

The Bioindicative Potential Evaluation of *Tabebuia alba* (Cham.) Sandwith, Bignoniaceae, in Urban Atmospheric Pollution

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ABSTRACT

This study aimed to evaluate the existence of leaf anatomic characteristics in Tabebuia alba changed by air pollutants, which could be used as tool for a bioindication program. The quantification of mutagenic events on pollen grains also were measured. For this, median leaves and pre-anthesis flowers were collected from the adult plants from three places of Curitiba and one place in Araucaria, all nearby to the air monitoring stations. The comparison of the four study sites showed a reduction in leaf area, an increasing of stomatal density, subepidermic layer, epidermis in both faces and the amount of micronucleus. Also, there was reduction of chlorophyllian parenchymas at the site where there was the higher average for the ozone level. It was concluded that these modifications might be a consequence of the effect of tropospheric pollution on T. alba plants. However, further studies with this species would be necessary to confirm its potential for bioindication.

Key words: Air quality, Tropospheric pollution, Bioindication, Vegetal morphoanatomy, Morphometry, Mutagenesis

INTRODUCTION

The air pollution in the big urban centers is one of the environmental problems, with dangerous implications in population health, especially in the children, elderly and patients with respiratory diseases (IBGE 2010). This factor is the cause of approximately two million of premature deaths in the world every year (WHO 2008). The monitoring of these tropospheric pollutants is measured by the stations, strategically located in the cities that recognize the rate of some pollutants in the air and also meteorological aspects of the region such as temperature, relative moisture,

global radiation, pressure, velocity and direction of the wind (IAP 2011). These results measured by the stations, however, cannot be used directly to predict the impacts of pollution in living organisms. For this, sensitive organisms must be introduced as biomonitors (plants), as they respond quickly to environmental modifications (stress factor – pollution), showing easy observing short-term qualitative and quantitative differences.

It is important to emphasize the relationship between the atmospheric data and the effect observed in the organisms, because they respond in an integrated form (Alves et al. 2001; Anicic et al. 2009; Nali et al. 2009; Kardel et al. 2010; Markert et

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al. 2011; Soto et al. 2011). This might be obtained by bioindication, which used exposed organisms in some environmental conditions to evaluate their effects (Arndt et al. 1995; Markert et al. 2011). It is known that the biomonitoring is the evaluation of some environmental quality in determined area, using organisms, or an amount of it, sensitive to pollution as a stress factor that respond changing their functions/vital processes (indicators by answers), or accumulating toxic compounds (indicators by accumulation) (Larcher 2000). The passive form of this evaluation focuses on the species that already exist at the place of the study, saving time and resources and showing the true impact of environment conditions (Górecki and Namiesnik 2002).

The exposure of the organisms to pollutants depends on the way of the contact and intrinsic susceptibility (Greim 2001). In plants, the relation between gas exchange and foliar structure works to verify if the plant anatomy changes in response to the environmental condition that acts upon it (Justo et al. 2005). Thus, plant species that live in anthropic places might show modifications in their structural organization to achieve a better development. Despite the effects observed in the plants, they cannot be used exactly by human populations, the results of the experiments with plants can be consider for this purpose, considering the big sensibility of these organisms, even in situations of very low rates of air contamination (Carneiro 2004). Thus, it is considered that if a pollutant doesn't affect most of the sensitive plants, it also won't affect other species, including humans (Guimarães et al. 2000). The genotoxicity of the tropospheric air can be measured by cytogenetic analysis that show micronucleus. The micronucleus indicates damage in DNA, which can be caused by the atmospheric pollutants (Guimarães et al. 2004; Meireles et al. 2009). There is a tendency in urban environments that the rate of micronucleus increases when the place is more anthropic (Meireles et al. 2009; Savóia et al. 2009; Villarini et al. 2009)

Tabebuia alba (Cham.) Sandwith, Bignoniaceae, known as ipê-amarelo is native of Brazil (Lorenzi 2000) and widely used in Curitiba's urban forestry. This was used in this study as was easy to collect for the passive biomonitoring and also because of few works relating it with air pollution. Curitiba holds the second largest fleet of cars per inhabitant in Brazil, showing, therefore, many problems related to air pollution. Araucária has a

high rate of heavy trucks that travel on BR 476. Thus, the aim of this study was to evaluate the efficacy of *T. alba* (Cham.) Sandwith for the passive bioindication of some tropospheric pollutants in different locations of the municipalities of Curitiba and Araucária, PR, Brazil.

MATERIALS AND METHODS

Study Points and Material Collect

The plant material was collected from four places with different traffic intensities, without a control area. From each place, in April 2011, 60 mature and healthy leaves of *T. alba* were collected (20 leaves per tree, following standardization), from three adult plants each near air quality monitoring station. Monitoring stations were:

- Santa Cândida (STC) 25°22'50'' S, 49°13'03'' O: automatic, located in Santa Cândida neighborhood, Curitiba. Aims to monitor the emissions of SO₂ and O₃ in residential areas.
- Santa Casa (SC) 25°26'10'' S, 49°16'26'' O: manual, located in Curitiba's downtown, Rui Barbosa square. Aims to monitor vehicle emissions (smoke, SO₂, Total suspended particulates-PTS and NH₃).
- Pardinho (PAR) 25°26'38'' S, 49°16'18'' O: automatic, located in Curitiba's downtown, Ouvidor Pardinho square. Monitors SO₂, CO, O₃, PTS, Inhalable particles - PI e HCT1.
- UEG (UEG) 25°34'04'' S, 49°24'03'' O: automatic, located in the side of BR 476, Araucária. Monitors the industrial emissions (SO₂, CO, O₃, PI, NO, NO₂) at the central region of this city.

Data related to atmospheric pollution from Oct/2010 to Mar/2011 where *T. alba* existed in vegetative stage were collected from Instituto Ambiental do Paraná (IAP).

Laboratory Tests

For the test of visible injuries, 10 middle folioles from the 10 leaves located from 4th stem node in which plant were observed naked eye for the presence of injuries (chlorosis, necrosis) and/or foliar damage were selected. The foliar specific area (FSA) was measured by the modified method of Balasooriya et al. (2009). For this, from one of the three plants selected from each location, 10 middle folioles from the un-damaged and fully

expanded leaves were collected. The FSA ($\text{cm}^2 \text{g}^{-1}$) were calculated dividing the fresh leaf area (FLA) from just one side of the leave (measuring with ImagePro-Plus program) by its dry biomass (BS) (60°C for 7 days).

For chlorophyll measurement was carried out using the method of Barnes et al. (1992). For this, 28 mg of fresh leaves was extracted with 5.0 mL of dimethylsulfoxide. The absorbance readings were made in spectrophotometer (UV-Visible Spectrophotometer UV-1601 Shimadzu) at 648 nm and 665 nm. The samples for the structural organization analysis were collected from 4th stem node, in the central region of the foliole. They were fixed in FAA 70 (Johansen 1940) for 72 h and stored in ethanol 70%. For stomata analysis, the epidermis in both the faces were separated according to the method of Franklin (1946). The semi permanent slides were stained with 0.05% aqueous toluidin blue (Sakai 1973), prepared in glycerin and sealed with varnish (Purvis et al. 1964). Ten leaves from each tree were analyzed the number of stomata was counted per 1 mm^2 using photonic microscope (Olympus – CX41RF) attached to a camera lucida (40x).

For the permanent slides preparation, the samples were included in metacrilatoaglicol (JB-4), following the factory specifications (Polysciences Inc.). The sectioning was made in Leica's manual microtome (RM2125), with $7 \mu\text{m}$ thickness, stained with 0.05% aqueous toluidin blue (Sakai 1973). The slides were prepared with Entellan® synthetic resin. The images and measurements were obtained using photomicroscope (Olympus BX-41) with image captured by the ImagePro-Plus software in the chosen foliar tissue layers (adaxial epidermis, subepidermic layer, palisade parenchyma, spongy parenchyma, abaxial epidermis and level of stomata elevation).

The evaluation of genotoxicity exerted by the tropospheric pollutants in the plant were observed by micronucleus spontaneous frequency. The study was performed according to the modified method of Sisenando et al. (2009). For this, the floral buds were collected from the young inflorescences and fixed in ethanol/acetic acid (3:1) – Carnoy's solution – for 24 h and then stored in ethanol (70%) at 4°C to continue with the cytogenetic preparation and microscope analysis. The young flowers were dissected for the anthers. These were smashed and stained with 2% acetic carmin, followed by a fast heating for 8-15 seconds on heater board (70°C) in order to

highlight the visualization of micronucleus. The tetrads were observed in optic microscopy under an objective of 40x. From each sampling place, 300 tetrads were analyzed. The presence of micronucleus was discriminated according to the total number of micronucleus per tetrad.

Statistical Analysis

The statistical analysis of quantitative data was made by the BioEstat software, version 7.1 (StatSoft 2005). The data normality and variances homogeneity were verified by Shapiro-Wilk and Levene tests, respectively. As the hypotheses of normality and homogeneity were satisfied for the parameters of foliar specific area, stomata density, chlorophyll A and B and palisade parenchyma thickness, the variance analysis was applied (ANOVA), followed by Tukey test at 5%. For the other parameters such as leaf area, total chlorophyll, adaxial epidermis, subepidermic layer, spongy parenchyma, abaxial epidermis and level of stomata elevation, which did not reach the normality assumptions, the Kruskal-Wallis nonparametric was applied, followed by Dunn at 5%.

RESULTS AND DISCUSSION

The mesophyll of *T. alba* was dorsiventral (Fig. 1a). The cuticle was approximately $5 \mu\text{m}$ (Fig. 1b). The cells from the adaxial epidermis were small, quadrangular, with rounded vertices, and were disposed in a single layer (Fig. 1b). Internally, the subepidermic tissue was with 1-2 rectangular, rounded, and bigger size layers (Fig. 1b). The palisade parenchyma had 2-3 layers of cells and the spongy parenchyma had compact rectangular cells (2-3 layers) (Fig. 1b). The abaxial epidermis was similar to the adaxial; however, the cells were smaller and had thin walls (Fig. 1b). The stomata had a little elevation when comparing to adjacent epidermic cells (Fig. 1b).

The average pollutants concentration, total suspended particles, global radiation, temperature and relative moisture, related to the period that *T. alba* was in a vegetative stage (October/2010 to March/2011) as shown in Table 1. During the study period, there was only once that exceeded the limit of $150 \mu\text{g m}^{-3}$ established as a secondary pattern for MPTS (Brasil, 1990). At SC station on October 20, 2010, it was $152 \mu\text{g m}^{-3}$ as a daily average.

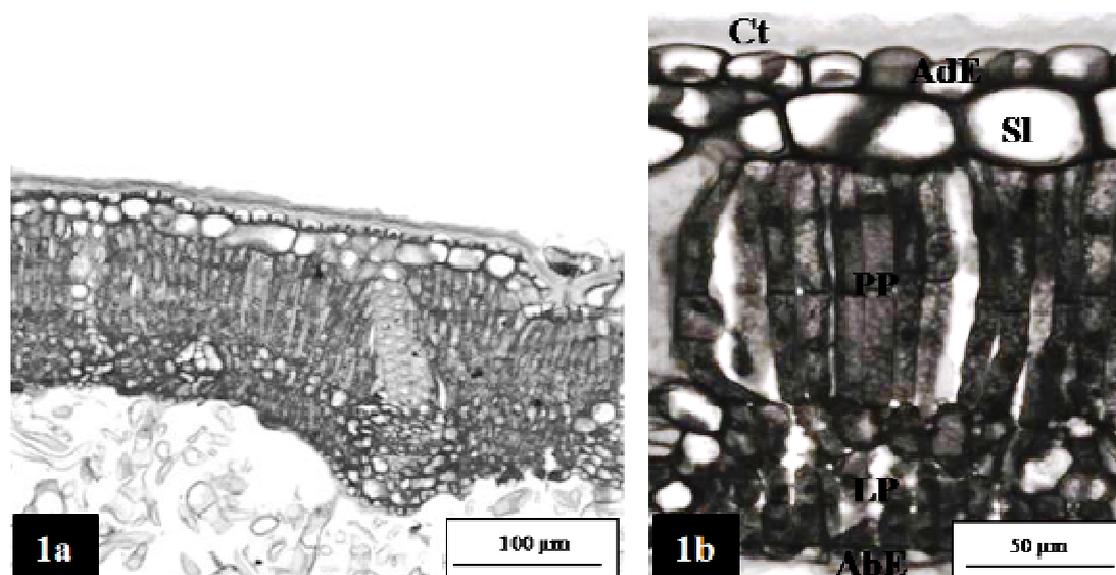


Figure 1 – Transversal section from *Tabebuia alba*'s leaf. (a) Overall vision of the tissues; (b) Detailed tissues (Ct – cuticle; AdE – adaxial epidermis; SI – subepidermic layer; PP – palisade parenchyma; LP – spongy parenchyma; AbE – abaxial epidermis).

Table 1 – Mean of the measurements from the four selected stations between October/2010 and March/2011.

	PAR	SC	STC	UEG
UMID (%)	83.9	*	84.8	*
RADG ($W m^{-2}$)	180.2	*	*	223.8
MPTS ($ug m^{-3}$)	14.3	50.5	*	*
SO ₂ (ppb)	1.5	3.0	1.5	0.6
NO ₂ (ppb)	20.0	*	6.7	15.4
PM ₁₀ ($ug m^{-3}$)	10.8	*	*	22.2
O ₃ (ppb)	16.1	*	17.8	14.1
TEMP (°C)	*	21.7	19.6	18.9

Relative moisture (UMID), Global radiation (RADG), Total suspended particle material (MPTS), Sulfur dioxide (SO₂), Nitrogen dioxide (NO₂), Smaller particles than 10 µm (PM₁₀), Ozone (O₃) and Temperature (TEMP). Source: IAP (2011). *Not monitored.

Visible injuries such as necrotic areas and abrasion were observed in the leaves collected from SC station, whereas those from UEG region showed only herbivore signals. There was also the presence of red punctuations in the adaxial side of leaves collected from the STC and PAR sites. Pina et al. (2007) reported little red punctuations on the adaxial surface from the old leaves of *Psidium guajava* induced by O₃ in São Paulo city. Apparently the red punctuations observed in *T. alba* were related to a bigger exposure to O₃, since this symptom was observed at STC and PAR sites, where there was a higher incidence of O₃.

The stomatal density from the leaves of UEG site was statistically smaller than other environments

(Table 2). Thus, SC had the bigger stomatal density (937.07 ± 180 est mm^{-2}), whereas UEG had the smaller one (744.53 ± 129 est mm^{-2}). It is known that air pollution affects the rising of stomata number per unit area as a strategy to compensate the smaller leaf area (Alves et al. 2008; Balasooriya et al. 2009; Kardel et al. 2010; Saadabi 2011). Alves et al. (2001) studied changes in *Tradescantia* anatomy arising from the polluted urban area and found larger stomatal density in the polluted regions (Cerqueira César 35.7 est mm^{-2} ; Congonhas 36 est mm^{-2}), which corroborated the results of the present study.

For leaf area (Table 2), Curitiba's downtown, specially SC place, showed small leaves areas

($35.23 \pm 9.57 \text{ cm}^2$), which could be a sign that the leaf size was affected due to pollution. Alves et al. (2008) noticed the leaves' reduction of length and width for *Eugenia uniflora* L. in two regions with constant emissions of primary and secondary pollutants. Balasooriya et al. (2009) observed a leaf area reduction of 37% in urban areas and 39% in the forest urban areas compared to another place less polluted. Under this aspect, foliar specific area was bigger while the leaf area decreased at SC point. This suggested that the plants invested less energy in their leaves as their longevity decreased due to the low quality of the environment (Balasooriya et al. 2009). Small leaves decrease their contact area with the atmosphere, which helps the reduction of harmful gases absorption besides reducing the particulate material accumulation on the leaf surface (Balasooriya et al. 2009). The fertile and sterile leaves of *Microgramma squamulosa* have small area in urban environment, which can be assigned to the presence of primary pollutants, as well particulate material. In this environment, there are suspended particles and SO_2 , pollutants which, according to some studies, interfere on the leaf size (Sharma and Tyree 1973; Eleftheriou 1987; Fares et al. 2006).

The UEG point has shown a statistically higher amount of total chlorophyll than STC (Tables 2 and 3). Balasooriya et al. (2009) demonstrated that the content of chlorophyll a in a forest urban region was fairly higher than the industrial regions, which was in contrast to the result found in this study. However, the results for chlorophyll a are less reliable, because they are influenced by many factors such as water availability, nutrients, air temperature, luminous intensity among others. It is possible that these factors act in a positive way for the observation of higher average found for total chlorophyll in UEG.

In relation to the foliar tissues thickness, the leaves presented the palisade parenchyma thinner at STC (Table 3) and the lacunous parenchyma was thicker at SC and UEG in relation to PAR and STC (Table 3). Adaxial epidermis was significantly thinner in the plants from UEG (Table 3). The subepidermic layer showed similar thickness between the leaves obtained from PAR and SC, which was significantly thicker than STC and UEG and also equal to each other (Table 3). The abaxial epidermis thickness was statistically higher at SC (Table 3). With this in view, both epidermis sides were thicker in SC point.

Reig-Armiñana et al. (2004), in a study about O_3 effect on *Pistacia lentiscus* observed that the presence of a thick epidermis and a high foliar specific area made injury symptoms less apparent than in the plants with thinner epidermis and leaves. The SC station presented the higher amount of necrotic symptoms, besides thick epidermis and high foliar specific area. Pompéia (1997), in a study about the acute effects of SO_2 , through the process of fumigation in laboratory, indicated that the dosage of 1 ppm caused internerval chlorosis and necrotic spots in *Cecropia hololeula* and *Tibouchina pulchra*, also inducing the stomata opening and loss of water from the cells in contact with guard cells. The same author observed the impact of industrial dust accumulation on the plants from the Atlantic rainforest, inferring a high osmotic effect by causing dehydration, plasmolysis and leaf necrosis. Probably the joint action of suspended particulate material and sulfur dioxide, which were high for the SC station (Table 1) caused necrosis and injuries in *Tabebuia alba*, not only O_3 .

The subepidermic layer thickness was similar between the leaves from PAR and SC points, which presented values significantly higher than STC and UEG, also equal to each other. Since the highest thickness was observed in the leaves from the SC point ($31.57 \pm 11.33 \mu\text{m}$), it was possible that the presence of more cell layers compensated the smaller leaf area observed ($35.23 \pm 9.57 \text{ cm}^2$). It's known that the subepidermic layer develops an important role for the plant since one of its main functions is to retain water (Esau 1974). The opposite was observed at STC point, which had large area leaves ($66.64 \pm 26.47 \text{ cm}^2$) and thin subepidermic layer ($21.09 \pm 7.27 \mu\text{m}$).

For the palisade parenchyma, the point with the highest cell height was SC ($103.40 \pm 15.14 \mu\text{m}$) and the smallest height was observed at STC ($84.58 \pm 15.69 \mu\text{m}$). Godoi et al. (2010), in a study about BTEX concentrations in Curitiba's public areas and its impacts on *T. stricta* observed that the bigger the pollutants concentration was, the smaller was the thickness of chlorophyll parenchyma. In *T. alba*, the chlorophyllian parenchyma included the palisade and lacunous parenchyma and if combined, it showed clearly that more polluted areas had bigger thickness of these tissues (SC = $154 \mu\text{m}$; UEG = $148 \mu\text{m}$ and PAR = $144 \mu\text{m}$) in conytrast to the less polluted ones (STC = $131 \mu\text{m}$). It is possible that O_3 , which had the highest average at STC (Table 1) could

interfere on the growth and development of chlorophyllian parenchymas, since this compound had a great phytotoxic action after entering in the leaves through the stomata, reacting with

substomatic cavity compounds and producing the species, which reacted to oxygen (EROs) and could oxidize vital molecules (Moraes 2009).

Table 2 – Average standard deviation from leaf morphometry data of *Tabebuia alba* (Cham.) Sandwith, (Bignoniaceae).

Parameters	PAR	SC	STC	UEG
Foliar specific area (cm ² g ⁻¹)	64.67 ± 10.03 ^a	68.76 ± 6.54 ^a	66.73 ± 7.56 ^a	63.77 ± 7.30 ^a
	F= 2.35; gL= 3; p= 0.08			
Stomatal density (est mm ⁻²)	897.07 ± 186 ^a	937.07 ± 180 ^a	856.00 ± 129 ^a	744.53 ± 129 ^b
	F= 8.21; gL= 3; p< 0.01			
A Chlorophyll	8.86 ± 3.16 ^a	8.87 ± 2.73 ^a	8.24 ± 3.04 ^a	9.30 ± 2.11 ^a
	F= 2.35; gL= 3; p=0.08			
B Chlorophyll	2.98 ± 2.28 ^a	3.36 ± 2.32 ^a	2.68 ± 2.48 ^a	3.47 ± 1.63 ^a
	F= 0.80; gL= 3; p= 0.49			
Palisade parenchyma (µm)	98.93 ± 14.48 ^a	103.40 ± 15.14 ^{a,c}	84.58 ± 15.69 ^b	94.09 ± 21.48 ^{a,d}
	F= 27.21; gL= 3; p< 0.01			

Pardinho (PAR), Santa Casa (SC), Santa Cândida (STC), UEG (UEG), F (ANOVA), gL (degrees of freedom), p (significance). Different letters in the same line differ statistically by Tukey test at 5%.

Table 3 – Average standard deviation from leaf morphometry data of *Tabebuia alba* (Cham.) Sandwith, (Bignoniaceae).

Parameters	PAR	SC	STC	UEG	H	p
Leaf area (cm ²)	60.84 ± 22.25 ^a	35.23 ± 9.57 ^b	66.64 ± 26.47 ^a	64.46 ± 21.37 ^a	39.51	< 0.01
Total chlorophyll	11.84 ± 2.98 ^{a,b}	12.22 ± 3.03 ^{a,b}	10.92 ± 2.33 ^a	12.78 ± 2.17 ^b	9.65	< 0.05
Adaxial epidermis (µm)	9.59 ± 1.53 ^a	9.85 ± 1.91 ^a	9.21 ± 1.60 ^a	8.08 ± 1.56 ^b	69.85	< 0.01
Subepidermic layer (µm)	28.46 ± 10.57 ^a	31.57 ± 11.33 ^a	21.09 ± 7.27 ^b	20.06 ± 9.55 ^b	119.84	< 0.01
Spongy parenchyma (µm)	46.41 ± 6.41 ^a	51.95 ± 7.03 ^b	47.23 ± 5.98 ^a	54.22 ± 9.44 ^b	84.13	< 0.01
Abaxial epidermis (µm)	6.64 ± 1.25 ^a	7.38 ± 1.44 ^b	6.19 ± 1.24 ^c	6.59 ± 1.33 ^{a,c}	44.57	< 0.01
Stomata elevation (µm)	10.65 ± 3.22 ^a	10.40 ± 3.35 ^a	10.34 ± 3.53 ^a	11.34 ± 3.23 ^a	8.26	> 0.05

Pardinho (PAR), Santa Casa (SC), Santa Cândida (STC), UEG (UEG), H (Kruskal-Wallis), p (significance). Different letters in the same line differ statistically by Dunn test at 5%.

The spongy parenchyma was thicker at SC and UEG points and was significantly bigger than the values observed for PAR and STC. As it was exposed to the chlorophyllian palisade parenchyma, the lacunous might have had development difficulties due to the higher rate of O₃ on the plant, as higher averages of the compound and the smaller heights were observed at PAR and STC points. Alves et al. (2001), however, did not observe the existence of significant differences between the lacunous

parenchymas from *Tradescantia* in higher or less polluted sites.

For the genotoxicity examination, data revealed a higher amount of micronucleus at UEG (Table 4). The tropospheric pollution in Araucaria (UEG) probably induced the chromosome breaking and emergence of more micronucleus, since high polluted environments produced high genotoxic damages and could affect the ecosystem, human beings, plants and bacterial health (Isidori et al. 2003).

Table 4 – Genotoxicity examination data correlating the rate of micronucleus with air quality monitoring stations.

Parameter	PAR	SC	STC	UEG
Micronucleus frequency (MCN/300 tetrads)	138.00	133.00	140.00	173.00

Micronucleus (MCN), Pardinho (PAR), Santa Casa (SC), Santa Cândida (STC) e UEG (UEG).

The analysis of *T. alba* (Cham.) Sandwith leaves revealed morphometry differences, which could be attributed to urban atmospheric pollution. The leaf area parameter was easily viewed and measured since the places with higher taxes of sulfur dioxide and suspended particulate material (SC station) showed smaller leaves and with more visible injuries such as necrosis and abrasion. Other parameters were also valid such as the reduction of chlorophyllian parenchymas and the appearing of red punctuations for O₃ on the leaves from Santa Cândida and Ouvidor Pardiniho's stations. There was an increase of stomatal density in the central and residential areas of Curitiba whereas the rise of thickness of subepidermic layer and abaxial epidermis was observed only downtown. Furthermore, genotoxicity examination proved to be promising by showing an increase in the chromossome breaking at the industrial sites.

CONCLUSION

T. alba (Cham.) Sandwith has potential for bioindication, emphasizing the parameters of leaf area, stomatal density, visible injury, subepidermic layer and epidermis. However, further and more advanced studies are required in order to make the development of an air quality biomonitoring program in Curitiba and Metropolitan region possible, ensuring the population welfare, protection of the environment and economic development.

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