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Evaluation the Urban Atmospheric Conditions Using Micronuclei Assay and Stomatal Index in *Tradescantia pallida*

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Abstract: Air pollution substantially damages ecosystems and public health and is one of the major challenges for air quality monitoring management. The use of the plant bioindicator *Tradescantia pallida* (Rose) D. R. Hunt has shown excellent results in terms of determining the effect of airborne contaminants in urban environments, complementing conventional methods. The present study seeks to determine the air quality in the Ivinhema Valley, MS, using the variation in MCN frequency and stomatal indices of *T. pallida* as air pollution biomarkers. The biomonitoring tests were performed monthly by collecting floral and leaf buds during the summer, autumn, winter, and spring of 2021 in Angélica, Ivinhema, and Nova Andradina. The stomatal leaf density, influence of vehicle flow, and environmental variables such as altitude, temperature (°C), relative humidity (RH), and rainfall in the three cities under study with different urban vehicle intensities were analyzed. A significant increase in MCN was observed for the cities of Nova Andradina and Ivinhema in summer and spring. On the other hand, the city of Angélica had a low frequency of MCN throughout the experimental period. A seasonal and spatial pattern was also observed for the stomatal index, with significantly higher values for the city of Angélica in autumn and winter. Our data allowed observing that the MCN showed the greatest association with vehicular flow. The mutagenic effects observed in *T. pallida*, through the MCN frequency, constituted an important biomarker of air pollution, explained mainly by the relationship with the flow of vehicles.

Keywords: woodpecker; air pollution; stomatal index; seasonality

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1. Introduction

Increasing industrialization and urbanization have contributed to the increased rate of atmospheric pollution, causing numerous impacts on human health and the environment [1]. In urban centers, the increase in the vehicle fleet and decrease in green areas have increased the concentration of toxic, mutagenic, genotoxic, and carcinogenic [2,3] compounds that are formed in the combustion process, contributing to the formation of heat islands that hinder the dissipation of pollutants [4].

The One Health approach encompasses and interrelates human health, animal health, and their interaction with the environment as a single and inseparable factor for the preservation and maintenance of the global balance [5], focusing on the importance of ecosystem stability and biodiversity conservation in relation to epidemiological health [6,7]. By associating the concept of individual health and the triad of human, animal, and environment, the propagation and dispersion of toxic compounds and chronic exposure to them becomes a worrisome factor at the biological scale with direct and indirect impacts on organisms, plants, and humans [8]. In short, collaborative efforts of various disciplines working locally, nationally, and globally are required to achieve optimal health for humans, animals, and our environment.

The impacts of natural resource destruction and changes in air quality associated with systemic, critical, and complex climate changes can result in environmental disasters such as floods, severe drought, and extreme temperature increases with lethal heat waves that threaten humans [9]. These events affect the dynamics of ecosystem food chains and webs, amplifying the trophic cascade effect in terms of propagating vectors of emerging diseases [10] and contributing to the occurrence of zoonoses [11], new infectious and respiratory diseases, epidemics, and pandemics [12]. In this same context, urban expansion driven by population growth has also generated deleterious effects on air quality, mainly due to toxic gases resulting from increased vehicular flow.

Several studies highlight air pollution as one of the main risk factors for mortality and morbidity worldwide, triggering respiratory [13,14] diseases such as asthma, bronchitis, and allergic rhinitis and causing irritation and discomfort of the eyes and skin [15]. Air pollution has also been correlated with pathological conditions associated with severe acute respiratory syndrome, one of the complications of COVID-19 [16], in addition to influencing neurological [17] and cardiac damage [18–20] and increased carcinogenic changes in populations [21,22]. Thus, the adoption of biological markers or biomonitoring of atmospheric pollution as a factor to control related comorbidities is emerging.

The use of higher plants integrated with environmental biomonitoring is an effective tool for measuring the air quality in urban areas, as addressed in several scientific articles [23–29]. The main reservoir of gaseous pollutants in plants is the leaf surface, with gases commonly entering through the stomata, an important structure in the gas exchange between plants and the atmosphere [26,27]. Stomata can be used more efficiently than physicochemical methods to provide data [28], allowing assessments of toxicological risks to public health, as stomata present cellular characteristics with genetic complexity similar to that of humans [29]. Therefore, they complement the mathematical evaluations that are based only on estimates of the rate of pollutants dissolved in the air and do not express the effects caused in the biotic environment [30,31].

Among the plant species with wide application in atmospheric biomonitoring, *Tradescantia pallida* var *purple* (Rose) DR Hunt stands out [32,33]. Known popularly as the purple heart, purple-throated pearl, or "trapoerabão", it is an exotic plant in Brazil [34] that is herbaceous and succulent, easily colonizes and adapts to diverse environments, and is widely used as a tropical ornamental plant [34–36].

Studies have shown the efficiency and sensitivity of mutagenic bioassays of *T. pallida* pollen grain tetrads by estimating the micronucleus (MCN) frequency (TRAD-MCN (TRAD-MCN)) and its relationship with pollutant compounds in inducing damage in meiotic cell division [35–37]. The TRAD-MCN test is a mutagenicity parameter widely used in environmental biomonitoring studies that allow the quantification of chromosomal aberrations during meiosis resulting from whole or fragmented chromosomes not incorporated into the nucleus after cell division with a tendency to have greater amplitudes under conditions of environmental disturbance [38–40].

Research also suggests that anatomical leaf analyses, such as stomatal density (SD), the stomatal index (SI) [26,29], and changes in epidermal, cuticle, and mesophilic thickness, are parameters related to the environmental conditions of urban centers that measure the air pollution impacts and can be associated with different variables, such as vehicle flow and environmental conditions. That is, according to environmental toxicity, plants can increase or decrease their photosynthetic area [41] and may exhibit variations in SD, stomatal distribution, and stomatal morphology [42,43]. To mitigate the damage caused by contaminants, changes in stomatal thickness, tissue size, and parameters are strategies found in some species exposed to stressors [44,45].

The territory of Mato Grosso do Sul is an important Brazilian agricultural region with high levels of pollutants. The state borders countries such as Bolivia and Paraguay and several states such as Goiás, Minas Gerais, Mato Grosso, Paraná, and São Paulo. One of the main causes of the increase in atmospheric contaminants in this territory is the growing number of vehicles along highways and urban roads, with more than 1.5 million vehicles, both for passengers (cars and motorcycles) and cargo (trucks and buses) [46]. However, the commonly adopted physical and chemical atmospheric indicators do not reflect the cumulative effects of certain pollutants on the tissues of plants and animals, which may lead to the aggravation of chronic diseases with deleterious effects on human health.

Studies conducted in cities in the state of Mato Grosso do Sul in its the southwestern mesoregion [26], Dourados microregion [23,29,47,48], some municipalities of greater Dourados [27], and Bodoquena region [24,25] have shown that toxic substances released by motor vehicles and weather conditions can lead to genetic and anatomical leaf damage to *T. pallida* in urban environments, indicating the applicability of this methodology for evaluating air quality and its negative implications for humans.

The region of the Ivinhema Valley, southeastern part of Mato Grosso do Sul state, has focused its regional economic growth on the sugarcane, agricultural, and beef processing sectors, which are strongly linked to the agribusiness sector. In addition to the productive economic sector, there is intense vehicle traffic in the region along the highways that connect São Paulo state and Paraná to Mato Grosso do Sul with a high number of vehicles grain transport, thus emitting large amounts of atmospheric contaminants that degrade the environment and affect human life in these locations. Given these impacts, the present study seeks to determine the air quality in this region using the variation in MCN frequency and SI of *T. pallida* as a bioindicator.

2. Materials and Methods

2.1. Study Area

Air quality biomonitoring was performed in three municipalities in the Ivinhema Valley: Angélica, Ivinhema, and Nova Andradina (Figure 1). The Ivinhema Valley Territory is located in the southeastern part of Mato Grosso do Sul state, Brazil. It contains ten municipalities, namely, Anaurilândia, Angélica, Bataguassu, Batayporã, Brasilândia, Ivinhema, Nova Andradina, Novo Horizonte do Sul, Santa Rita do Pardo, and Taquarussu. It comprises an area of 29,627.90 km^2, which corresponds to almost 8% of the state's area. The area of the municipalities within the territory varies between 6141.62 km^2 (Santa Rita do Pardo) and 849.12 km^2 (Novo Horizonte do Sul) [49].

The sampling area was random considering strategic points such as easy access, plants in good condition, existing in the place for some time (more than 2 months), in two areas with different urban vehicle densities. Sampling was performed monthly between January and September during the summer, fall, winter, and spring seasons in 2021.

2.2. Test Procedures (TRAD-MCN)

For each city and biomonitoring point, high-flow and low-flow sites were adopted using *T. pallida* biomonitors already existing at the evaluation sites (passive method). The test (TRAD-MCN) was developed according to the appropriate protocol [39]. Seven young *T. pallida* flower buds were collected monthly in the summer, fall, winter, and spring seasons of 2021 in the regions of high and low flow, totaling fourteen samples, for each city.

The inflorescences were fixed in Carnoy's solution (3 ethyl alcohol:1 acetic acid), and after 24 h, they were transferred to an alcohol solution at a concentration of 70%. From the sampled flower buds, 6 slides were prepared for each site according to the methodology proposed by Ma (1981) [40]. In each slide, 300 young tetrads of pollen grain mother cells were calculated, and the MCN number was recorded using an optical microscope at a

400× magnification (Nikon YS2; Tokyo, Japan). The results were expressed as percentages (MCN frequency in 100 tetrodes).

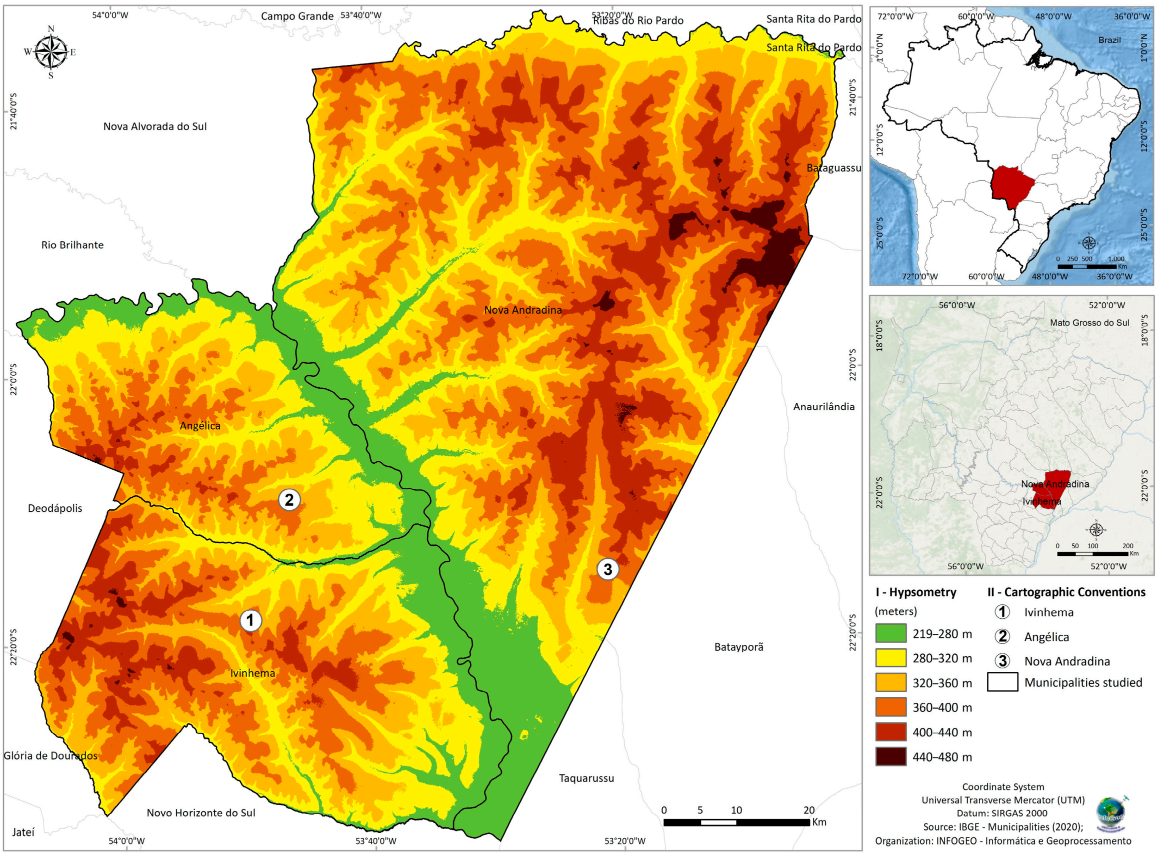


Figure 1. Map of the altitudes of the cities evaluated in the Ivinhema Valley in the southeastern region of Mato Grosso do Sul, highlighting the sampling points.

2.3. *Stomatal Anatomical Analysis*

Stomatal analyses were performed with fresh plant material fixed in FAA (formol, alcohol, glacial acetic acid) 50 solution and preserved in 70% ethanol (*v*/*v*). The samples were formed into paraffin sections in the laboratory by hand, and semipermanent slides were prepared with the bleached sections in 2% sodium hypochlorite solution, washed in distilled water, and stained with 1% safranin and Astra blue 1% solution at a 9:1 ratio [50]; then, the slides were mounted between the slide and the coverslip with glycerinated gelatin.

For each point (high and low flow) and sampling period (month), five leaves from each sampling, totaling 10 leaves, were cut into paradermal sections by hand with a razor blade. For each slide, 10 fields were analyzed, totaling 100 fields. The stomatal index (SI) was calculated and estimated using the formula SI = NS/(EC + NS) × 100 where NS is the number of stomata and EC is the number of epidermal cells [51].

2.4. *Analysis of Vehicle Flow in Cities with Low and High Flow and Environmental Variables*

The influence of vehicle flow was evaluated monthly concomitant with the collection period, adopting the quantitative method of determining traffic by counting the vehicles that circulated through the sampling points and monitoring during the hours of high flow, from 8:00–9:00 in the morning and from 11:00–12:00 and 17:00–18:00 in the afternoon. Subsequently, the results were expressed as the mean of the regions where traffic was measured (high flow and low flow). Roads with intense flow of vehicles, commercial areas (high flow), and roads with low flow of vehicles, residential were considered.

Data from environmental variables of the three cities, such as temperature (°C), relative humidity (RH), and rainfall data were obtained from weather stations in Nova Andradina from January to September (https://www.cemtec.ms.gov.br/boletim-mensal/ accessed on 4 January 2021).

2.5. Mapping of the Evaluated Areas

From the analysis of the MCN frequency in *T. pallida*, the mapping of the most affected regions in terms of air quality was performed using a hypsometric map and spatial interpolation. The hypsometric map consisted of digital elevation model data provided by the TOPODATA (Geomorphometric Database of Brazil; accessed on 05 October 2021) project of the National Institute of Space Research (INPE) and processed in the Quantum Geographic Information System (GIS) application, version 2.6. The spatial interpolation map of the sampled cities was obtained based on the MCN frequency using the kernel interpolation technique, allowing an image of the air quality conditions in the analyzed cities. The pollution indicator triad consists of a three-axis graphical interface where each axis represents an analyzed index; its interpretation is provided by checking the position of each end of the polygon formed on the axis in question; the closer to the outer edge, the more representative the indicator is at the studied point. In this study, four pollution indicators (population estimate, cardiorespiratory diseases, MCN frequency, and vehicle flow) were used. The indicators were standardized to the same scale (new index (NI)), from the sum of the RTM (ratio to maximum value), according to the equation:

$$〖RTM〗 V_{yi} = V_{yi}/V_{my} \qquad NI = 〖RTM〗_i / 〖RTM〗_0$$

where V_{yi} is the value of the evaluated parameter (SI, MCN frequency, and vehicle flow) at Station i; V_{my} is the maximum average value of the parameter among all sampling stations; NI is the new index calculated for each component of the analysis (population estimate, cardiorespiratory diseases, MCN frequency, and vehicle flow), which at the reference station is equal to 1 for each variable; RTMi corresponds to the RTM of the station under analysis; and RTM0 is the corresponding RTM of the reference station. As a reference area, we used a fragment of Atlantic forest (Mata Azulão) far from the vehicles and other stressors.

2.6. Statistical Analysis

The experiment was conducted in a completely randomized design with a factorial scheme of 3 cities × 9 months with 10 repetitions for the stomatal analysis and 6 repetitions for MCN frequency. The data were evaluated by the F test at 5% probability. When statistically significant differences were detected, the means were compared by Tukey's test at 5% probability.

The vehicular flow in the cities was determined by means (high and low flow). It was considered 3 cities and 2 collection points in each city for the three different times analyzed. The data were analyzed in a completely randomized design and the means were compared by Tukey's test at 5% probability. For the SI, the evaluation of the significant interaction between cities and seasons was compared by Tukey's test at 5% probability.

The environmental data, vehicle flow, MCN frequency, and SI were transformed (data observed–mean)/standard deviation and compared by principal component analysis (PCA) through the "rda" function in the "vegan" package in the R program (R Development Core Team. R: a language and environment for statistical computing. R Foundation for Statistical Computing; Vienna, Austria. ISBN3-900051-07-0, Available in: http://www.r-project.org/ accessed on 4 September 2021).

3. Results

3.1. Mutagenicity and Micronuclei (MCN)

In Figure 2, it is possible to identify significant seasonal and spatial patterns in the frequency of MCN: values three to nine times higher between June and September (winter and early spring) for the cities of Ivinhema and Nova Andradina and low values throughout the year in the city of Angélica. There was also a significant increase in MCN for the cities of Nova Andradina and Ivinhema from the beginning of the experimental period (January—summer) to its end (September—spring); the city of Angélica had low MCN throughout the experimental period.

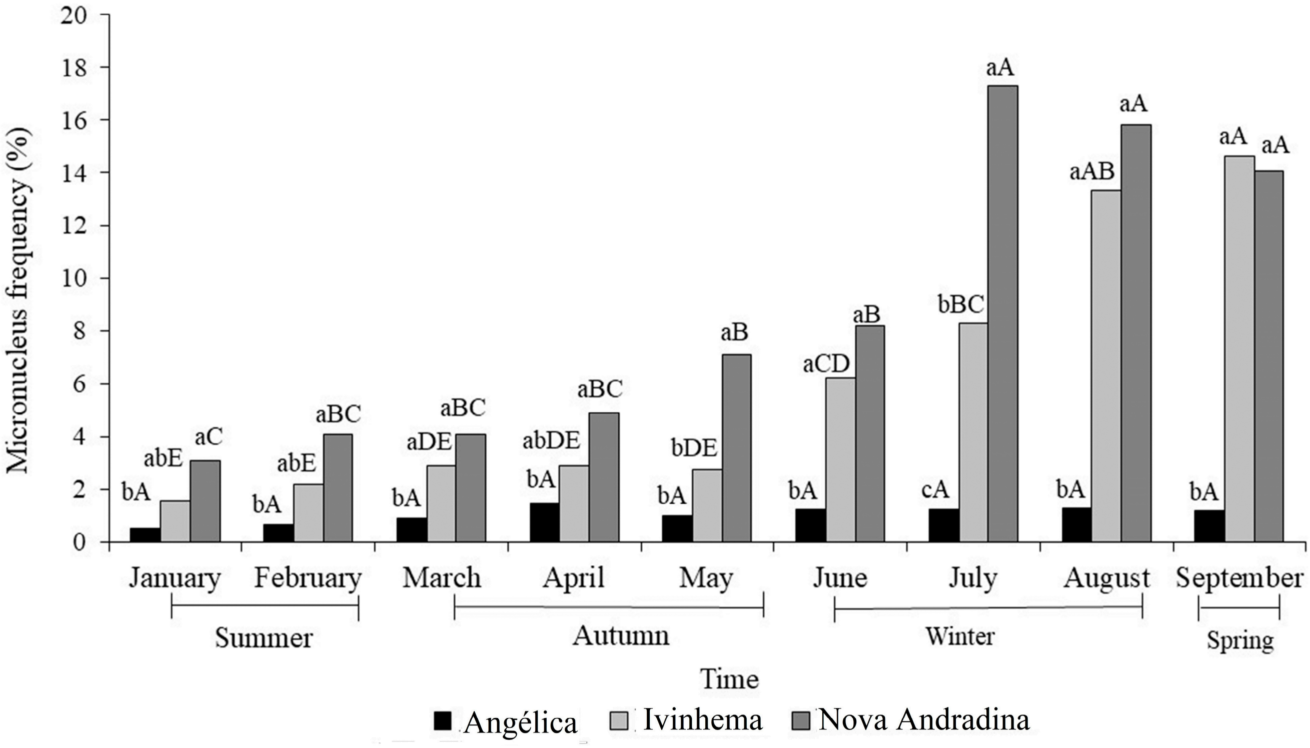


Figure 2. Micronucleus frequency in tetrads of *Tradescantia pallida* (Trad-MCN) in the cities and in the months evaluated. Lowercase letters compare cities within a month, and uppercase letters compare a city within the different months. Columns indicated with different letters differ statistically from each other by Tukey's test at 1% probability.

Nova Andradina and Ivinhema cities had high vehicular traffic (average of 20.55 and 16.44, respectively), differing significantly from Angélica (average 5.00).

In cities, in general, it was found that the highest vehicle flow occurred between 5:00 pm and 6:00 pm in the cities analyzed. In between 11:00 am and 12:00 pm, a significant difference in vehicle flow was observed for the cities of Angélica when compared to Ivinhema and Nova Andradina. Statistical differences were identified between the traffic schedules for the city of Angélica ($p = 0.001$), with the morning (08:00–09:00 h) and the afternoon (17:00–18:00 h) being the hours of higher traffic peaks with higher mean flow in the month of August, differing statistically from the period of 11:00–12:00 (Figure 3A,B). No differences were identified between the times for the city of Ivinhema ($p = 0.11$) (Figure 3C,D). However, in July, higher values of vehicular flow were recorded. In Nova Andradina, there was a significant difference between the times ($p = 0.007$), showing intense vehicle flow between 17:00 and 18:00, with higher averages in February (Figure 3E,F).

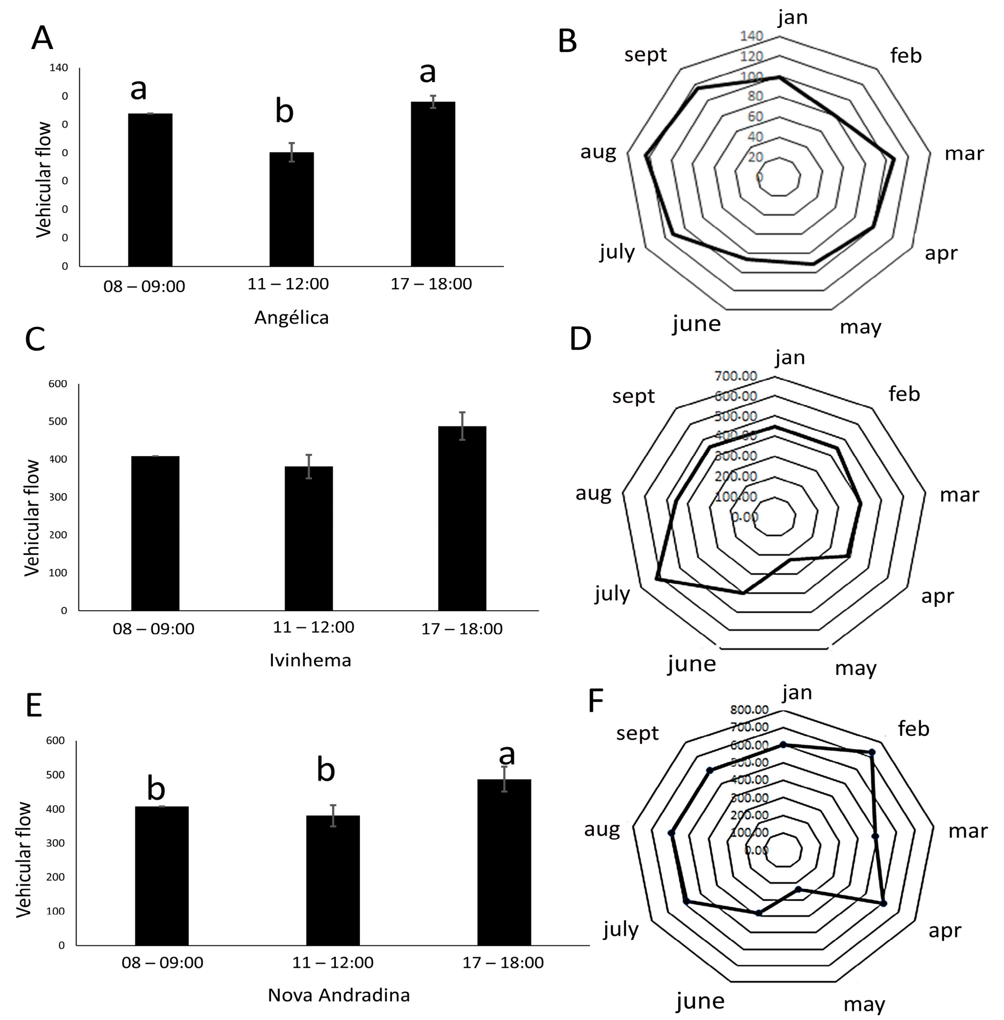


Figure 3. Average vehicle flow for the cities evaluated (**A**,**C**,**E**) and average vehicle flow throughout the sampling periods (**B**,**D**,**F**). Lowercase letters (a, b) in (**A**,**E**), differ statistically, from each other by Tukey's test at 1% probability.

3.2. Leaf Epidermal Characteristics

It was possible to observe a significant seasonal and spatial pattern for the SI: when comparing the cities within each month, significantly higher Sis were observed for *T. pallida* in the city of Angélica in the months of April, June, July, and August, which was autumn and winter; when comparing the months for each city, only Ivinhema and Nova Andradina showed a significant drop in the SI in the months of June to August, which was exclusively during the winter (Figure 4).

3.3. Environmental Variables, Micronuclei, and Stomata

Figure 5 shows the interactions between the indicators shown in Figures 2–4; Axis 1 accounts for 57.01% of the interactions between the MCN frequency and vehicle flow variables. Axis 2 accounts for 23.63% of the factors, with the SI variable in the inverse position to the MCN frequency. The environmental variables of temperature, RH, and rainfall showed low associations with the frequency of micronucleus and vehicular flow, this reinforces our findings considering that the vehicular flow is indicative of the emission of polluting gases and was what most correlated with the frequency of micronuclei. In summary, the MCN frequency and the flow of vehicles were associated, inferring for air pollution. On the other hand, the SI was not considered an adequate biomarker to infer air pollution due to the low association with the aforementioned factors.

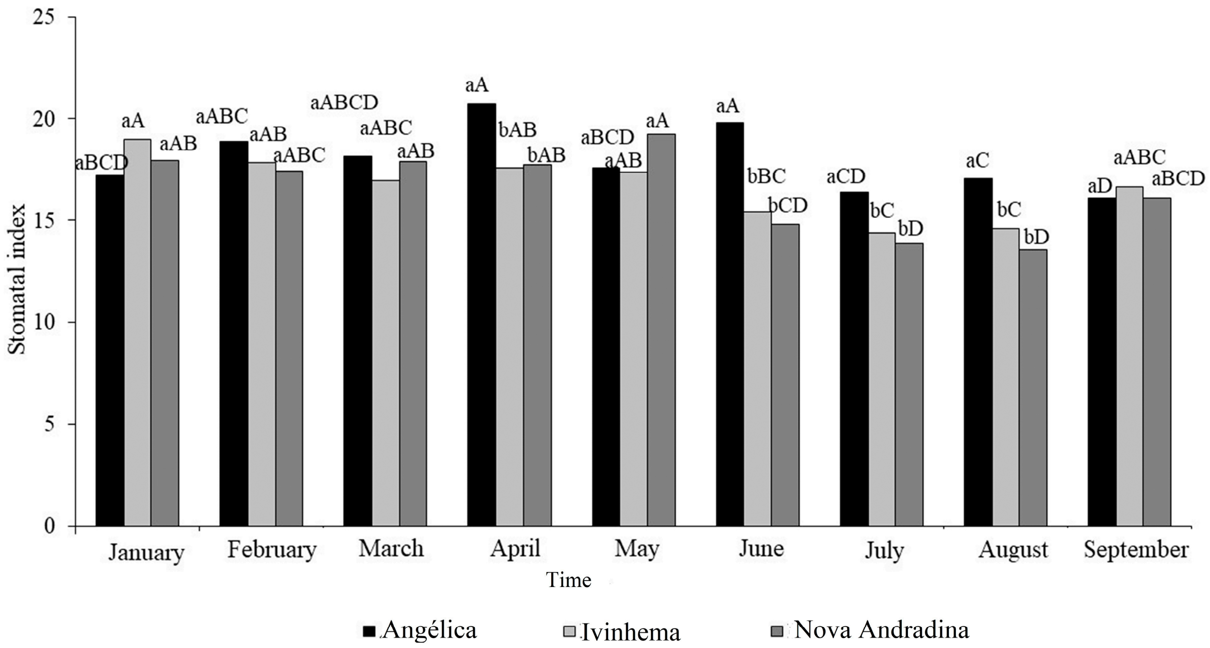


Figure 4. Means of the stomatal index of *Tradescantia pallida* from three cities in the Ivinhema Valley in the southwestern region of the state of Mato Grosso do Sul-MS. Lowercase letters compare cities within a month, and uppercase letters compare a city within the different months.

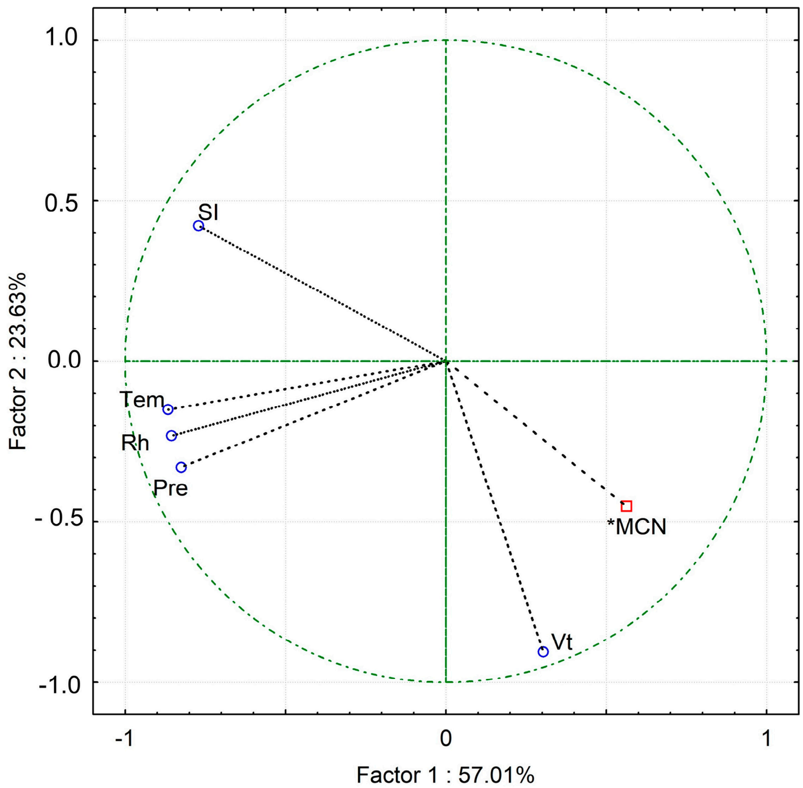
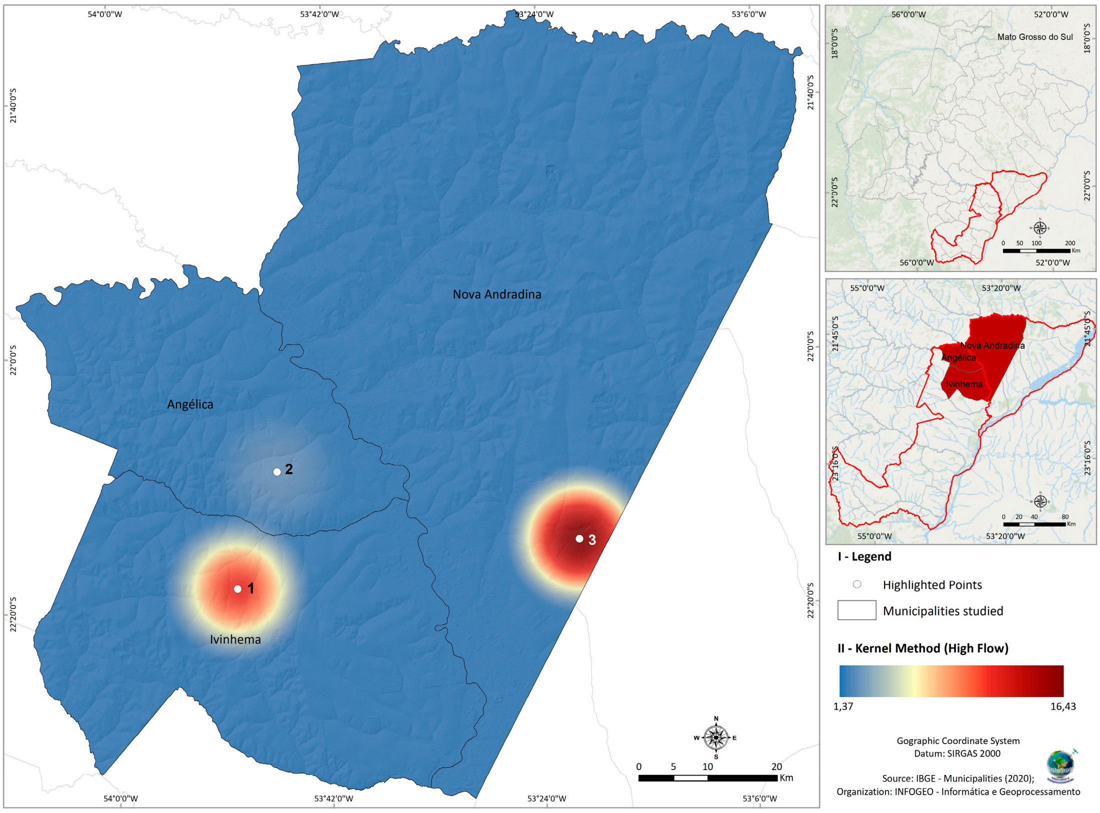


Figure 5. Principal component analysis (PCA) of the environmental variables temperature (Temp), relative humidity (Rh) and precipitation (Pre), in addition to vehicle traffic (Vt), frequency of micronuclei (*MCN) and stomatal index.

3.4. Mapping the Risk Areas

The kernel map showed a spatial geographic pattern of the MCN frequencies (Figure 6 above). The areas in red represent the regions of Nova Andradina and Ivinhema, with Nova Andradina being the region with the highest MCN frequency. The area in blue represents city of Angélica, where we identified a smaller area characterizing the lowest MCN frequency observed. The triad of pollution indicators (Figure 6 below), which took into account the SI, MCN, and vehicle traffic (FV) variables, showed the strength of each indicator compared to a reference environment. In the city of Angélica, all three indicators were very close to the reference value, while in the cities of Ivinhema and Nova Andradina, only the SI was close to the reference values (outside line of graphical representation). These results indicate that the SI was not a good indicator of pollutants at the sampled sites.



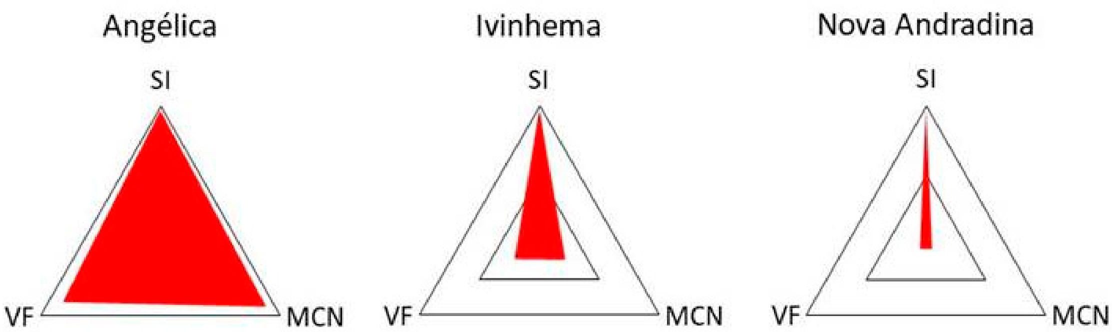


Figure 6. Kernel interpolation map showing the spatial distribution of pollution intensity in the cities: Angélica, Ivinhema and Nova Andradina, southeastern part of the state of Mato Grosso do Sul (**upper image**). Analysis of the triad of pollution indicators for the stomatal index (SI), micronucleus (MCN) frequency, and vehicle flow (VF) represented in the radar chart on the map (**lower image**).

4. Discussion

The data obtained in the study show the influence of high vehicular flow, when comparing the cities among themselves, on the induction of genetic damage in *T. pallida*. According to literature data [38,52–54], it is possible to infer that this damage was attributed to the large amount of toxic particles released by vehicles' engines during the burning of fossil fuels, and these particles stimulated the occurrence of mutations in urban centers. On the other hand, the data obtained with the SI did not show a robust relationship with vehicle flow and, indirectly, with aspects related to air pollution.

Of the cities, Nova Andradina and Ivinhema had the highest means of vehicles (536.11; 425.97 in the three hours, respectively) when compared to Angélica (101.48 vehicles at three times) (Figure 3), i.e., indicators that infer atmospheric pollution. It is the eighth largest city in the state of Mato Grosso do Sul and known as the "Capital of the Ivinhema Valley", with 55.224 inhabitants and a large economic and livestock sector for Brazil. It is located 308 m in altitude, at 22°14′6″ S, 53°19′54″ W [55]. This city has state and federal highways that connect the states of São Paulo and Paraná and are an important route for the flow and export of agribusiness. In addition to these implications, among the cities evaluated, this city is the only one that has several traffic lights at its central point to control and organize transportation flow and urban movement, promoting the expansion of contaminants emitted by vehicle exhaust and exhaust gases at the monitored points.

At intersections with traffic lights and signals, drivers tend to decrease their speed, stopping, and then continuing with sudden acceleration, and it is possible to infer that the gradual emission of exhaust gases, from the engine and combustion with a high concentration of toxic elements, especially carbon monoxide (CO), particulate matter (PM), hydrocarbons (PAHs), nitrogen oxide (NO), and sulfur (SO_2), lead to mutations in the somatic and germ cells of *T. pallida* and favor the incidence of MCN [29,33,35,56,57].

The city of Ivinhema also had high MNF and vehicle traffic values, suggesting high levels of atmospheric pollutants. These observed mutagenic effects may have been related to the state highway (BR-276), which connects several cities, facilitating access to the urban perimeter. In addition to this factor, the city contains sugar and ethanol plants in the region that require a large fleet of trucks and heavy vehicles for transportation of the products.

The city of Angélica had relatively low values of vehicle traffic, which conferred a low MCN frequency. According to Brazilian Institute of Geography and Statistics (IBGE) data from 2021 [46], Angélica also has the lowest estimated population (11,081 humans) compared to that in Ivinhema (23,277 humans) and Nova Andradina (56,057 humans). The results for vehicle flow and MCN frequency associated with population data indicated low or moderate air pollution. Angélica has a single-access road to the municipality, which results in reduced vehicle circulation and consequently minimal emissions of exhaust gases from the combustion engine with a high concentration of toxic elements. Godoy et al. [23] also showed that the high MCN frequencies in *T. pallida* were mainly related to the high vehicle flow and high population density.

Other studies in the literature on different regions of Brazil corroborate our results and the patterns found between vehicle flow and its mutagenic effect in *T. pallida*. In a study on the influence of pollutants on *T. pallida*, the authors [27,29] identified a positive association between genetic effects in pollen grains of inflorescences exposed to a high volume of cars in urban intersections. Similar results were found in regions with different fleet volumes in the municipalities of Ribeirão Preto in southeastern Brazil [36] and São Leopoldo in southern Brazil [58] and in the Dourados microregion in central-western Brazil [23], where areas with high vehicle emissions were correlated to higher frequencies of MCN. These regions have minimum and maximum distances from each other of approximately 900 and 1400 km, respectively; however, they showed consistent patterns when inferring vehicle flow at the MCN frequency.

Our results indicated that the SI had an imprecise relationship between pollution (vehicle flow) and climatic stressors. The months corresponding to the winter period, characterized by significant drops in temperature and RH, also showed decreases in the SI values for cities with high vehicle traffic. Similar studies suggest that these two factors may aggravate their substantial risks to human health and urban biodiversity [26,48]. These climatic conditions are likely related to the process of thermal inversion and the formation of heat islands that provide less wind circulation and dispersion of gaseous particles [59], thus intensifying the oxidative action of pollutants in terms of genetic changes. We observed that during the winter and spring seasons, in periods with lower temperatures and RH, the MCN frequency increased for cities with higher vehicle traffic. These results are similar to those found in other studies [26,27], which found an inversely proportional relationship between the increase in the frequency of MCN in *T. pallida* specimens and environmental variables, such as RH and temperature.

In our study, the altitude variable was not related to the increase in the frequency of chromosomal damage in the samples, possibly due to the low variability between the municipalities in the Ivinhema Valley (380 to 358 m). This trend was also shown by Spósito et al. [27] in cities of the Mato Grosso do Sul microregion, with similar vertical distances to sea level (318 to 470.2 m). However, studies conducted in other regions of the state with substantial variations in altitude (206 and 658 m) found that cities with higher altitudes had a positive correlation with the MCN frequency in *T. pallida* plants, indicating an influence on air quality and clastogenic effects [26].

The increase in the MCN frequency in different seasons was probably an adaptive response of the *T. pallida* plants to atmospheric mutagenic and genotoxic agents dispersed by vehicle sources, demonstrating their possible effects and damage to exposed living organisms, including humans. In a recent study, ref. [23], the authors related the frequency of micronuclei of environmental conditions, cardiorespiratory diseases, number of hospitalizations, and pathologies to vehicle flow in some cities of Mato Grosso do Sul. This same study also identified that municipalities with high MCN frequencies exhibited the highest number of cardiorespiratory diseases. In birds present in forest fragments, the effect of urbanization and vehicle traffic influenced the increase in the frequency of mutations due to the absorption of polluted air as a causative agent of genetic toxicity (MCN) in erythrocytes of birds close to urban habitats [60]. Both studies highlighted the impacts of toxic compounds from the air and the drastic damage in the short- and long-term as a result of exposure in organisms previously exposed to the action of toxicological agents, emphasizing their risks and effects through mutagenic analyses.

In contrast, the observations found for the leaf epidermal parameters were not as robust as the MCN data in terms of indicating air pollution. The SIs indicated that stomatal and epidermal cell reduction may be adaptive strategies in *T. pallida* that allow it to tolerate toxic stress while also being adaptive responses to climate change. This adaptive mechanism mitigates the physiological deterioration and losses from gas exchange in response to stressors, and *T. pallida* may limit its metabolism and trigger protective mechanisms. Studies conducted in the state of Mato Grosso do Sul corroborate these observations and indicate a correlation between low SIs and high-altitude regions [26]. The authors associated these data as biological responses to prevent the diffusion of atmospheric pollutants from intense vehicle use to leaves in the cities evaluated. This adaptation mechanism was also described by Alves et al. [61] when observing stomatal reduction in the hybrid clone 4430 of *T. pallida* exposed in polluted areas in the city of São Paulo.

The mean annual monitored risks in the areas on the kernel interpolation map show the deleterious mutagenic effects in the cities of Nova Andradina and Ivinhema, places with high vehicle flow. The city of Angélica, with low vehicle flow, has a low intensity of pollutants. To corroborate these results, the triad of environmental quality shows how close the city of Angélica is to the reference values for an area with good and/or excellent environmental quality in relation to atmospheric pollution. The results presented in the triad of environmental quality for vehicle flow and MCN frequency were consistent for the three regions sampled. On the other hand, the results of the Sis were not visually associated with the other indicators. The high SI values in all regions with markedly different vehicle flow amounts and mutagenicity effects in *T. pallida* suggest the need for further studies to understand the adaptive mechanisms of this species. Variations in SI are one of the main necessary anatomical adaptations of plants needed to survive in habitats with toxic atmospheric particles [45,62,63] and intrinsic climatic characteristics [26]. However, our results were inconclusive in terms of supporting such patterns related to atmospheric pollution stress.

In summary, the mutagenic effects observed in *T. pallida* through the MCN frequency constituted an important biomarker of air pollution, mainly explained by the relationship with the vehicle flow. Such mutagenic effects were also highlighted by the chronic environmental effects on *T. pallida* resulting from atmospheric pollutants.

In short, the findings of this study provide information on the impact of pollution as a tool for assessing atmospheric quality and preventing and controlling urban vehicle emissions. Vehicular flow is the main inducing activity of oxidative stress in *T. pallida*, signaling how the growing vehicle fleet deteriorates atmospheric quality, causing biological injuries in plants and consequently in humans.

5. Conclusions

The MCN frequency was positively associated with vehicular flow, which was considered in this study as the main activity responsible for air pollution. However, the comparative analysis between vehicular flow, MCN frequency and SI showed that MCN is a more sensitive biomarker to indicate the deleterious effects of air pollution. The SI requires further study since climatic oscillations had an adaptive effect on the test plant masking the possible effects of atmospheric pollutants. The cities of Nova Andradina and Ivinhema were the regions with the highest MCN frequency and lowest SI, distinguishing them from Angélica, which showed low variation throughout the evaluations.

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