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Original Article

Biomonitoring of air quality in the Bodoquena microregion, Mato Grosso Do Sul: mutagenic and morphoanatomical alterations in *Tradescantia pallida* (rose) D.R. Hunt var. *purpurea*

Biomonitoramento da qualidade do ar na microrregião da Bodoquena, Mato Grosso Do Sul: mutagênese e alterações morfonatomicas em *Tradescantia pallida* (Rose) D.R. Hunt var. *purpurea*

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Abstract

The objective of this study was to assess air quality in relation to vehicular traffic flow in cities located at different elevations in the Bodoquena microregion, state of Mato Grosso do Sul, Brazil. To do so, a micronucleus test was carried out using the TRAD-MCN bioassay on young *Tradescantia* buds collected from February to November 2018 in seven cities of the microregion with different traffic flow intensities. Meteorological parameters were evaluated, and vehicular traffic was counted to determine traffic flow in each city. With data from the Shuttle Radar Topography Mission (SRTM) and processing in Esri ArcGIS® software version 10.5.1, the regions was mapped based on an Elevation Model. Morphoanatomical analyses were performed according to standard methodology. Measurements were taken of thickness, length and width of tissues and structures, including the upper and lower cuticle, upper and lower epidermis, hypodermis and mesophyll. The greatest traffic flow was found in the cities of Bodoquena, Guia Lopes da Laguna, Jardim, and Porto Murtinho, with the period from 5:00 to 6:00 p.m. showing the highest traffic flow. The greatest frequency of mutagenic alterations was found in the city of Guia Lopes da Laguna, although the results did not differ significantly from Bonito, Caracol, and Jardim. Throughout the biomonitoring, the summer and autumn seasons showed the greatest micronuclei frequencies in all evaluated cities. Variations in the tissue/structure thickness was observed across cities and seasons, but with a decrease in thickness during autumn. In general, the tissues/structures were smaller for the cities of Nioaque and Porto Murtinho, while the anatomical and morphological characteristics of leaf length and thickness showed no differences among cities. We found limited correlation between micronuclei frequency and traffic flow, supporting the hypothesis that although mutagenic alterations are observed in *T. pallida*, in this microregion the changes are numerically lower when compared to other regions of the state. In light of the genotoxic and morphoanatomical factors assessed herein, the Bodoquena microregion appears to be well preserved in terms of air quality, presenting low micronuclei frequency and a limited reduction in tissues and leaf structures, regardless of the season.

Keywords: bioindicator, micronucleus, automotive vehicles, air pollution.

Resumo

O objetivo deste trabalho foi avaliar a qualidade do ar com base no fluxo veicular das cidades localizadas em diferentes altitudes na microrregião da Bodoquena, no estado de Mato Grosso do Sul, Brasil. Para tal, foi realizado o teste de micronúcleo, por meio do bioensaio TRAD-MCN em botões jovens de *Tradescantia* coletadas no período entre fevereiro a novembro de 2018 em sete cidades da microrregião da Bodoquena, com diferentes intensidades de fluxo veicular. Foram avaliados os parâmetros meteorológicos, os veículos foram contados para determinar o tráfego de veículos em cada cidade e altitude. A partir da topografia Shuttle Radar (SRTM) e processamento no software Esri ArcGIS® versão 10.5.1 foi possível mapear a área com base no Modelo de Elevação. As análises morfoanatômicos foram realizadas conforme metodologia padrão. As mensurações de espessura, comprimento, largura dos tecidos e estruturas como a cutícula superior, cutícula inferior, face superior e face inferior da epiderme, hipoderme e mesófilo foram avaliadas. O maior fluxo veicular foi encontrado nas cidades de Bodoquena, Guia Lopes

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da Laguna, Jardim e Porto Murtinho. O horário das 17:00 às 18:00h foi o que apresentou maiores fluxo de veículos. A maior frequência de alterações mutagênicas foi encontrada na cidade de Guia Lopes, não diferindo de Bonito, Caracol e Jardim. Ao longo do biomonitoramento observou-se que as estações de verão e outono foram as que apresentaram maiores frequências de micronúcleo independente da cidade avaliada. Observou-se que a correlação entre a frequência de micronúcleos e o fluxo veicular foi baixa, apoiando a tese de que essa microrregião, embora apresente alterações mutagênicas em *T. pallida,* as alterações numericamente são pequenas quando comparadas a outras regiões do estado de Mato Grosso do Sul. Observou-se uma variação na espessura dos tecidos/estruturas que é variável entre as diferentes cidades e estações do ano. De forma geral os tecidos/estrutura apresentaram redução na espessura para as cidades de Nioaque e Porto Murtinho quanto aos aspectos anatômicos e morfológicos, sendo que, para o comprimento e espessura foliar não foi observado diferenças entre as cidades. Em relação as estações do ano, observou-se que no outono a espessura dos tecidos/estruturas são menores. Diante dos fatores genotóxicos e morfoanatômicos aqui avaliados, a microrregião da Bodoquena parece estar bem preservada em termos de qualidade do ar, apresentando baixa frequência de micronúcleos e redução limitada de tecidos e estruturas foliares, independentemente da estação do ano.

Palavras-chave: bioindicador, micronucleos, veículos automotivos, poluição do ar.

1. Introduction

Air pollution is a consequence of growing urbanization, agricultural development, and poorly controlled vehicular traffic density, studied by Andrade Júnior et al. (2008); Pereira et al. (2013), Cassanego et al. (2015), Costa et al. (2016) and Alves et al. (2020) in the various brazilian states. These factors generate genotoxic agents (carbon, nitrogen and sulfer oxides, and organic compound such as hydrocarbons, volatile organic compounds (VOCs) and partícles that when released into the atmosphere combine with other compounds in the air, affecting the quality of life of the population (Braga et al., 1999; Brauer et al., 2002; Perera et al., 2002; Pope III et al., 2002; Saldiva et al., 2002; Ribeiro and Cardoso, 2003; Daumas et al., 2004) ausing harmful effects on living organisms (Batalha et al., 2002; Rivero et al., 2005; Claxton and Woodall Junior, 2007; Pereira et al., 2010; Brito et al., 2013).

With a view to reducing the effects of pollution and contamination by chemicals on the health of the population, since 2015, the United Nations (UN) 2030 agenda has defined 17 Sustainable Development Goals (SDGs) to be achieved with the participation of several countries. Among them, Brazil has an important role to play through the development of innovative strategies, especially in terms of public policies that attempt to integrate the economic, social, and environmental dimensions of sustainable development.

The plant *Tradescantia pallida* (Rose) D.R. Hunt. var. *purpurea* Boom offers good results in monitoring air pollution it presents a simple methodology, in which the sample material is easily accessible, and has high sensitivity to genotoxic agents (Carvalho-Oliveira et al., 2005; Carreras et al., 2009; Spósito et al., 2017) besides has been adopted in Midwest Brazil for this purpose because in this region, economic development is intrinsically linked to the agricultural sector (Crispim et al., 2012, 2014; Spósito et al., 2015, 2017; Rocha et al., 2018). This is particularly the case for the state of Mato Grosso do Sul, which is divided into four mesoregions (Pantanal, Center North, East and Southwest) and eleven microregions (Upper Taquari, Aquidauana, Lower Pantanal, Bodoquena, Campo Grande, Cassilândia, Dourados, Iguatemi, Nova Andradina, Paranaíba, and Três Lagoas).

Mato Grosso do Sul (MS) occupies a strategic position, as it borders five states (Mato Grosso, Goiás, Minas Gerais, São Paulo, and Paraná) and two countries (Bolivia and Paraguay). The main economic activity in the state is agriculture (soy, corn, cotton, rice, and sugarcane) and cattle husbandry, along with mining and food processing (Fagundes et al., 2017). Products from these sectors are transported to other states and countries via highway. As a result of the flow of agricultural products from MS to other states, the traffic of heavy vehicles has increased. Consequently, changes in air quality have been found through air biomonitoring studies performed by Rocha et al. (2018) in the mesoregions of southwest MS, by Crispim et al. (2012, 2014) in the microregion of Dourados and by Spósito et al. (2015) using micronuclei (TRAD-MCN) and comet assays (Spósito et al., 2017) on *Tradescantia pallida*.

Although some regions in southwest MS have been studied by Rocha et al. (2018), air quality in the Bodoquena microregion was not included in the analysis. This region attracts many tourists and researchers as it offers a range of eco and historical tourism, while also occupying an extremely strategic position in Brazil and Latin America. Green areas corroborate with the improvement of air quality in urban environments, and consequently for the health of the local population (Rocha and Mussury, 2020), showing that climatic conditions probably do not interfere with MCN frequencies in forest fragments, as they are less extreme than in urban areas Savoia et al. (2009) and Cassanego et al. (2015).

Thus, this study sought to demonstrate the air quality in the region, so that improvements can be made to the well-being and health of the population (SDG 3). It also aimed to inform appropriate public policies that help to improve the quality of life of the population, as linked to SDG 11 to make cities and communities sustainable by 2030 by reducing the negative environmental impact per capita of cities, particularly in terms of air quality and municipal waste management. Therefore, the objective of this study was to assess the air quality in the Bodoquena microregion, Mato Grosso do Sul, based on genotoxic and morphoanatomical characteristics of *Tradescantia pallida* in the different seasons.

2. Material and Methods

2.1. Study sites

The study was carried out in the municipalities of Bodoquena (elevation of 132 m), Bonito (315 m), Caracol

(212 m), Guia Lopes da Laguna (272 m), Jardim (259 m), Nioaque (200 m), and Porto Murtinho (90 m) (Figure 1). Within each city, sample locations were classified into two categories: urban area with high levels of vehicular traffic flow and urban area with low levels of traffic flow. Subsequently, the average traffic flow was calculated for each city. The sampling points were close to the avenues for cars circulation and in flowerbeds that measured, on average, from 5 to 10 m2.

Tradescantia pallida (Rose) Hunt var. *purpurea* Boom was used to test air quality. The species was planted in cities where it does not naturally occur, and data were collected bimonthly in all four seasons (summer, autumn, winter and spring). The sample collection locations were marked with a GPS (Garmin e Trex Legend HCx). *Tradescantia pallida* var. *purpurea* were grown in pots of height: 20 cm; Mouth width: 20 cm; Bottom width: 11 cm, with a capacity of 3.5 liters, containing ravine soil + sand + semi-composted chicken litter (1: 1: 1) (v: v) and kept under a dark greenhouse 50% being watered three times a week; after 15 days of cultivation, they received biostimulant applications (0.2mL / plant), commercial product Stimulate® that contains plant regulators such as indolbutyric acid (Auxin) 0.005%, kinetin (Cytokinin) 0.009% and gibberellic acid (Gibberellin) 0.005% and also chelated mineral salts traces (Stoller do Brasil, 1998). The biostimulant was applied with a spray bottle purchased from the pharmaceutical handling trade.

2.2. Trad-MCN bioassay

The Trad-MCN bioassay was conducted according to the protocol of Costa et al. (2016). Fifteen young *T. pallida* flower buds were collected bimonthly beginning in the summer of 2017 and during the autumn, winter, and spring of 2018. Inflorescences were fixed in Carnoy's solution (3 ethyl alcohol: 1 acetic acid) for 24 hours. The inflorescences were then transferred to an alcohol solution (70% concentration). From the sampled floral buds, six slides were made for each study location following the methodology proposed by Ma (1981). The micronuclei frequency was calculated by observing 300 tetrads per slide, using an optical microscope at 400x magnification (Nikon YS2; Tokyo, Japan). The results are expressed in percentage (micronuclei frequency in 100 tetrads), following Ma et al. (1994) (Figure 2).

2.3. Morphoanatomical analysis

We conducted a morphoanatomical analysis on 20 of the most expanded leaves from distinct plants at each sample location in the studied cities. The leaves were collected and separated in groups of 10 for each sampling location, divided into areas of high- and low-traffic flow for each city.

The collected leaves were stored in 70% alcohol and transported to the Botanical Laboratory of the School of Biological and Environmental Sciences (FCBA) at the Federal University of Grande Dourados (UFGD). Subsequently, leaves were sectioned transversally by hand. The sections were clarified with 20% sodium hypochlorite and then submitted to a double staining process with astra blue (1%) and aqueous safranin (1%) at a proportion 9:1 (Johansen, 1940), and then mounted on 66% glycerin (Alves et al., 2001).

The thickness, length, and width of tissues and structures were measured with a digital pachymeter (DIGIMESS - 100,176 BL PLUS), including the upper (ADC) and lower

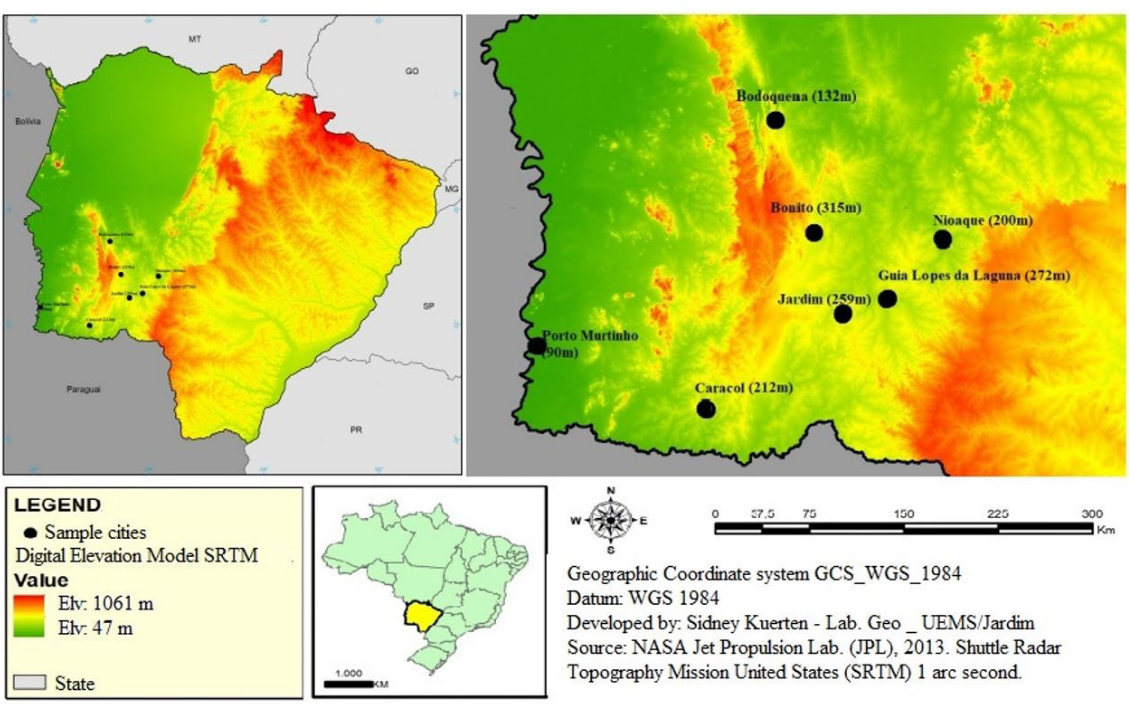

Figure 1. Map showing the elevation of the studied cities in the Bodoquena microregion Mato Grosso do Sul, Brazil.

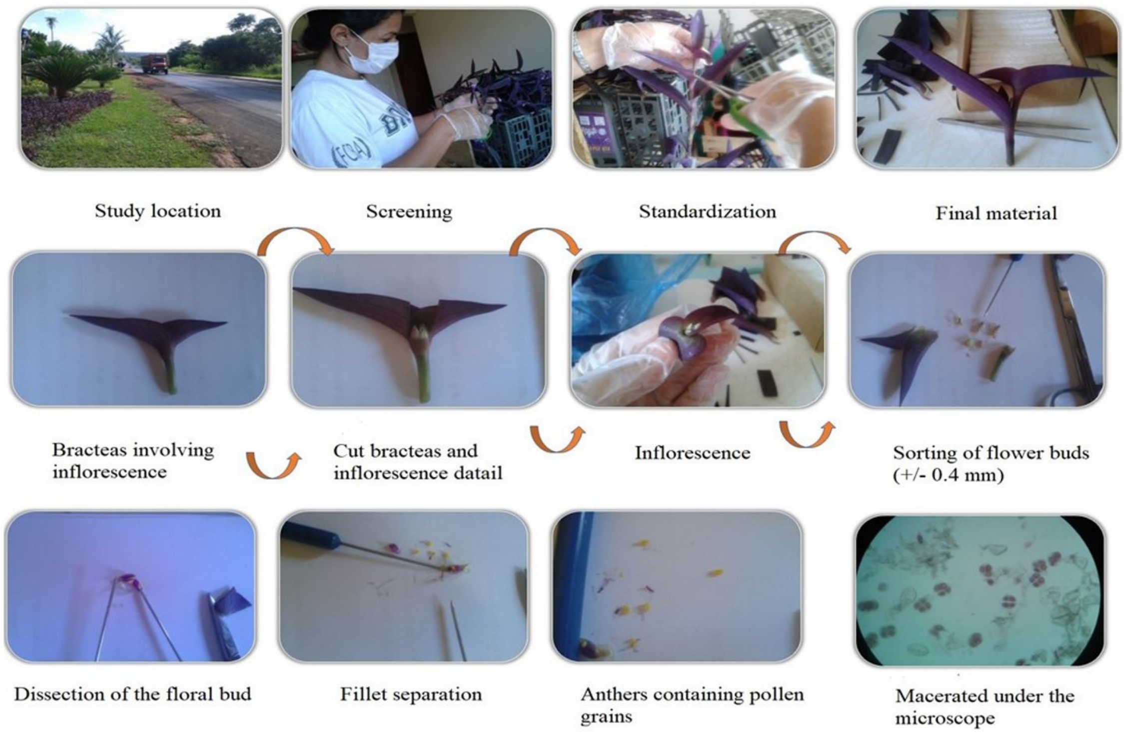

Figure 2. The methodology schematic representation used for the evaluation of micronuclei frequency.

cuticle (ABC), upper (ADE) and lower epidermis (ABE), hypodermis (HYPO) and mesophyll (MESO) (Figure 3). Structures were evaluated using a binocular microscope coupled with a camera and the image capture program Moticam 2300 3.0MP live Resolution.

2.4. Evaluation of vehicular traffic flow and environmental conditions

The traffic flow was obtained by counting the vehicles (passengers cars, trucks and motorcycles) that circulated past the collection point on each sampling day, at three times a day: 8:00-9:00 a.m.; 11:00 a.m.-12:00 p.m.; and 5:00-6:00 p.m. From this, the average traffic flow per city was calculated (Table 1).

The hypsometric map of Mato Grosso do Sul was produced using data from the Shuttle Radar Topography Mission (SRTM) and processed in Esri ArcGIS®, version 10.5.1. The elevation intervals were defined based on the maximum and minimum values for the topography of the state and represented by a standard scale (IBGE). With this data, the elevation of MS was spatialized by means of an Elevation Model map (EM) (Figure 1 and Table 1). The EM is the reproduction of a section of the surface, given by an array of pixels with planimetric coordinates and a pixel amplitude value compatible with elevation (Silva Junior and Fuckner, 2010).

Throughout the experiment, in addition to collecting biological samples, we also recorded meteorological data related to temperature (°C) and relative humidity (RH), which were obtained using an Instrutemp® Digital Thermo-Hygrometer ITHT 2250.

2.5. Statistical analyses

The experimental design to Trad-MCN bioassay was entirely randomized in a factorial scheme, with 7 cities x 4 seasons and 12 repetitions. The normality of the data was verified by the Shapiro-wilk test. The average micronucleus frequency was evaluated by the F test at 5% probability. After verifying significant differences, the averages were compared by Duncan's test at 5% probability. The traffic flow at different times and cities were analyzed using a completely randomized design with factorial scheme of 7 (cities) x 3 (times) with four repetitions. The averages were compared using the Tukey test at 5% probability. All data has been transformed to the root of x+0.5. The sample's standard deviation was calculated.

Pearson's correlation coefficient between environmental variables, traffic flow, and micronuclei frequency was also performed (Snedecor and Cochran, 1989).

The experimental design to morphoanatomical analysis was completely randomized in a factorial scheme, with 7 cities x 4 seasons and 20 repetitions. The averages were compared using the Scott-Knott test at 5% probability (Snedecor and Cochran, 1989).

3. Results

The data obtained from the climatic variables area presented in Table 1.

Based on analysis of variance, there were significant differences for the isolated factors, city and season, but the

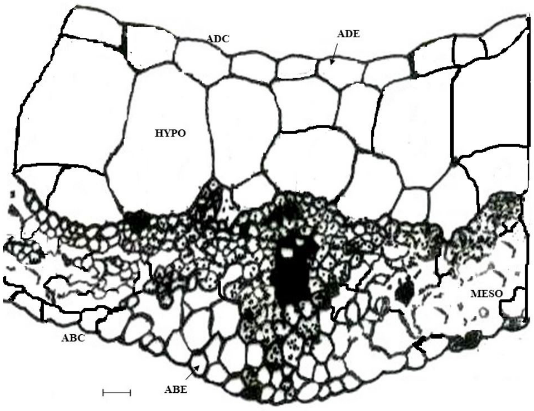

Figure 3. Leaf anatomy of *Tradescantia pallida* (cross section) where: **ABC**: Abaxial cuticle, **ADC**: Adaxial cuticle, **ABE:** Abaxial face of the epidermis, **ADE:** Adaxial face of the epidermis, **HYPO**: Hypodrmis, **MESO**: Mesophyll. Scale bar 100µm.

interaction between these two factors was non-significant (Table 2).

In relation to the micronuclei frequency in *T. pallida* in the different cities analyzed, Guia Lopes da Laguna presented the highest MCN frequency, but the results did not differ statistically from Jardim, Bonito, and Caracol. Lower MCN frequencies were found for Bodoquena, Nioaque and Porto Murtinho (Figure 4).

When the seasons were analyzed, we found that the MCN frequency was highest in the summer and autumn seasons, with minimal variation in winter, and lowest frequency in spring (Figure 5).

No significant interaction was observed between the factors city and time of traffic flow, but we did find significant variation for city and time as isolated factors. The cities with the highest average vehicular flow were Bodoquena (177.4 cars), Guia Lopes da Laguna (295.50 cars), Jardim (272.00 cars) and Porto Murtinho (294.04 cars), Bonito (346.70 cars) and Nioaque (172.8 cars) differed significantly with lower average traffic flow, and the city of Caracol (78.16 cars) showed the lowest traffic flow (Figure 6).

Regarding the time of day, the period from 5:00 to 6:00 p.m. showed the greatest traffic flow (271.86), which was significantly different from the periods between 8:00 to 9:00 a.m. (219.52) and 11:00 a.m. to 12:00 p.m. (210.01) (Figure 7).

A positive correlation between micronucleus frequency and vehicular flow are observed throughout the experimental period presented in Table 3.

The results presented in Table 3 indicate a positive and significant correlation for the vehicle flow and the micronuclei frequency, indicating a direct relationship between the two variables and, in relation to humidity and temperature, a negative and significant relationship was observed.

Variation in the thickness of tissues and structures was observed between the studied cities. Figures 8 and 9 show this variation and the differences across cities and seasons.

In general, tissues and structures of *T. pallida* samples from Nioaque and Porto Murtinho were smaller, with

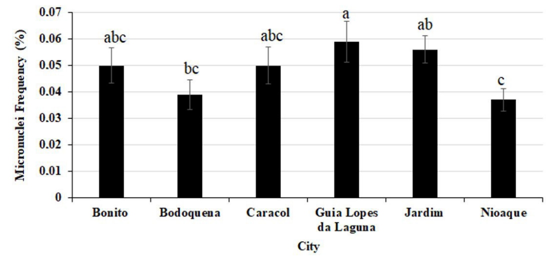

Figure 4. Micronuclei frequency in the cities evaluated. Columns with the same letter do not differ statistically according to the Duncan's test at 5% probability (Transformed data to root of x + 0.5).

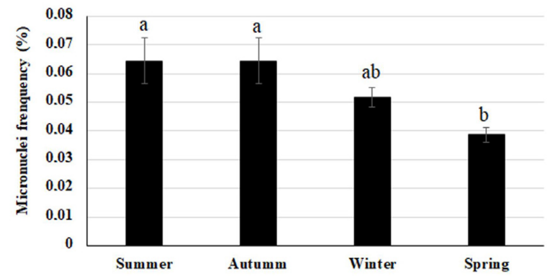

Figure 5. Micronuclei frequency during the different seasons of the year considering the total frequency in the 7 cities evaluated. Mean values with the same letter do not differ statistically based on Duncan's test at 5% probability. (Transformed data to root of x + 0.5).

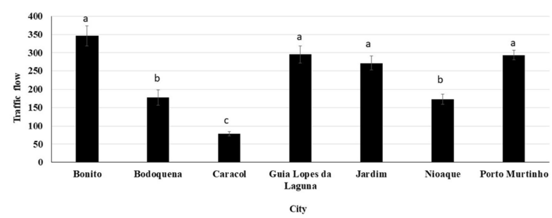

Figure 6. Vehicular traffic flow total in cities of the Bodoquena microregion, considering the three sampling times. Cities with the same letter do not differ statistically based on Duncan's test at 5% probability. (Transformed data to root of x + 0.5).

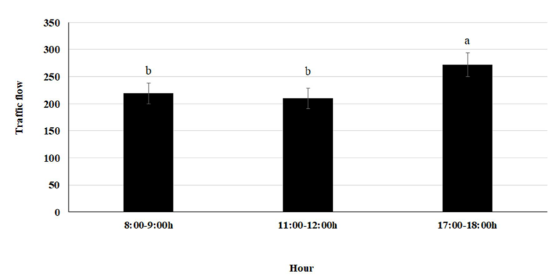

Figure 7. Vehicular traffic flow at different times of the day in cities of the Bodoquena microregion. Mean values with the same letter do not differ statistically based on Duncan's test at 5% probability. (Transformed data to root of x + 0.5).

Table 1. Data collected in the cities analyzed: Elevation in meters (Elv.); Average of Vehicular Flow per hour (VF); Monthly Average Relative Air Humidity % (RH); Monthly Average Temperature (T°C); and Micronuclei Frequency (MCN).

City	Elv	Environmental variables by season															
		Summer				Autumn				Winter				Spring			
		February				June				September				November			
		VF	RH	TºC	MCN	VF	RH	TºC	MCN	VF	RH	TºC	MCN	VF	RH	TºC	MCN
Bonito	315	1340	92	22.5	0.062	1170	72	20.1	0.049	798	81	11.5	0.044	852.5	94	20.1	0.042
Bodoquena	132	382.5	83	27.9	0.065	310	84	22.6	0.03	693.5	25	31	0.032	742.5	96	19.8	0.023
Caracol	212	242.5	51	19.7	0.053	227.5	52	11.9	0.069	215	25	33	0.039	253	91	23	0.032
Guia Lopes da Laguna	272	915	92	21.3	0.074	583	84	16.7	0.081	934.5	69	14	0.049	1113.5	92	20.7	0.032
Jardim	259	580	87	23.2	0.067	847.5	84	16	0.067	857.5	91	10	0.055	979	95	20.3	0.035
Nioaque	200	665	69	28.4	0.03	453	61	20.4	0.046	500.5	29	33	0.044	455	92	19.3	0.028
Porto Murtinho	90	950	84	23.8	0.03	825	94	13.5	0.049	881.5	26	31.9	0.039	872	92	22.6	0.035

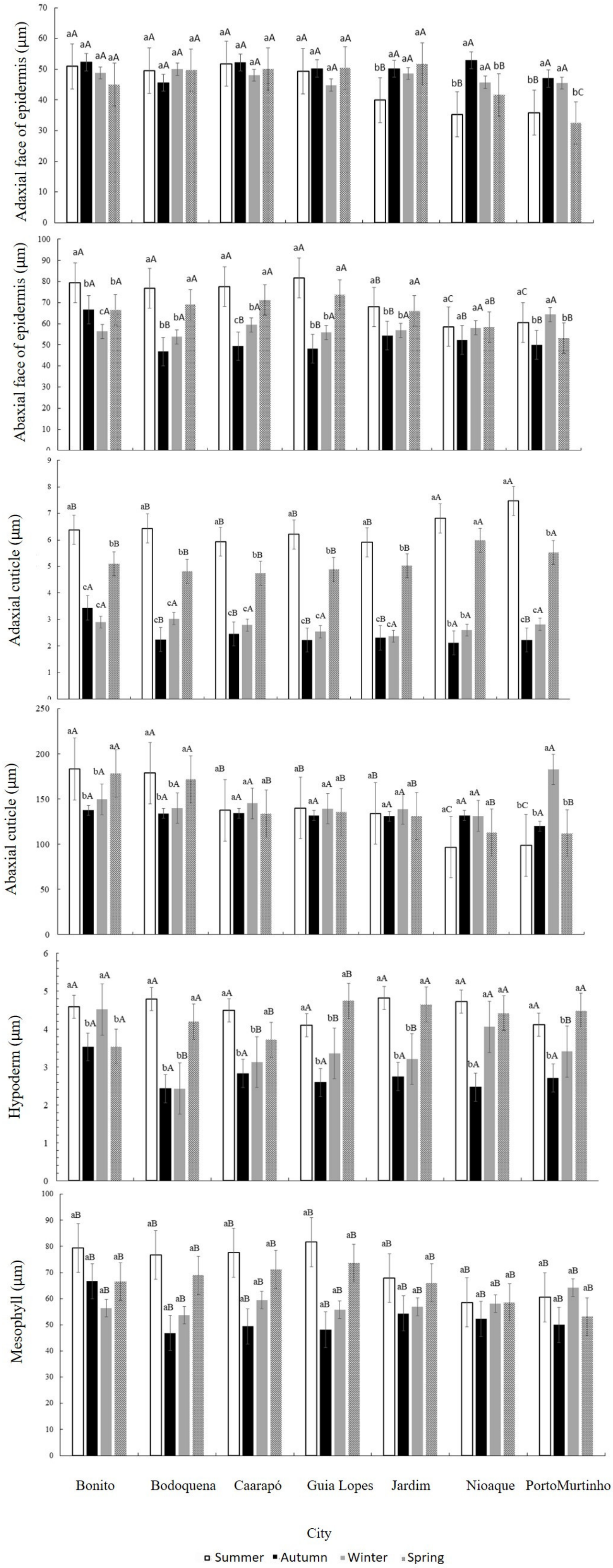

Figure 8. Thickness of foliar tissue/structure in *Tradescantia pallida*. Different lowercase letters indicate statistical differences between seasons for the same city and uppercase letters indicate difference between cities considering the season.

a marked difference for hypodermis in summer when compared to other cities (Figure 8). For the *T. pallida* mesophyll, a reduction in thickness was observed in samples from Nioaque (Figure 8). We did not observe significant differences in relation to leaf width and thickness between the municipalities, with the lowest leaf thicknesses being observed in the city of Nioaque in the summer, and leaf width in the winter, when compared within the season (Figure 9). The leaf length in Nioaque (120 mm) and Porto Murtinho (127 mm), and the variations in leaf length in other cities varied between 140 mm and 148 mm.

Regarding the seasons, in autumn the tissues/structures were thinner than during other times of the year.

4. Discussion

The intense vehicular flow was observed in the greater ecotourism cities as Bodoquena micro-region, being Bonito, Guia Lopes da Laguna, Jardim and Porto Murtinho. However, we observed higher micronucleus frequency values for the cities of Guia Lopes da Laguna, Jardim, Caracol and Bonito, the observed values are very low when compared to other cities in the state. Establishing a reference value for the analysis of the micronucleus frequency observed in the literature, we have that in the studies condected by Klumpp et al. (2004) and Pereira et al. (2013), frequencies of up to 2,0 MCN, are considered to result from spontaneous mutations when the plants are kept in na unpolluted environment. Considering the studies carried out in the MS, it appears that the polluted environments literature reference value is about 10,0 MCN (Rocha et al., 2018); greater than 20,0 fot the city of Dourados (Crispim et al., 2012, 2014; Spósito et al., 2015). Thus, it appears that within the cities that compose the Mato Grosso do Sul Southwest mesoregion, the Bodoquena microregion presented lower MCN frequency values when compared to the analyzed cities, with the vehicular flow in the cities analyzed by Rocha et al. (2018) showed, in some cities, twice the numbers of cars traveling. This observation, added to the MCN frequency results in the presente study, leads us to believe that the micro-region of Bodoquena, even with the ecotourism and historical appeal is preserved in terms of air quality.

The Bodoquena microregion is located in a strategic zone in MS; not only is it in the center of Latin America, but it also shares borders with important Brazilian states and two South American countries. The cities that make up the Bodoquena microregion have well-developed eco and historical tourism sectors (Guimarães and Silva, 2018) and as such an elevated amount of traffic was expected.

The study conducted by Guimarães and Silva (2018) highlights the ecotourism potential of Bonito; however, the authors also note that while Bodoquena and Jardim may present this same capacity, they only act as support for Bonito. As such, we expected to find a greater vehicular flow and, consequently, a higher MCN frequency in these cities, higher than in other cities in the microregion.

Among the cities analyzed herein, the MCN frequency is highest in the cities of Bonito and Jardim, nevertheless the values found are far below those observed by Spósito et al. (2017) and Rocha et al. (2018). Therefore, although mutagenic alterations are observed in *T. pallida*, in this microregion the alterations are less significant when compared with other regions of Mato Grosso do Sul.

The data found corroborate the studies by Rocha and Mussury (2020), which inferred less mutagenic potential in urban areas when it presents a green space. Therefore, the area of preservation and ecotourism use mitigates the action of polluting gases. Cassanego et al. (2015) in their study showed that in the municipality of Caraá, with less demographic density than two other municipalities studied, Taquara and Campo Bom, located within the metropolitan region of Porto Alegre, presented the lowest number of vehicles in the urban area and was accompanied by lower micronuclei frequencies. The authors also mention that for riparian forest fragments, no significant relationship between the TRAD-MCN frequency, temperature, humidity relative and precipitation was observed. The findings show that climatic conditions are unlikely to interfere with MCN frequencies in forest fragments, as they are less extreme than in urban areas. Rocha-Uriartt et al. (2015)

Table 2. Analysis of variance for the micronuclei frequency in different cities and seasons.

Cause of variation	DF	MS	F
City	6	0.0016	2.38*
Season	3	0.0043	6.23**
City x season	18	0.00064	0.92
CV(%)	3.6		

DF: degrees of freedom; CV: coefficient of variation; MS: mean square; and F: F test. **significant at 1% probability ($p < 0.01$). *significant at 5% probability ($p < 0.05$).

Table 3. Pearson's correlation coefficient between the analyzed variables: Temperature (Temp °C); micronuclei frequency (MCN); humidity (RH %); elevation (ELV); vehicular traffic flow (Flow).

	Temp	MCN	RH	ELV	Flow
Temp	1	-0.32 ns	-0.56**	-0.29 ns	-0.18 ns
MCN	-0.32 ns	1	0.07 ns	0.35 ns	0.047 *
RH	-0.56**	0.07 ns	1	0.12 ns	0.36 ns
ELV	-0.29 ns	0.35 ns	0.12 ns	1	-0.11 ns
Flow	-0.18 ns	0.047*	0.36 ns	-0.11 ns	1

**significant at 1% probability (p <0.01). *significant at 5% probability (0.01 = < p < 0.05). ns = not significant (p > = 0.05).

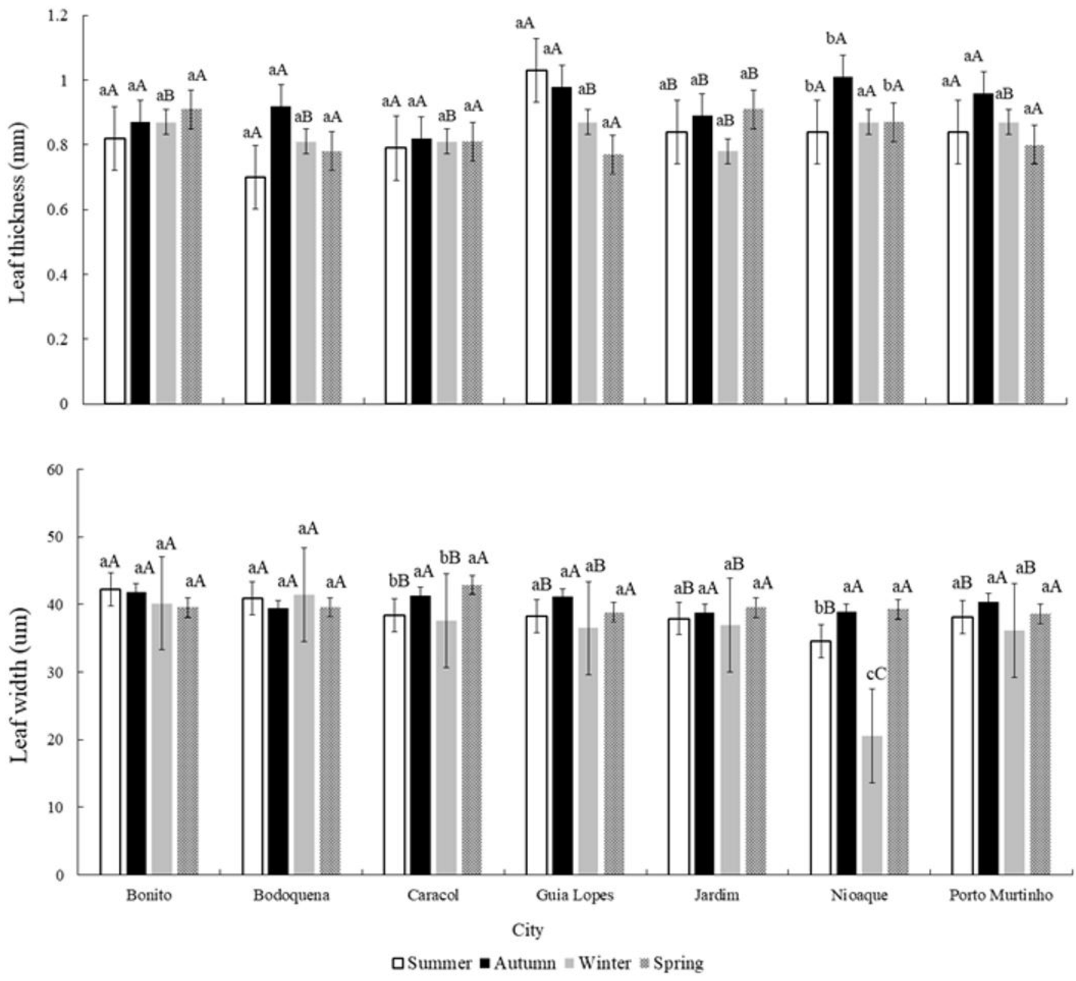

Figure 9. Morphological appearance of the *Tradescantia pallida* leaf. Different lowercase letters indicate statistical differences between the seasons for the same city and uppercase letters indicate difference between cities considering the seasons.

evaluating the environmental quality of fragments of riparian forest in Rio dos Sinos, Rio Grande do Sul, covering the municipalities of Caraá, Taquará and Campo Bom, using botanical, meteorological and genetic parameters, found that in Caraá the riparian forest presented a larger number of trees, taller, with a larger basal area, supporting epiphytic richness, associated with favorable meteorological conditions, with a volume of rainfall above Taquará and Campo Bom, in addition to low air genotoxicity. Thus, the authors observed that the indicators used to assess environmental quality allowed pointing out the Caraá riparian forest as a reference area, evidenced by the richness of epiphytes and the absence of genotoxicity in *T. pallida*. Thus, it appears from the studies presented that the integrated assessment of factors, based on the response of living organisms, should be used in the analyzes and, therefore, the morphoanatomical analyzes are added to the genotoxic ones in the present study.

The parameters that can maximize pollution include weak winds, low elevation, and low levels of relative humidity. In analyzing the seasons, we found that the MCN frequency was higher in the summer and lower in spring. However, the micronuclei frequency in winter was also high, which may have occurred due to lower temperatures which leads to increased humidity, a factor that contributes to the dispersion of pollutants, however, according to Pereira et al. (2013) studies conducted with *T. pallida* in Uberlandia, MG, showed that changes in relative humidity have no clear effect on mutation rates. For Klumpp et al. (2004), the air low relative humidity and high temperatures seem to stimulate the stomata opening, absorption increasing and genotoxic substances transport to the target cells. For riparian forest fragments, no significant relationship between the micronuclei frequency in *T. pallida*, temperature, relative humidity and precipitation was observed and thus, no influence of climatic conditions on the genotoxic response of exposed plants was observed (Savóia et al., 2009; Cassanego et al., 2015). As for relative humidity, the highest micronuclei frequency occurred during winter, being negatively correlated (Pereira et al., 2013). In the present study, the variable that had clear effects on the micronuclei frequency was vehicular traffic, a result in line with several authors (Batalha et al., 1999; Isidori et al., 2003; Klumpp et al., 2006; Savóia et al., 2009; Crispim et al., 2012, 2014; Spósito et al., 2015; Costa et al., 2016; Rocha et al., 2018; Alves et al., 2020).

Regarding the morphoanatomical analysis, the observed results reinforce the idea that the Bodoquena microregion may be a preserved region of Mato Grosso do Sul in terms of air quality. Other authors have found that an increase in the concentration of pollutants caused a decrease in leaf thickness (Eleftheriou, 1987; Evans and Miller, 1995; Alves et al., 2001). In studies conducted by Roman (2015) in the microregion of Dourados and Rocha (2017) in the southwestern mesoregion of Mato Grosso do Sul, the authors found a marked variation in the micronuclei frequency and in the thickness of tissue/structures for cities with greater traffic flow. In the present study, these variations were not evident, which supports the hypothesis of environmental preservation in the Bodoquena microregion. Nevertheless, longer-term studies must be conducted to ensure that morphoanatomical modifications are captured.

Thus, this study sought to demonstrate air quality in the region with a perception of improving the well-being and population's health (SDG 3), and to inform appropriate public policies that will improve the population's quality of life (SDG 11). Thus, in view of the founded results, we can suggest as a path of maintaining the conditions presented here the investment in public transport, which is always productive when thinking about the easiest displacement of people and incentive to cyclomobility policies.

5. Conclusion

In light of the genotoxic and morphoanatomical factors assessed herein, the Bodoquena microregion appears to be well preserved in terms of air quality, presenting low micronuclei frequency and a limited reduction in tissues and leaf structures, regardless of the season.

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