correlation between PM and mutations was calculated for all the selected stations. The results of the Pearson correlation (r) test between mutations and PM_{10}, $PM_{2.5}$ data from the selected monitoring points are shown in Figure 10.

Figure 10 shows the r values of the Pearson correlation, showing a positive relationship, with the r values ranging from a weak correlation to a strong correlation. In the work by Guimarães *et al*. (2004) the Trad-SHM bioassay was performed with the KU-2 clone to evaluate the toxicity of PM_{10} in two cities of São Paulo (capital and country side) and the mutation frequency observed in the flowers of the country side city was lower in relation to the capital, where the mean PM_{10} in the capital is 64μg/m^3 against 14μg/m^3 in the interior and the correlation between PM and mutations was positive (r = 0.47).

Conclusions

The present work shows the procedure of data collection and evaluation of particulate matter concentration in Curitiba and Araucária, distributed in eight points plus the control point, as well as the mutation frequency in stamen hair by the Trad-SHM bioassay in *Tradescantia* sp. clone 4430. Furthermore, this research investigated if the concentrations of PM impact the result of the Trad-SHM bioassay. The particulate matter was considered a proxy for air pollution (Carreras *et al.*, 2006; Puett *et al.*, 2014).

Daily mean concentrations of PM at the monitoring points exceeded the limits specified by WHO (2021). $PM_{2.5}$ is more critical than PM_{10} as there were 16 days during the entire studied period when the mean $PM_{2.5}$ daily average was bigger than 15μg/m^3, considering all the sampling points. This happened mostly in seasons when atmospheric dispersion is less favorable, thus causing a higher concentration of pollutants in the atmosphere. Therefore, there is an urge to increase monitoring and to detect the most critical points so that political actions can be taken.

There was an occurrence of mutation frequency in stamen hair at all points studied, being more frequent in the periods between autumn and spring. For the same period of the particulate matter, a tendency can be noticed between the results. Comparisons between PM concentration and

mutations in stamen hair of *Tradescantia* sp. clone 4430 showed that the results followed the same trend of increase and decrease in relation to different periods of the year.

The correlations between particulate matter and mutation frequency showed positive values, ranging from weak to strong correlation. The mean correlation between particulate matter and mutation frequency was r = 0.53 for PM_{10} and r = 0.43 for $PM_{2.5}$ (moderate correlation), with a maximum value of 0.61 and a minimum of 0.31. The use of *Tradescantia* sp. clone 4430 with the Trad-SHM bioassay showed sensitivity to the environments in which it was exposed, and this study reveals that biomonitoring may be an important tool for understanding the effects of pollutants on the ecosystem, especially facing increasing urbanization globally. We suggest for future work the comparison of mutation frequency with the concentration of other polluting gases, such as CO, SO_x, O_3 and NO_x.

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