

Biomonitoration of Areas Producing Ceramic Manufactures in Two Municipalities of The South of Brazil

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Abstract

Industrial and urban development, together with population growth, has contributed to the increase of atmospheric pollution, and causes the air of urban centers to present more and more substances that are harmful to living beings. In southern Santa Catarina, Morro da Fumaça and Sangão are the highlights in the production of red ceramics (bricks and tiles), these industries use combustion processes for the manufacture of their product and releases air pollutants. The exposure period of the organism was from June to October 2015, at Points A and B in Morro da Fumaça and C, D and E in Sangão. Collections of the young inflorescences of the exposed plants were carried out weekly. After collection, inflorescences were fixed in ethanol / glacial acetic acid solution (3: 1) for 24 hours, transferred to 70% ethanol solution and stored under refrigeration. These were submitted to staining with acetic carmine and the number of micronuclei in 300 tetrads was quantified for each inflorescence, and blades with 5 inflorescences / week were made for each point studied. The results were expressed as mean \pm standard deviation and median and range. In the present study it can be verified that the bioindicator *Tradescantia pallida* shows to be a highly sensitive plant and an excellent test organism, considered an essential tool in the biomonitoring of polluted environments. The results show that in the month of August, the frequency of micronuclei was significantly higher in the plants of the sampling points.

Keyword: *Tradescantia pallida*; Mutagênese; Cerâmicas; Poluição atmosférica.

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Abstract

Industrial and urban development, together with population growth, has contributed to the increase of atmospheric pollution, and causes the air of urban centers to present more and more substances that are harmful to living beings. In southern Santa Catarina, Morro da Fumaça and Sangão are the highlights in the production of red ceramics (bricks and tiles), these industries use combustion processes for the manufacture of their product and releases air pollutants. The exposure period of the organism was from

June to October 2015, at Points A and B in Morro da Fumaça and C, D and E in Sangão. Collections of the young inflorescences of the exposed plants were carried out weekly. After collection, inflorescences were fixed in ethanol / glacial acetic acid solution (3: 1) for 24 hours, transferred to 70% ethanol solution and stored under refrigeration. These were submitted to staining with acetic carmine and the number of micronuclei in 300 tetrads was quantified for each inflorescence, and blades with 5 inflorescences / week were made for each point studied. The results were expressed as mean \pm standard deviation and median and range. In the present study it can be verified that the bioindicator *Tradescantia pallida* shows to be a highly sensitive plant and an excellent test organism, considered an essential tool in the biomonitoring of polluted environments. The results show that in the month of August, the frequency of micronuclei was significantly higher in the plants of the sampling points.

Keywords: *Tradescantia pallida*. Mutagênese. Ceramics. Air pollution.

1. Introduction

Industrial and urban development, coupled with population growth and the comfort generated by the advancement of technology, have contributed to increased air pollution, causing urban air to increasingly show harmful substances to living beings (Cançado et al. (2006).

In southern Santa Catarina, the municipalities of Morro da Fumaça and Sangão stand out in the production of red ceramics (bricks and tiles), these industries use combustion processes for the manufacture of their product, and release atmospheric pollutants such as particulate matter (MP), nitrogen oxides (NO_x) sulfur (SO_x) and carbon monoxide (CO). Their release occurs due to the combustion and composition of the feedstock and fuel used (United States Environmental Protection Agency 1997).

In this context, the monitoring of air pollution is an important tool, since it helps control measures, reduces the substances expelled by the industry chimneys and the vehicular fleet (Silva 2005; Teixeira; Barbério 2012).

Since the middle of the last century, the use of living organisms has begun as a method to aid in the detection of dangerous changes in the quality of the environment, which has been called biomonitoring (Aksoy and Örtürk 1997; Garty et al., 1998; al. 1998).

Biomonitoring can be defined as an indirect experimental method of verifying the existence of pollutants in a certain area, using living organisms that respond to the stress to which they are subjected by changes in the vital cycles or by the accumulation of pollutants (Rossbach et al. al. 1999; Carreras and Pignata 2001). *Tradescantia pallida* (Rose) D.R. Hunt belongs to the Family Commelinaceae, a perennial herbaceous, rustic plant with succulent foliage (Ribeiro et al. 2010), widely used in the evaluation of the genotoxic effects of atmospheric pollutants and other toxic substances.

In the bioindicator *Tradescantia pallida* (Rose) D.R. Runt occurs the fragmentation of genetic material and causes micronuclei to be formed (Teixeira and Barbério 2012). This plant has as main characteristic the facility of identifying the micronuclei due to the existence of six large pairs of chromosomes (Carvalho 2005).

The micronucleus test with *Tradescantia pallida*, also known as Trad-MCN, is considered by many

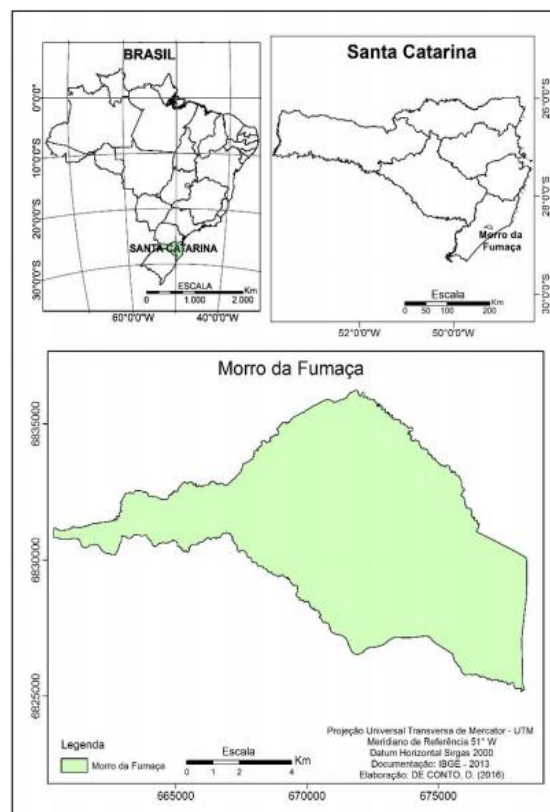
researchers as a valuable tool due to the methodological simplicity and sensitivity of the plant when exposed to genotoxic and mutagenic agents, which are four haploid cells that later (Alves et al 2001, Sisenando et al., 2009).

Taking into account the large number of ceramics in the municipalities of Morro da Fumaça and Sangão, the emission of several pollutants that such activity produces and the health consequences of any biota, the present study aimed to evaluate the mutagenicity through the bioindicator *Tradescantia pallida* (Rose) DR Hunt, exposed in locations close to polluting sources.

2 Material and methods

2.1 STUDY AREA

The study was carried out in the municipalities of Morro da Fumaça ($28^{\circ} 39' / 0''$ - Latitude - and $49^{\circ} 12' / 3''$ - Longitude) and Sangão ($28^{\circ}38' / 16''$ - Latitude - and $49^{\circ}07' / 45''$ - Longitude) (Fig. 1 and 2), Santa Catarina, Brazil, from June to October 2015. Morro da Fumaça has a total area of 82.94 km² and houses a population of 17213 inhabitants; Sangão has a territorial extension of 83.06 km² with approximately 10,400 residents (Brazilian Institute of Geography and Statistics 2016). The municipalities have a subtropical, humid mesothermic and hot summer climate, with an mean temperature of 19.7°C and an mean annual rainfall of 1,375 mm (Climate-data 2016). These municipalities have a total of 47 and 31 red ceramics (bricks and tiles), respectively, being the highest percentages of the southern region of Santa Catarina (Camara 2012).



Source: Authors, 2016.

Figure 1 - Geographic location represented on a map of the municipality of Morro da Fumaça, Santa Catarina, Brazil.

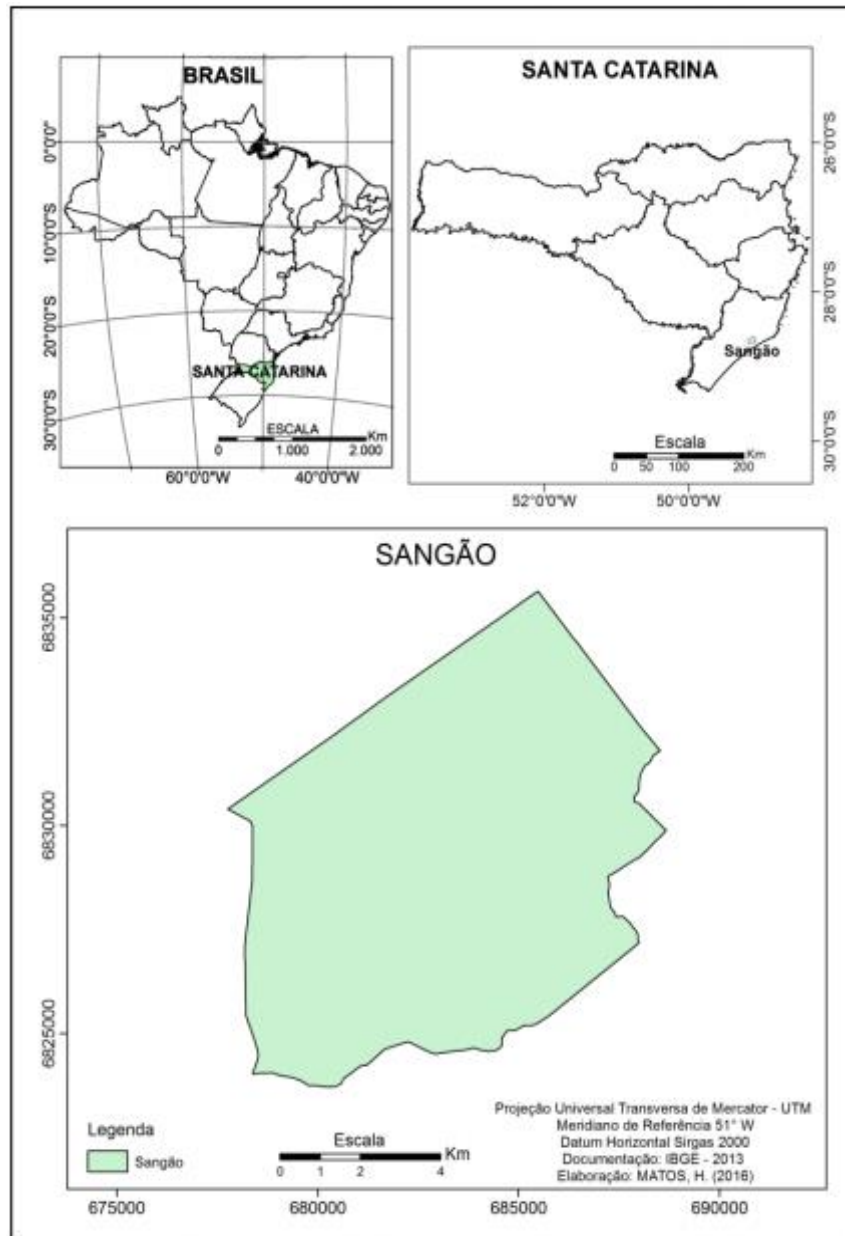


Figure 2 - Geographic location represented on map of the municipality of Sangão, Santa Catarina, Brazil. Source: Authors, 2016.

2.2 Methodology

The bioindicator specimens were exposed in five sample points, two of them (Point A - Biázio Maragno Municipal Basic School, Point B - Vicente Guollo Municipal Basic School) in Morro da Fumaça and the others in Sangão (Point C - Pre-School Balloon Magician, Point D - Preschool Child Happy and Point E - Fundamental Education Center João Manoel).

The choice of points was based on the survey of the sources of pollution of the municipalities, where the presence of municipal schools very close to these places was verified. With the authorization of the Education Department of these municipalities were placed five flowerpots with a total of 15 specimens of

Tradescantia pallida, in each of the municipal schools. The flowerbeds received the same substrate and were positioned in the best ventilation area.

The material was collected weekly between June and October 2015, on the same days and times. The bioindicator analysis was carried out at the Laboratory of Pharmacognosy, Homeopathy and Phytotherapy, University of the Extreme South of Santa Catarina (UNESC). After collection the material in ethanol / glacial acetic acid solution (3: 1) was fixed for 24 h, transferred to 70% ethanol solution and kept under refrigeration until analysis. Also, submitted to staining with acetic carmine, according to a protocol (Ma 1981), for each site studied 5 blades were made per week. Micronucleus frequencies were recorded on each slide (number of micronuclei in 300 tetrad).

The data were analyzed with the Statistical Package for the Social Sciences (SPSS). The data are presented in tables with means and standard deviation and, median and range.

The distribution of data were examined with Shapiro-Wilk and Kolmogorov tests.

When the independent variable was categorical, with two categories, because the dependent variable was not normally distributed in the groups, data were compared with the Mann Whitney U Test.

When the independent variable was categorical, with three or more categories, and the dependent variable was normally distributed in all groups, data were compared with a one-way analysis of variance (ANOVA) using Tukey post hoc tests. When the dependent variable presented non-parametric distribution data were compared with Kruskal Wallis H Test.

The degree of correlation between quantitative data were expressed by Spearman's correlation coefficient. Significance was predetermined at $P < 0.05$ (Zar 2010).

3 Results and discussion

The mean number of micronuclei (MCN) in the points of table 1, relative to the municipality of Morro da Fumaça ranged from 1.31 to 1.60, where $p = 0.467$, was not statistically significant. Table 2 shows a variation from 1.06 to 3.75, where $p < 0.001$ was statistically significant. The mean MCN shows that Point D presented a significant difference in relation to the others. A study carried out in the municipality of Taubaté, in the Paraíba Valley, São Paulo, observed the frequencies of micronuclei ranging from 0.8 to 2.3 (Texeira; Barbério 2012). According to a study carried out in the city of Santo André, in São Paulo, frequencies of micronuclei ranging from 0.5 to 4.8 were observed, in this sense, the results obtained in the present research are in agreement with the literature (Savóia 2007).

Table 1 - Number of micronuclei per sampling point (schools) in the municipality of Morro da Fumaça.

School	n *	Mean \pm SD	Md (min - max)	P-value
Biázio Maragno (A)	211	1,31 \pm 1,11	1,0 (0 – 4)	0,467
Vicente Guollo (B)	169	1,60 \pm 1,38	1,5 (0 – 5)	

* Total number of inflorescences analyzed.

SD = Standard Deviation; Md = Median; Min = Minimum; Max = Max.

** Value obtained after application of the Mann-Whitney U test.

Source: Research Data (2016) .

Table 2 - Number of micronuclei per sampling point (schools) in the municipality of Sangão.

School	n*	Mean \pm SD	Md (mín – máx)	P-value
João Manoel (C)	125	2,02 \pm 1,14 ^b	2,0 (0 – 4)	<0,001
Balão Mágico(D)	165	3,75 \pm 1,94 ^a	3,5 (0 – 8)	
Preschool (E)	97	1,06 \pm 1,06 ^b	1,0 (0 – 3)	

* Total number of inflorescences analyzed.

SD = Standard Deviation; Md = Median; Min = Minimum; Max = Max.

** Value obtained after one-way ANOVA test.

^{a,b}Distinct letters are significantly different (Tukey post hoc test $p < 0.05$).

Source: Research Data (2016).

In a second approach, statistical analyzes on the MCN frequency were performed, in relation to the months of collection, table 3, for the municipality of Morro da Fumaça, we have the value of $p < 0.01$, significant, that is, June 1,94, July 1.45, August 2.5, September 1.36, October 0.81. The MCN frequency was highest in August.

The results found in this analysis, when related to the climatic conditions of the winter season under the frequency of micronuclei, corroborated with studies carried out in Uberlândia (MG), where there was an increase in the frequency of micronuclei in individuals of *Tradescantia pallida* in winter, in which (Table 2). These results suggest that these results can be elucidated by the thermal inversion phenomenon, which occurs in the winter season (Campos, Kerr 2009). The difficulty of the dispersion of pollutants occurs mainly in winter, since thermal inversion, temperature decrease and, consequently, the lower dilution of the gases in the atmosphere, resulting in a low dispersion of particulate matter (Copelli 2011).

Table 3 - Mean of micronuclei in each month in which individuals were collected from the bioindicator in the municipality of Morro da Fumaça.

School	n *	Mean \pm SD	Md (min - max)	P-Value**
June	84	1,94 \pm 1,18 ^a	2,0 (0 – 4)	0,010
July	84	1,45 \pm 1,13 ^{a,b}	2,0 (0 – 3)	
August	35	2,50 \pm 1,87 ^a	2,5 (0 – 5)	
September	64	1,36 \pm 1,29 ^{a,b}	1,0 (0 – 4)	
October	113	0,81 \pm 0,75 ^b	1,0 (0 – 2)	

* Total number of inflorescences analyzed.

** Value obtained after one-way ANOVA test.

^{a,b}Distinct letters are significantly different (Tukey post hoc test $p < 0.05$)

SD = Standard Deviation; Md = Median; Min = Minimum; Max = Max.

Source: Research Data (2016).

Table 4, which shows the information regarding the municipality of Sangão, the value of $p = 0.924$ was not significant. However, it can be observed that in all months there were micronuclei, June 2,75, July 2,25,

August 2,86, September 2,24, October 2,48.

Table 4 - Mean of micronuclei in each month in which individuals from the bioindicator were collected in the municipality of Sangão.

School	n*	Mean \pm SD	Md (mín – máx)	P-Value**
June	62	2,75 \pm 2,34	2,5 (0 – 8)	0,924
July	27	2,25 \pm 0,96	2,5 (1 – 3)	
August	45	2,86 \pm 1,57	3,0 (1 – 6)	
September	128	2,24 \pm 1,61	2,0 (0 – 5)	
October	125	2,48 \pm 2,13	2,0 (0 – 7)	

* Total number of inflorescences analyzed.

** Value obtained after one-way ANOVA test.

SD = Standard Deviation; Md = Median; Min = Minimum; Max = Max.

Source: Research Data (2016).

Tables 5 and 6 present the result of the number of micronuclei related to the weather conditions in the municipalities of Morro da Fumaça and Sangão, respectively. In table 5, the value of $p = 0.709$, not significant, however with the following values in the sunny weather 1.60, overcast 1.40, rainy 1.24. In table 6, the value of $p = 0.965$ shows that there is no significant difference between the presented climatic conditions, in sunny weather 2,38, cloudy 2,47, rainy 2.53..

Table 5 - Relationship between micronuclei and time conditions in the municipality of Morro da Fumaça

Time	n *	Mean \pm SD	Md (min - max)	P-Value**
Sunny	207	1,60 \pm 1,38	1,00 (0 – 5)	0,709
Cloudy	26	1,40 \pm 1,67	1,00 (0 – 4)	
Rainy	147	1,24 \pm 0,93	1,00 (0 – 3)	

* Number of inflorescences analyzed with the recorded climate.

** Value obtained after application of the Kruskal H-test .

SD = Standard Deviation; Md = Median; Min = Minimum; Max = Max.

Source: Research Data (2016) .

Table 6 - Relationship between micronuclei and time conditions in the municipality of Sangão .

Time	n*	Mean \pm SD	Md (mín – máx)	P-Value**
Sunny	95	2,38 \pm 1,67	2,5 (0 – 5)	0,965
Cloudy	108	2,47 \pm 2,32	2,0 (0 – 7)	
Rainy	184	2,53 \pm 1,74	2,0 (0 – 8)	

* Number of inflorescences analyzed with the recorded climate.

** Value obtained after one-way ANOVA test.

SD = Standard Deviation; Md = Median; Min = Minimum; Max = Max.

Source: Research Data (2016).

In a micronucleus test carried out in researches with *T. pallida*, some authors take into account meteorological factors in which the effects of genotoxic agents are reduced during rainy periods (Savóia et al., 2008). However, a study by Silva Dias et al. (2012) shows the non-relation between rainy season and micronucleus occurrence.

Tables 7 and 8 show the correlations between wind, rainfall and micronuclei in the municipalities of Morro da Fumaça and Sangão, respectively.

In Table 7, the correlation between wind and the MCN frequency has a significant value of $p < 0.01$, where the directions of the same during the sampling period influenced the frequency of micronuclei found in the analysis of the bioindicator.

Table 7 - Correlation between wind, rainfall and micronuclei in the municipality of Morro da Fumaça.

Climate conditions	r_s	P-value
Wind	-0,317	0,010*
Rain	-0,063	0,619

* Statistically significant correlation by Spearman's coefficient ($p < 0.05$).

Source: Research Data (2016).

The predominant wind direction in the south of the state of Santa Catarina, regardless of the time of year, is SE (southeast), followed by NE direction (Silveira et al., 2014). The SE wind stream comes straight from the ocean and passes through the polluting sources, which causes the pollutants to flow towards the collection points (Guerra 2016). The rainfall data did not show a significant p value, and indicates that the rain did not cause interference in the frequency of micronuclei.

In Table 8, with the value of r_s negative, it is verified that the wind did not influence the MCN frequency. On the other hand, rainfall, presenting the value of positive r_s , indicates that in more rainy periods the occurrence of micronuclei is greater. The correlation between the wind and micronuclei occurrence was negative in a research done in 2013, indicating that in the period with greater intensity of winds the pollutants are dispersed with greater efficiency, which contributes to the lower assimilation by the bioindicator (Silva et al., 2013).

Table 8 - Correlation between wind, rainfall and micronuclei in the municipality of Sangão.

Climate conditions	r_s	P-Value
Wind	-0,201	0,102
Rain	0,047	0,706

Source: Research Data (2016).

It is worth noting that the direction of the winds is also a determining factor, since it is a dispersant of

pollutants (Savóia et al., 2008). Wind currents carry the particulate material to other places and decrease the occurrence of MCN. In relation to rain, the value of p is not considered significant, however, the value of r_s positive indicates that rain contributed to the occurrence of micronuclei.

In the analyzes related to the points studied, the influence of the wind currents is observed, which confirms the idea that they carry the pollutants and take them, even in a less concentrated way, to points other than those of their origin.

In the present study it can be verified that the bioindicator *Tradescantia pallida* shows to be a highly sensitive plant and an excellent test organism, considered an essential tool in the biomonitoring of degraded environments, which is also confirmed in the literature.

It is observed that in the winter (August) the frequency of micronuclei was significantly higher in the plants of the sampling points. This study was the first carried out in the region, however, new studies are underway in other localities to obtain more data on the subject.

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