http://dx.doi.org/10.1590/1519-6984.05514 Original Article

Biomonitoring the genotoxic potential of the air on *Tradescantia pallida* var. *purpurea* under climatic conditions in the Sinos River basin, Rio Grande do Sul, Brazil

*M. B. B. Cassanego[a,b], M. H. Sasamori[b], C. T. Petry[b] and A. Droste[a,b]**

[a]Programa de Pós-graduação em Qualidade Ambiental, Universidade Feevale – FEEVALE, Rodovia ERS-239, 2755, CEP 93352-000, Novo Hamburgo, RS, Brazil

[b]Laboratório de Biotecnologia Vegetal, Universidade Feevale – FEEVALE, Rodovia ERS-239, 2755, CEP 93352-000, Novo Hamburgo, RS, Brazil

*e-mail: annette@feevale.br

Received: May 10, 2014 – Accepted: September 11, 2014 – Distributed: November 30, 2015
(With 2 figures)

Abstract

The present study evaluated the genotoxic effects of the atmospheric air on *Tradescantia pallida* var. *purpurea* in urban areas with different intensities of vehicular traffic and in riparian forest fragments in the Sinos River Basin (Rio Grande do Sul, Brazil), considering the influence of climatic conditions prevailing in these environments. Bimonthly, from May 2012 to March 2013, cuttings with flower buds were exposed for 8 h in urban and riparian forest environments in the municipalities of Caraá, Taquara and Campo Bom in the upper, middle and lower sections, respectively, of the Sinos River Basin. Simultaneously, negative controls were made and climatic data were recorded. Micronuclei (MCN) frequencies were determined in young tetrads of pollen mother cells and expressed as MCN/100 tetrads. Significantly higher MCN frequencies were observed in buds exposed in urban and riparian forest environments in Taquara (up to 7.23 and 4.80, respectively) and Campo Bom (up to 4.90 and 4.23, respectively) than in buds exposed in Caraá (up to 2.90 and 2.50, respectively), in the majority of samplings, and in relation to the negative control (up to 1.93) in all months. Over the course of the period monitored, there were significant variations in MCN frequencies at all sampling points, with the exception of the urban environment in Caraá. For the urban environments, relation between the MCN frequency, vehicular traffic and mean temperature was observed. For the riparian forest fragments, there was no association between MCN frequency and climatic factors. *Tradescantia pallida* var. *purpurea* can be considered a useful tool to point out areas with increased atmospheric pollution, since the exposure of plants under severe climatic conditions is avoided to minimize their negative influence on the formation of micronuclei.

Keywords: abiotic factors, vehicular emissions, bioindicator, environmental quality, micronucleus.

Biomonitoramento do potencial genotóxico do ar em *Tradescantia pallida* var. *purpurea* sob condições climáticas na bacia do Rio dos Sinos, Rio Grande do Sul, Brasil

Resumo

O presente estudo avaliou os efeitos genotóxicos do ar atmosférico sobre *Tradescantia pallida* var. *purpurea* em áreas urbanas com diferentes intensidades de tráfego veicular e em fragmentos de mata ciliar na Bacia do Rio dos Sinos (Rio Grande do Sul, Brasil), considerando a influência de condições climáticas prevalecentes nesses ambientes. Bimensalmente, de maio de 2012 a março de 2013, ramos com botões florais foram expostos por 8 h em ambientes urbanos e de mata ciliar nos municípios de Caraá, Taquara e Campo Bom nos trechos superior, médio e inferior, respectivamente, da Bacia do Rio dos Sinos. Simultaneamente, foram realizados controles negativos e levantados dados climáticos. Frequências de micronúcleos (MCN) foram determinadas em tétrades jovens de células-mãe de grãos de pólen e expressas como MCN/100 tétrades. Frequências de MCN significativamente superiores foram observadas em botões expostos nos ambientes urbanos e de matas ciliares em Taquara (até 7,23 e 4,80, respectivamente) e Campo Bom (até 4,90 e 4,23, respectivamente) do que em botões expostos em Caraá (até 2,90 e 2,50, respectivamente), na maioria das amostragens, e em relação ao controle negativo (até 1,93) em todos os meses. Ao longo do período monitorado, ocorreram variações significativas nas frequências de MCN em todos os pontos amostrais, com exceção do ambiente urbano de Caraá. Para os ambientes urbanos, foi observada relação entre a frequência de MCN, tráfego veicular e temperatura média. Para os fragmentos de mata ciliar, não houve associação entre a frequência de MCN e fatores climáticos. *Tradescantia pallida* var. *purpurea* pode ser considerada uma ferramenta útil para apontar áreas com poluição atmosférica aumentada, desde que, sob condições climáticas severas, a exposição das plantas seja evitada, para minimizar o efeito destas sobre a formação de micronúcleos.

Palavras-chave: fatores abióticos, emissões veiculares, bioindicador, qualidade ambiental, micronúcleo.

1. Introduction

High demographic densities, industrialization and intense vehicle traffic are the primary causes of degradation of air quality. Atmospheric air pollution is primarily the result of emissions of gases and particulate material, causing negative effects on living organisms and the environment (Savóia et al., 2009; Teixeira et al., 2012). Among air pollutants, mobile sources comprising motorized vehicle traffic, and stationary sources which include industries, are noteworthy. The major atmospheric toxic substances released by mobile and stationary sources are carbon dioxide, carbon monoxide, sulfur oxides, nitrogen oxides, ozone, particulate material, volatile organic compounds, and polycyclic aromatic hydrocarbons (Rocha et al., 2004; Teixeira et al., 2012).

The association of atmospheric pollutants and climatic conditions may cause significant effects on the ecosystem, due to various synergistic or antagonistic interactions (Bytnerowicz et al., 2007). Further, atmospheric emissions remain not restricted to this compartment because of pollutant volatilization, dispersal and deposition mechanisms (Jahnke et al., 2007; Merlo et al., 2011). Despite this, the majority of risk assessment studies of atmospheric pollutants are based on concentrations detected by chemical analyses for simple substances and the synergic and genotoxic effects of complex mixtures of these compounds are still poorly known (Savóia et al., 2009; Merlo et al., 2011). Over recent years, studies have begun to integrate biological indicators with physical and chemical parameters, with the goal of identifying genetic changes that may occur in organisms as a result of the environmental conditions in which they live or after exposure as active indicators in environments that are supposedly polluted (Meireles et al., 2009; Savóia et al., 2009; Carreras et al., 2013; Pereira et al., 2013).

Tradescantia pallida (Rose) D.R. Hunt. var. *purpurea* Boom presents high sensitivity to genotoxic agents and has been used in biomonitoring of atmospheric air quality in urban environments with different intensities of vehicular traffic, in industrial areas, and in rural environments with preserved vegetation (Meireles et al., 2009; Costa and Droste, 2012; Pereira et al., 2013). The influence of the combination of climatic factors with atmospheric pollutants on the response of *T. pallida* var. *purpurea* has been investigated, warning of the possible interference of certain weather conditions in micronuclei formation (Meireles et al., 2009; Savóia et al., 2009; Pereira et al., 2013).

The Sinos River Basin, in the State of Rio Grande do Sul, Brazil, has undergone a high degree of ecosystem degradation (FEPAM, 2013) since 94.02% of the inhabitants live in urban areas (IBGE, 2013). Increasing urbanization and industrialization especially of municipalities that are part of the Porto Alegre metropolitan area has caused degradation and fragmentation of forests, to the extent that only a forestry mosaic interspersed with urban areas remains (Teixeira et al., 1986; Figueiredo et al., 2010). Therefore, the forest remnants that are left in the basin are vulnerable to atmospheric pollutants mainly released by industry and intense vehicle traffic (Costa and Droste, 2012).

The present study evaluated the genotoxic effects of the atmospheric air on *Tradescantia pallida* var. *purpurea* in urban areas with different degrees of vehicular traffic and in riparian forest fragments in the Sinos River Basin, considering the influence of climatic conditions prevailing in these environments.

2. Material and Methods

2.1. Study area

The Sinos River Basin is located in the East of the State of Rio Grande do Sul, Brazil (Figure 1), between coordinates 29º 20' to 30º 10' S and 50º 15' to 51º 20' W, occupying an area of 3,800 km^2 (COMITESINOS, 2013). The basin comprises 32 municipalities, with a population of around 300 inhabitants/km^2 (IBGE, 2013). The Sinos River is the main watercourse in the basin. It has a length of 190 km, supplying water to a population of approximately 1.5 million people, and is divided into thirds for the purposes of hydrological classification (FEPAM, 2013). The Sinos River Basin region has a Cfa climate, according to the Köppen classification, since it is subtropical (C), humid all year round (f) and the mean temperature of the hottest month is higher than 22 °C (a). Rainfall is regularly distributed throughout the year (Moreno, 1961; Buriol et al., 2007). The basin's vegetative cover comprises remnants of dense and mixed ombrophylous forest specifically located around the springs of the Sinos River (Backes, 2012; Becker et al., 2013), and seasonal semideciduous forest fragments, mostly located along the margins of the river and its tributaries (Teixeira et al., 1986; Schmitt and Goetz, 2010), belonging to the Atlantic Forest biome.

2.2. Sampling points

The study was conducted in urban environments and in riparian forest fragments in Caraá, Taquara and Campo Bom, which are municipalities located in the upper, middle and lower sections of the basin, respectively (Figure 1, Table 1). Monitoring was conducted bimonthly from May 2012 to March 2013. The population of the Caraá municipality is 7,742 inhabitants, distributed across an area of 294,323 km^2, with a demographic density of 24.84 inhabitants/km^2, primarily living in the rural area (IBGE, 2013). The municipality's economy is predominantly agricultural, producing primary cultures of sugar cane, maize, beans and rice, plus market gardening and poultry farming (FEE, 2013). The fleet in Caraá totals 2,262 vehicles (IBGE, 2013), and vehicle traffic on the state RS 030 and federal BR 290 highways passes at a distance of 10 and 12.5 km, respectively, from the urban sampling point in the municipality. The riparian forest of Caraá comprises an environmental protection area (Backes, 2012). The municipalities of Taquara and Campo Bom are both within the metropolitan area of Porto Alegre (PROSINOS, 2013), which is the capital city of Rio Grande do Sul. The population of Taquara is 56,896 inhabitants, distributed across an area of 457,855 km^2, with a demographic

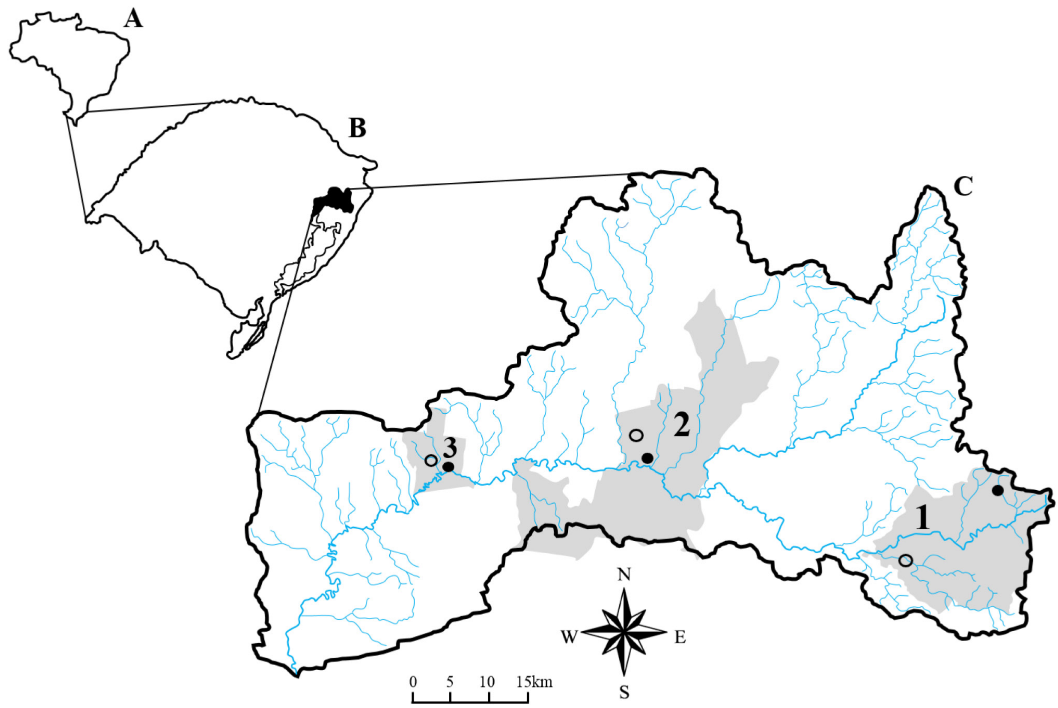

Figure 1. Location of the sampling points in urban environments (○) and riparian forest fragments (●) in the municipalities of Caraá (1), Taquara (2) and Campo Bom (3) in the Sinos River Basin (C), Rio Grande do Sul (B), Brazil (A).

Table 1. Location and characterization of sampling points in the Sinos River Basin (Rio Grande do Sul, Brazil).

Municipality	Sampling point	Coordinates	Location	Distance (km) between*	
				Points	Municipalities
Caraá	Urban environment	29º 47’ 32.1” S 50º 26’ 04.9” W alt. 42 m	São Cristovão church square, town center	16.82	
	Riparian forest fragment	29º 42’ 25.0” S 50º 17’ 27.8” W alt. 424 m	Area around the source of São Miguel stream Area: ≈ 8,932 ha 50 m inside the forest		37.4
Taquara	Urban environment	29º 38’ 59.8” S 50º 47’ 00.4” W alt. 29 m	Bandeira square, town center	3.84	
	Riparian forest fragment	29º 40’ 46.8” S 50º 45’ 57.0” W alt. 20 m	Area around falls on Tucano stream Area: ≈ 2.60 ha 50 m inside the forest		26.8
Campo Bom	Urban environment	29º 40’ 46.6” S 51º 03’ 29.5” W alt. 25 m	João Blos square, town center	2.40	
	Riparian forest fragment	29º 41’ 23.1” S 51º 02’ 06.0” W alt. 11 m	Area around falls on Schmidt stream Area: ≈ 3.65 ha 50 m inside the forest		

*Straight-line distance measured on Google Earth.

density of 119.35 inhabitants/km^2, primarily living in the urban area (IBGE, 2013). The municipality’s economy is based on footwear manufacturers, metalworking plants and small-scale livestock and agricultural farming (FEE, 2013).

Taquara has a fleet of 28,469 vehicles (IBGE, 2013), and intense vehicle traffic on state highways RS 020, RS 115 and RS 239 passes through the urban perimeter at distances of approximately 0.7, 1.16 and 1.06 km respectively from the urban sampling point. The population of Campo Bom is 63,339 inhabitants, distributed across an area of 60,510 km^2, with a demographic density of 992.79 inhabitants/km^2, primarily living in the urban area (IBGE, 2013). The leather, footwear and metalworking industries are the mainstay of the municipality's economy (FEE, 2013). Campo Bom has a fleet of 34,493 vehicles (IBGE, 2013), and intense vehicle traffic on state highway RS 239 and federal highway BR 116 passes 3.38 and 8.72 km respectively from the urban sampling point.

2.3. Vehicular traffic and climatic data recording

Bimonthly, from May 2012 to March 2013, at each day and sampling point of *Tradescantia pallida* var. *purpurea* exposure in the urban environments, the number of vehicles in circulation was counted for one hour. There is no vehicular traffic along the riparian forest fragments. Simultaneously, microclimatic data were recorded for each sampling point of the urban environments and the forest fragments, including temperature and relative air humidity at three times of day (9:00 am, 1:00 pm and 5:00 pm) using a thermohygroanemometer (Instrutherm®, Thal-300). Data on accumulated rainfall on the day of exposure plus three days preceding the day of exposure were obtained using a mobile meteorological station (Davis®) for Caraá, from the civil defense agency (Rio Grande do Sul, 2013) for Taquara, and from the Meteorological Station no. 83961(29º 41' S; 51º 03' W) for Campo Bom.

2.4. Tradescantia pallida var. purpurea bioassay

Tradescantia pallida var. *purpurea* plants were grown in pots (37 cm × 20 cm × 20 cm) containing 4 kg of commercial soil from the same batch, at the university campus following the method described by Thewes et al. (2011). The plants were watered three times a week and fertilized with 100 mL of an N-P-K solution (10-10-10 w/w/w) once a month. All of the plants were derived from vegetative propagation.

Air genotoxicity tests were conducted bimonthly, as follows: 20 cuttings (10 to 15 cm long) with flower buds were collected from the plants cultivated at the campus and partially immersed in vessels containing 2 L of distilled water (Cassanego et al., 2014) and left for 24 h to adapt in a climate-controlled room. The vessels containing the adapted cuttings were then transported in cool boxes to the sampling points, where they were exposed for 8 h (9:00 am to 5:00 pm) in urban environment and riparian forest fragment in each municipality. The cuttings were then recovered for 24 h in distilled water (Cassanego et al., 2014) in a climate-controlled room. Negative controls were set up simultaneously in a climate-controlled room, following the same methodology.

2.5. Inflorescence fixation, slide preparation and micronuclei score

The flower buds were fixed in ethanol:acetic acid (3:1 v:v) for 24 h and then stored in 70% ethanol under refrigeration (4 °C). Slide preparation and analysis of cells and micronuclei (MCN) were as follows: flower buds were dissected and the anthers were squashed in 1% acetocarmine stain. Three hundred cells at the tetrad phase were counted per slide and the number of micronuclei was recorded, for a total of 10 slides per point per sampling period, using an optical microscope at 400x magnification (Olympus CX4). Micronuclei frequencies were expressed as MCN/100 tetrads (Thewes et al., 2011).

2.6. Statistical analyses

The MCN frequencies obtained in the bioassays were subjected to the Shapiro-Wilk test of normality. Analysis of variance (ANOVA) was then conducted and differences between means were subjected to the Tukey test. Mean MCN frequencies were compared for the urban environment and the riparian forest in each municipality and period using Student's *t* test. A multiple linear regression analysis was performed to test for relation between MCN frequency, vehicular traffic and climatic variables (mean temperature, mean relative air humidity, and accumulated rainfall) in the urban environment (tree sampling points; six exposures: n=18). Data were analyzed through the stepwise method and the regression curve was adjusted so that only the factors that significantly contributed to the MCN variances were maintained in the model. The Pearson correlation coefficient was used to analyze the association of MCN frequency in the riparian forest and climatic variables (tree sampling points; six exposures: n=18). All statistical analyses were conducted using SPSS version 20, and the significance level was set on 5%.

3. Results

Comparison between data for urban environments and negative controls showed that all samples of flower buds exposed to the air in Taquara and Campo Bom and samples exposed in Caraá during May 2012 and January and March 2013 had significantly higher MCN frequencies than were observed in controls. Buds exposed in Taquara had significantly higher MCN frequencies than were observed in Campo Bom in November 2012 and January and March 2013. On the other hand, in July of 2012, the frequency recorded for Taquara was significantly lower than observed for Campo Bom, and no statistical differences between the MCN frequencies recorded for Taquara and Campo Bom were observed in May and September 2012. The MCN frequencies recorded for Caraá were significantly lower than observed for Taquara and Campo Bom, with the exception of July 2012, when they did not differ statistically from those in Taquara, and January and March of 2013 with relation to Campo Bom. Over the course of the months sampled, there was significant variation in MCN frequencies observed in flower buds exposed at sampling points in Taquara and Campo Bom and in negative controls (Table 2).

For riparian forest fragments and negative controls, the comparison showed that all samples of buds exposed in Taquara exhibited significantly higher MCN frequencies than were observed for Caraá and in controls. Buds exposed to the air in Campo Bom also exhibited frequencies significantly higher than were observed for Caraá (with the exception of July 2012 and March 2013) and in negative controls for the whole period. In Taquara, MCN frequencies were significantly higher than observed for Campo Bom, in January and March 2013. Over the course of the months sampled, there was a significant variation in the MCN frequencies observed in flower buds exposed in sampling points and in negative controls (Table 2).

When the two types of environment in each municipality were compared, flower buds exposed in the urban environment in Caraá exhibited significantly higher MCN frequencies in relation to buds exposed in the riparian forest for May (t=–2.623; p=0.017) and September 2012 (t=–2.690; p=0.015) and for January 2013 (t=–2.481; p=0.023). In Taquara, the MCN frequencies observed in buds exposed in the urban environment were statistically higher than for riparian forest samples from November 2012 (t=–3.523; p=0.002) and January 2013 (t=–2.427; p=0.026). In Campo Bom, the MCN frequencies observed in samples from the urban environment were also significantly higher than for riparian forest samples from May (t=–2.332; p=0.032) and July 2012 (t=–3.805; p=0.001) (Table 2).

The number of vehicles/hour in movement in the urban sampling points at the days of exposure of *Tradescantia pallida* var. *purpurea* varied from 225 to 308 in Caraá, from 720 to 1438 in Taquara and from 505 to 900 in Campo Bom (Figure 2). The climatic data recorded at the same points and days were typically of Cfa climate that is characteristic for the Sinos River Basin (Table 3).

For urban environments, multiple linear regression indicated a relation between MCN frequency, vehicular traffic and mean temperature. The analysis model showed that the movement of vehicles was responsible for 93.8% of the coefficient of determination (r^2) (F=256.374, p<0.001) while the mean temperature added 2.7% to this coefficient (r^2=0,965; F=232.507, p<0.001). Therefore, the MCN frequency was predicted by the linear combination of these two variables, with the equation: MCN=0.489+0.003vehicular traffic+0.044mean temperature. For riparian forest fragments, there was no association between MCN frequency, mean temperature (r=0.438; p=0.690), mean humidity (r=–0.372; p=0.129) and accumulated rainfall (r=0.174; p=0.490).

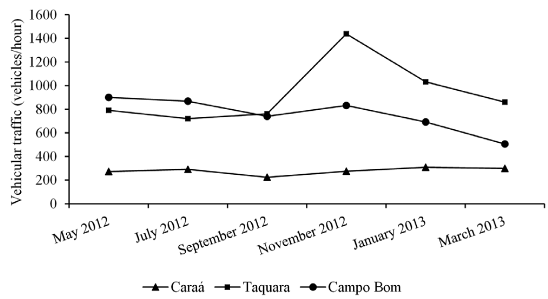

Figure 2. Number of vehicles per hour in circulation registered at each day and sampling point of *Tradescantia pallida* var. *purpurea* exposure in urban environments, from May 2012 to March 2013.

Table 2. Micronuclei frequencies in *Tradescantia pallida* var. *purpurea* flower buds exposed to the air in urban environments and riparian forest fragments in the municipalities of Caraá, Taquara and Campo Bom (Rio Grande do Sul, Brazil) and in negative controls, from May 2012 to March 2013.

Urban environment								
Municipality	**MCN frequency (mean ± standard deviation)**						**F**	**p**
	May 2012	**July 2012**	**September 2012**	**November 2012**	**January 2013**	**March 2013**		
Caraá	2.53 ± 0.85bA*	2.73 ± 0.75bcA	2.13 ± 0.57bA*	2.53 ± 0.50cA	2.90 ± 0.94bA*	2.83 ± 0.42bA	1.589	0.179
Taquara	3.97 ± 0.87aB	3.63 ± 0.89bB	3.67 ± 0.94aB	7.23 ± 2.08aA*	5.20 ± 0.92aB*	4.33 ± 1.02aB	13.205	<0.001
Campo Bom	4.90 ± 1.16aA*	4.73 ± 0.84aA*	4.03 ± 1.13aAB	4.53 ± 0.85bA	3.80 ± 1.24bAB	2.80 ± 0.65bB	5.991	<0.001
Control	1.33 ± 0.38cB	1.93 ± 0.66cA	1.60 ± 0.26bAB	1.57 ± 0.22cAB	1.53 ± 0.48cAB	1.33 ± 0.35cB	2.766	0.027
F	33.132	23.050	21.626	46.692	27.172	34.016		
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		
Riparian forest								
Municipality	**MCN frequency (mean ± standard deviation)**						**F**	**p**
	May 2012	**July 2012**	**September 2012**	**November 2012**	**January 2013**	**March 2013**		
Caraá	1.66 ± 0.61bBC	2.50 ± 0.82bcA	1.50 ± 0.47bC	2.26 ± 0.64bABC	2.10 ± 0.38cABC	2.43 ± 0.61bAB	4.587	0.001
Taquara	3.73 ± 0.89aBC	3.50 ± 0.80aBC	2.97 ± 0.55aC	4.80 ± 0.65aA	4.23 ± 0.86aAB	3.60 ± 0.94aBC	6.382	<0.001
Campo Bom	3.63 ± 1.26aAB	3.37 ± 0.76abAB	3.47 ± 0.83aAB	4.23 ± 1.30aA	3.37 ± 0.46bAB	2.60 ± 0.64bB	3.211	0.013
Control	1.33 ± 0.38bB	1.93 ± 0.66cA	1.60 ± 0.26bAB	1.57 ± 0.22bAB	1.53 ± 0.48cAB	1.33 ± 0.35cB	2.766	0.027
F	22.233	9.402	29.795	37.170	44.905	19.216		
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001		

Means followed by different lower case letters in the same column and means followed by different upper case letters in the same row differ significantly according to the Tukey test (p=0.05). Asterisks (*) indicate significant difference between the urban environment and riparian forest fragment in each municipality according to Student's *t* test (p=0.05).

Table 3. Climatic data recorded during the period of *Tradescantia pallida* var. *purpurea* exposure in the municipalities of Caraá, Taquara and Campo Bom (Rio Grande do Sul, Brazil) from May 2012 to March 2013.

Month/Year	Temperature* (°C)		Relative air humidity* (%)		Rainfall** (mm)
	Urban environment	Riparian forest fragment	Urban environment	Riparian forest fragment	Municipality
Caraá					
May 2012	22.0	19.4	61.5	69.9	0.0
July 2012	19.7	19.6	77.1	70.7	38.6
September 2012	19.6	19.3	58.3	60.3	0.3
November 2012	25.0	23.2	50.4	66.1	19.3
January 2013	28.0	27.7	61.3	66.1	0.0
March 2013	29.0	25.2	61.7	71.0	10.2
Taquara					
May 2012	17.8	17.3	56.8	59.5	20.0
July 2012	16.6	14.5	50.9	59.5	9.0
September 2012	17.7	17.6	55.5	58.8	2.0
November 2012	34.3	31.9	31.9	53.8	1.0
January 2013	28.3	26.9	63.4	70.4	40.0
March 2013	29.4	29.0	53.3	55.8	58.0
Campo Bom					
May 2012	26.6	25.6	46.2	57.8	0.0
July 2012	26.3	23.5	49.3	63.1	0.3
September 2012	24.2	23.4	71.8	73.2	106.8
November 2012	29.9	30.2	52.7	55.2	0.0
January 2013	30.9	30.4	58.0	63.4	52.9
March 2013	21.5	23.7	76.9	73.3	35.0

*Mean of three readings for temperature and relative air humidity during each exposure. **Accumulated rainfall on the day of exposure plus three days preceding the day of exposure.

4. Discussion

The *Tradescantia pallida* var. *purpurea* bioassay evidenced the genotoxic response of the plants to atmospheric air conditions in urban environments and in riparian forest fragments in the Sinos River Basin, since the flower buds exposed at the sampling points exhibited significant increase in MCN frequencies, compared with the negative controls. Frequencies obtained for negative controls remained below the background rate of up to 2.0 MCN/100 tetrads considered by Pereira et al. (2013) as the result of spontaneous mutations that can occur naturally in the species, even when plants are grown in environments free from interference of any type of pollutant. In order to minimize any environmental interference before exposure, it is important that plants be allowed to adapt in controlled conditions (Ma et al., 1994; Klumpp et al., 2004). This procedure was employed in this study, following Cassanego et al. (2014).

For urban environments, a relation between MCN frequencies, vehicular traffic and mean temperature was observed. The number of vehicles circulating at the sampling points in Taquara and Campo Bom, which was respectively 3.4 and 2.7 times higher than recorded for Caraá, was the main factor influencing the MCN frequencies in these municipalities. In Caraá, in general, the lower number of vehicles at the urban point was accompanied by lower MCN frequencies. These differences between Caraá and both Taquara and Campo Bom could be associated, at least partially, to a variety of potentially genotoxic substances present in the atmospheric air. While Caraá has a low demographic density, with an almost entirely rural population (IBGE, 2013) and lower anthropic impact, Taquara and Campo Bom are within the Porto Alegre metropolitan area, which is characterized by significant emission of atmospheric pollutants, from vehicle traffic and stationary sources such as steelworks, oil refineries and industrial complexes (Teixeira et al., 2008, 2012). The state highway RS 239 that passes through urban perimeters of Taquara and Campo Bom is characterized by intense vehicle traffic (≈ 626,000 vehicles/month) (EGR, 2013). Moreover, in

Taquara, the vehicular traffic on state highways RS 020 and RS 115 may be contributing to the MCN frequencies found. In the lower section of the Sinos River Basin, the federal BR-116 highway has an intense vehicular flow of approximately 150 thousand vehicles per day (Migliavacca et al., 2012), being the major link between Porto Alegre and the municipalities of the metropolitan area. Atmospheric pollutants including carbon monoxide, sulfur dioxide, ozone, oxides of nitrogen, hydrocarbons, polycyclic aromatic hydrocarbons, particulate material, heavy metals and metals oxides have been detected in Porto Alegre and Canoas, Esteio and Sapucaia do Sul, municipalities integrating the lower section of the basin (Teixeira et al., 2012).

To a lesser extent, the temperature also influenced MCN frequencies in urban environments, showing that extreme conditions of this climatic factor may induce spontaneous mutation rates. In most months, the temperature remained within the historical range of mean temperatures recorded for the region of the Sinos River Basin (Moreno, 1961; Buriol et al., 2007). However, in November 2012, the high temperature recorded in the urban environment of Taquara (mean of 34.3 °C) may have contributed to the increased MCN frequency. Temperature from 33 °C on under controlled conditions was reported as increasing spontaneous mutation rate of *Tradescantia* clone 4430 by Klumpp et al. (2004). Air pollutants and stressful climatic conditions caused effects on the response of *Tradescantia pallida* var. *purpurea* plants exposed in urban areas of the municipality of Santo André (São Paulo, Brazil), since the combination of temperature, relative humidity and pollutants predicted 17 and 26% of the MCN frequency in two exposure sites around downtown (Savóia et al., 2009). Pereira et al. (2013) showed that 88.01% of the MCN frequency was predicted by the combination of the number of vehicles and the relative humidity in the municipality of Uberlândia (Minas Gerais, Brazil), with the first variable contributing 83.92%. It is noteworthy that the climate in Rio Grande do Sul is humid all year long (Buriol et al., 2007), differently from the southeast region of Brazil where the other studies were conducted. This may be related to the fact that there was no relation between the MCN frequency and humidity.

The MCN frequencies observed in *Tradescantia pallida* var. *purpurea* plants exposed in urban environments corroborate data reported by Costa and Droste (2012), who observed MCN frequencies from 3.26 to 8.13 in Estância Velha, a municipality with leader and footwear industrial activity in the lower section of the Sinos River Basin. Savóia et al. (2009) recorded MCN frequencies of up to 4.6 in the municipality of Santo André (São Paulo), Meireles et al. (2009) observed up to 2.1 in Feira de Santana (Bahia) and Pereira et al. (2013) observed up to 5.0 MCN in Uberlândia (Minas Gerais). In an urban area with intense vehicle traffic in the Argentinean municipality of Córdoba Carreras et al. (2009) recorded 3.6 MCN, and genotoxicity was attributed to polycyclic aromatic hydrocarbons in atmospheric air (Carreras et al., 2013).

For riparian forest fragments, no significant relation between *Tradescantia pallida* var. *purpurea* MCN frequencies, temperature, relative humidity and precipitation was observed. Similarly, no influence of climatic conditions on the genotoxic response of *T. pallida* var. *purpurea* plants exposed in a natural park located far from the urban area of Santo André was reported by Savóia et al. (2009). The findings evidence that climatic conditions probably will not interfere in MCN frequencies in forest fragments as they are less extreme than in urban areas.

The low frequencies observed in plants exposed to the riparian forest fragment in Caraá, comparable to those of the negative controls, indicated less influence from genotoxic factors. This riparian fragment constitutes an environmental protection area still slightly impacted (Becker et al., 2013) contributing to a better quality of the air. On the other hand, the significant MCN frequencies recorded for Taquara and Campo Bom may be the result of the high degree to which the vegetation that borders the Sinos River has been degraded, particularly in the middle and lower sections of the river's basin (PROSINOS, 2013). Additionally, the differences between MCN frequencies may be related to the distance between the riparian forest fragments and the respective urban environments. In Caraá, this distance is four times greater than in both municipalities of Taquara and Campo Bom, diminishing the influence of the urban atmospheric emissions on this forest fragment.

This study focused on the influence of the atmospheric conditions on *Tradescantia pallida* var. *purpurea*, taking into account the synergic effect of pollutants and climatic variables. The bioindicator employed exhibited significant responses that allow for inferences about the genotoxic risks to which the organisms have been exposed in the Sinos River Basin. Considering the results and the environmental conditions prevailing in the basin, the increase of genetic damage in plants exposed to the air in urban environments, especially in the middle and lower sections of the basin, may be related mainly to the emission of atmospheric pollutants by vehicular traffic. The low MCN frequencies recorded in flower buds exposed to the air in the riparian forest fragment of Caraá indicate that this environment may still be referred to as a reference site. *Tradescantia pallida* var. *purpurea* can be considered a useful tool to point out areas with increased atmospheric pollution, since the exposure of plants under severe climatic conditions is avoided to minimize their negative influence on the formation of micronuclei.

Acknowledgements

The authors wish to thank: the Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS) for financial support (Edital FAPERGS 004/2012-PqG, Processo SPI nº 1989 12-8); the Universidade Feevale for the use of the laboratory and the grant awarded to C. T. Petry; the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the doctoral grant awarded to M. B. B. Cassanego; the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the grant awarded to M. H. Sasamori, and D. Endres Júnior for editing Figure 1

References

BACKES, A., 2012. Áreas protegidas no Estado do Rio Grande do Sul: o esforço para a conservação. *Pesquisas Botânica*, no. 63, pp. 225-356.

BECKER, D.F.P., CUNHA, S., GOETZ, M.N.B., KIELING-RUBIO, M.A. and SCHMITT, J.L., 2013. Florística de samambaias e licófitas em fragmento florestal da Bacia Hidrográfica do Rio dos Sinos, Caraá, RS, Brasil. *Pesquisas Botânica*, vol. 64, pp. 273-284.

BURIOL, G.A., ESTEFANEL, V., CHAGAS, A.C. and EBERHARDT, D., 2007. Clima e vegetação natural do Estado do Rio Grande do Sul segundo o diagrama climático de Walter e Lieth. *Ciência Florestal*, vol. 17, no. 2, pp. 91-100.

BYTNEROWICZ, A., OMASA, K. and PAOLETTI, E., 2007. Integrated effects of air pollution and climate change on forests: a northern hemisphere perspective. *Environmental Pollution*, vol. 147, no. 3, pp. 438-445. http://dx.doi.org/10.1016/j.envpol.2006.08.028. PMid:17034915.

CARRERAS, H.A., CALDERÓN-SEGURA, M.E., GÓMEZ-ARROYO, S., MURILLO-TOVAR, M.A. and AMADOR-MUÑOZ, O., 2013. Composition and mutagenicity of PAHs associated with urban airborne particles in Córdoba, Argentina. *Environmental Pollution*, vol. 178, pp. 403-410. http://dx.doi.org/10.1016/j.envpol.2013.03.016. PMid:23624338.

CARRERAS, H.A., RODRIGUEZ, J.H., GONZALEZ, C.M., WANNAZ, E.D., FERREYRA, F.G., PEREZ, C.A. and PIGNATA, M.L., 2009. Assessment of the relationship between total suspended particles and the response of two biological indicators transplanted to an urban area in central Argentina. *Atmospheric Environment*, vol. 43, no. 18, pp. 2944-2949. http://dx.doi.org/10.1016/j.atmosenv.2009.02.060.

CASSANEGO, M.B.B., COSTA, G.M., SASAMORI, M.H., ENDRES JÚNIOR, D., PETRY, C.T. and DROSTE, A., 2014. The *Tradescantia pallida* var. *purpurea* active bioassay for water monitoring: evaluating and comparing methodological conditions. *Revista Ambiente & Água*, vol. 9, no. 3, pp. 424-433.

COMITÊ DE GERENCIAMENTO DA BACIA HIDROGRÁFICA DO RIO DOS SINOS – COMITESINOS [online], 2013 [viewed 14 July 2013]. Available from: http://www.comitesinos.com.br

COSTA, G.M. and DROSTE, A., 2012. Genotoxicity on *Tradescantia pallida* var. *purpurea* plants exposed to urban and rural environments in the metropolitan area of Porto Alegre, Southern Brazil. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 72, no. 4, pp. 801-806. http://dx.doi.org/10.1590/S1519-69842012000500004. PMid:23295507.

EMPRESA GAÚCHA DE RODOVIAS – EGR, 2013 [viewed 2 September 2013]. Available from: http://www.egr.rs.gov.br/conteudo/1716/volume-de-trafego

FIGUEIREDO, J.A.S., DRUMM, E., RODRIGUES, M.A. and SPILKI, F.R., 2010. The Rio dos Sinos watershed: an economic and social space and its interface with environmental status. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 70, no. 4, suppl., pp. 1131-1136. PMid:21225153.

FUNDAÇÃO DE ECONOMIA E ESTATÍSTICA DO ESTADO DO RIO GRANDE DO SUL – FEE, 2013 [viewed 14 May 2013]. Available from: http://www.fee.tche.br/sitefee/pt/content/resumo/pg_municipios_detalhe.php/

FUNDAÇÃO ESTADUAL DE PROTEÇÃO AMBIENTAL HENRIQUE LUIZ ROESSLER DO ESTADO DO RIO GRANDE DO SUL – FEPAM, 2013 [viewed 30 June 2013]. Available from: http://www.fepam.rs.gov.br/

INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA – IBGE [online], 2013 [viewed 14 May 2013]. Available from: <http://www.ibge.gov.br/cidadesat/topwindow.htm?1

JAHNKE, A., AHRENS, L., EBINGHAUS, R. and TEMME, C., 2007. Urban *versus* remote air concentrations of fluorotelomer alcohols and other polyfluorinated alkyl substances in Germany. *Environmental Science & Technology*, vol. 41, no. 3, pp. 745-752. http://dx.doi.org/10.1021/es0619861. PMid:17328178.

KLUMPP, A., ANSEL, W., FOMIN, A., SCHNIRRING, S. and PICKL, C., 2004. Influence of climatic conditions on the mutations in pollen mother cells of *Tradescantia* clone 4430 and implications for the Trad-MCN bioassay protocol. *Hereditas*, vol. 141, no. 2, pp. 142-148. http://dx.doi.org/10.1111/j.1601-5223.2004.01806.x. PMid:15660975.

MA, T.H., CABRERA, G.L., CHEN, R., GILL, B.S., SANDHU, S.S., VANDENBERG, A.L. and SALAMONE, M.F., 1994. *Tradescantia* micronucleus bioassay. *Mutation Research*, vol. 310, no. 2, pp. 221-230. http://dx.doi.org/10.1016/0027-5107(94)90115-5. PMid:7523893.

MEIRELES, J., ROCHA, R., COSTA NETO, A. and CERQUEIRA, E., 2009. Genotoxic effects of vehicle traffic pollution as evaluated by micronuclei test in *Tradescantia* (Trad-MCN). *Mutation Research*, vol. 675, no. 1-2, pp. 46-50. http://dx.doi.org/10.1016/j.mrgentox.2009.02.005. PMid:19386247.

MERLO, C., ABRIL, A., AMÉ, M.V., ARGUELLO, G.A., CARRERAS, H.A., CHIAPPERO, M.S., HUED, A.C., WANNAZ, E., GALANTI, L.N., MONFERRÁN, M.V., GONZÁLEZ, C.M. and SOLÍS, V.M., 2011. Integral assessment of pollution in the Suquía River (Córdoba, Argentina) as a contribution to lotic ecosystem restoration programs. *The Science of the Total Environment*, vol. 409, no. 23, pp. 5034-5045. http://dx.doi.org/10.1016/j.scitotenv.2011.08.037. PMid:21925711.

MIGLIAVACCA, D.M., TEIXEIRA, E.C. and RODRIGUEZ, M.T.R., 2012. Composição química da precipitação úmida da região metropolitana de Porto Alegre, Brasil, 2005-2007. *Quimica Nova*, vol. 35, no. 6, pp. 1075-1083. http://dx.doi.org/10.1590/S0100-40422012000600002.

MORENO, J.A., 1961. *Clima do Rio Grande do Sul*. Porto Alegre: Governo do Estado do Rio Grande do Sul, Secretaria da Agricultura. 42 p.

PEREIRA, B.B., CAMPOS-JÚNIOR, E.O. and MORELLI, S., 2013. *In situ* biomonitoring of the genotoxic effects of vehicular pollution in Uberlândia, Brazil, using a *Tradescantia* micronucleus assay. *Ecotoxicology and Environmental Safety*, vol. 87, pp. 17-22. http://dx.doi.org/10.1016/j.ecoenv.2012.10.003. PMid:23116623.

PROGRAMA DE EDUCAÇÃO AMBIENTAL – PROSINOS [online], 2013 [viewed 25 May 2013]. Available from: http://www.portalprosinos.com.br/downloads.php/

RIO GRANDE DO SUL. Defesa Civil do Estado do Rio Grande do Sul [online], 2013 [viewed 23 June 2013]. Available from: http://www.defesacivil.rs.gov.br/SGDC/MPluviometros/PluviCons.asp

ROCHA, J.C., ROSA, A.H. and CARDOSO, A.A., 2004. *Introdução à química ambiental*. Porto Alegre: Bookman. 154 p.

SAVÓIA, E.J.L., DOMINGOS, M., GUIMARÃES, E.T., BRUMATI, F. and SALDIVA, P.H., 2009. Biomonitoring genotoxic risks under the urban weather conditions and polluted atmosphere in Santo André, SP, Brazil, through Trad-MCN bioassay. *Ecotoxicology and Environmental Safety*, vol. 72, no. 1, pp. 255-260. http://dx.doi.org/10.1016/j.ecoenv.2008.03.019. PMid:18571723.

SCHMITT, J.L. and GOETZ, M.N.B., 2010. Species richness of fern and lycophyte in an urban park in the Rio dos Sinos basin, Southern Brazil. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 70, no. 4, suppl., pp. 1161-1167. http://dx.doi.org/10.1590/S1519-69842010000600005. PMid:21225157.

TEIXEIRA, E.C., FELTES, S. and SANTANA, E.R.R., 2008. Estudo das emissões de fontes móveis na Região Metropolitana de Porto Alegre, Rio Grande do Sul. *Quimica Nova*, vol. 31, no. 2, pp. 244-248. http://dx.doi.org/10.1590/S0100-40422008000200010.

TEIXEIRA, E.C., MATTIUZI, C.D.P., FELTES, S., WIEGAND, F. and SANTANA, E.R.R., 2012. Estimated atmospheric emissions from biodiesel and characterization of pollutants in the metropolitan area of Porto Alegre-RS. *Anais da Academia Brasileira de Ciencias*, vol. 84, no. 3, pp. 655-667. http://dx.doi.org/10.1590/S0001-37652012000300008. PMid:22886159.

TEIXEIRA, M.B., COURA-NETO, A.B., PASTORE, U. and RANGEL-FILHO, A.L.R., 1986. Vegetação. In: INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA – IBGE. *Levantamento de recursos naturais*. Rio de Janeiro: IBGE, pp. 541-620. vol. 33.

THEWES, M.R., ENDRES JÚNIOR, D. and DROSTE, A., 2011. Genotoxicity biomonitoring of sewage in two municipal wastewater treatment plants using the *Tradescantia pallida* var. *purpurea* bioassay. *Genetics and Molecular Biology*, vol. 34, no. 4, pp. 689-693. http://dx.doi.org/10.1590/S1415-47572011005000055. PMid:22215975.