

# *Tradescantia pallida* cv. *purpurea* Boom in the Characterization of Air Pollution by Accumulation of Trace Elements

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## ABSTRACT

*Tradescantia pallida* cv. *purpurea*, a plant species widely employed for ornamentation in Brazil, has been successfully used for monitoring the genotoxicity of various agents by the micronucleus assay. To amplify knowledge about its suitability as a bioindicator species, its capacity for accumulating trace elements from urban air pollution was evaluated. *T. pallida* was rooted using standardized soil, and the vases were distributed in two highly polluted sites of the urban area of São Paulo, Brazil (Cerqueira César and Congonhas districts), and in one unpolluted control site situated approximately 50 km from downtown São Paulo (in Caucaia do Alto). Approximately six months after exposure to pollutants, adult leaves of this plant were collected monthly for 12 months. The leaves were washed with deionized water, dried, and ground for analyses. Characterization of element levels was carried out by neutron activation analysis. Powdered samples and standards were irradiated at the IEA-R1 nuclear reactor for short and long periods, and concentrations of As, Ba, Br, Ca, Ce, Cl, Cr, Co, Fe, K, La, Mn, Na, Rb, Sb, Sc, Sr, Th, and Zn were determined. Analysis of variance applied to the results indicated that samples from polluted sites present the highest concentrations of Ba, Ce, Cr, Co, Fe, La, Sb, and Sc ( $p < 0.05$ ). Discriminant analysis revealed that it

was possible to distinguish the two polluted areas with a precision of 97.5%, based on the amount of pollutant elements measured in the plants at each site. The results indicated the potential use of *T. pallida* as an accumulator plant for air pollution biomonitoring.

## INTRODUCTION

The determination of trace elements in samples from the environment or from living organisms such as particulate matter, soil, plants, animals, and water may provide valuable data about the general pollution level of a region. In areas where air pollution distribution changes rapidly, such as developing countries, the availability of conventional air pollution assessment networks is frequently surpassed by the necessity for air pollution measurements. In other words, traffic and industrial activities of growing economies may deteriorate air quality in areas devoid of pollution measurements, making it difficult to establish strategies of emissions control to preserve public health. In such a context, biomonitoring strategies—using either bioindicators or biomonitors—are important alternatives for environmental screening and air pollution evaluation.<sup>1-3</sup>

Generally, biological monitoring has a low cost, and neither sophisticated equipment nor special care is necessary; thus, it is suitable for long-term monitoring over large areas. Plants play an important role in the biological monitoring of air pollution. Plants can be used to monitor pollution by either passive or active methods. Passive methods analyze plants growing naturally in the investigated area. Active methods use plants that are transplanted into the study area for a defined period of time.<sup>2</sup> Plants and soils have been analyzed in various countries to obtain maps of the geographical distributions of pollutant concentrations.<sup>4-10</sup>

## IMPLICATIONS

Plants have played an important role in the biomonitoring of air pollution. *T. pallida* is a plant species frequently employed to detect the genotoxic potential of several air pollutants by the micronucleus assay. Now, the determination of levels of trace elements in this plant, using neutron activation analysis, indicates its potential as an accumulator plant for the evaluation of environmental pollution.

**Table 1.** Annual mean concentrations, obtained in 1999–2000, of the main air pollutants and their maximum value ranges at Cerqueira César and Congonhas stations, in São Paulo.<sup>a</sup>

	PM <sub>10</sub> (µg/m <sup>3</sup> )		CO (ppm)	SO <sub>2</sub> (µg/m <sup>3</sup> )		NO <sub>2</sub> (µg/m <sup>3</sup> )	
	Mean <sup>b</sup>	Daily Max	Hourly Max	Mean <sup>b</sup>	Daily Max	Mean <sup>c</sup>	Hourly Max
Cerqueira César	48/72	126/168	8.1/7.2	15/16	53/46	64/70	343/277
Congonhas	44/48	192/123	12.0/13.7	22/24	56/73	76/# <sup>d</sup>	255/# <sup>d</sup>

<sup>a</sup>Data from CETESB. <sup>b</sup>Mean based on daily values. <sup>c</sup>Mean based on maximum hourly event in each day. <sup>d</sup># = not measured.

Among several plants present in our ecosystem, *Tradescantia pallida* cv. *purpurea* was chosen in this work. *T. pallida* is a popular ornamental plant present in the gardens, roadsides, and streets of São Paulo, Brazil. This plant has a wide distribution and is easily propagated in São Paulo, even in regions with high levels of pollution. Previous studies from our group reported an increased frequency of mutation in the DNA of pollen mother cells in *T. pallida* samples exposed to urban air pollution in São Paulo.<sup>11,12</sup> In the present study, the feasibility of using *T. pallida* for the biomonitoring of air pollution was explored further, assessing the capability of this plant to accumulate air pollution within its leaves, acting as a biological pollution sampler. The specific aim of this study was to verify whether the elemental composition of leaves taken from *T. pallida* could characterize areas with different air pollution levels in São Paulo.

## MATERIALS AND METHODS

### Exposure Sites

São Paulo (latitude 23°32'51" S, longitude 46°38'10" W) is located at an altitude of 715–900 m, the annual precipitation is approximately 1300 mm, and the temperature varies from 15 to 23 °C. During the winter, corresponding to the months from June to August, low wind speeds and diminished rain precipitation cause frequent periods of low wind dispersion of pollutants.

Two sites in the urban area of São Paulo were selected: the garden of Cerqueira César of São Paulo University (Cerqueira César district) and a school along Bandeirantes Avenue (Congonhas district). Both sites are located in polluted areas, along side roads with heavy traffic of gasoline- and alcohol-powered vehicles, buses, and trucks. Congonhas, in particular, has heavier diesel-powered truck traffic than does Cerqueira César, and it is very close to São Paulo airport, the most important air traffic route in Brazil. The exposure sites were located adjacent to air pollution measurement stations of Companhia de Tecnologia de Saneamento Ambiental (CETESB), the state governmental agency responsible for air quality control, whose data provide hourly concentrations of CO and NO<sub>2</sub> and daily concentrations of particles with diameters less

than 10 µm (PM<sub>10</sub>) and SO<sub>2</sub>. Table 1 shows the mean levels of air pollutants measured during 1999–2000 for Cerqueira César and Congonhas.<sup>13</sup>

The third site (situated in Caucaia do Alto) is considered a clean region of the countryside situated approximately 50 km from the central area of São Paulo. Caucaia do Alto was a control region. Air quality data are not available for this rural site, but CETESB considers the air to be clean and unpolluted. Occasional measurements of CO and O<sub>3</sub> were made in Caucaia using colorimetric methods, which showed negligible levels of both pollutants (CO <0.1 ppm, O<sub>3</sub> ~40 µg/m<sup>3</sup>).<sup>12</sup>

### Cultivation and Collection of Samples

*T. pallida* was planted in 60 vases using the same commercial lot of soil, and subsequently 20 vases were distributed at each chosen site. The cultivation vases were maintained over wood platforms 50 cm above the soil. In São Paulo, plants were located approximately 150 m from the roads (in the internal garden of the school and in the garden of the Faculty of Medicine). In Caucaia, there are no roads with significant traffic in the vicinity (at least 3 km). The plants were maintained at the sites for six months before collection. Twenty adult leaves from the third node were collected manually using plastic gloves and placed in paper bags.<sup>14</sup> The collections were carried out monthly for 14 months in the polluted sites and for 12 months in the control site. Samples of soil from the vases also were taken for pH measurements.

### Treatment of the Samples for Analysis

*T. pallida* leaves were washed with deionized water and then dried in an oven at 37 °C for 24 hr. In this process, there was a mean weight loss of 94.3% for samples from Cerqueira César, 94.2% for those from Congonhas, and 95.6% for those from Caucaia. These dried leaves were placed in clean polyethylene bags and sent to the Radiochemistry Division of the Nuclear Energy Research Institute for analysis.

### Standards

Stock solutions of elemental standards were prepared by dissolving high-purity metals, oxides, or salts of elements

with appropriate reagents. Diluted solutions containing one or more elements were prepared from these stock solutions. Aliquots of these diluted solutions were pipetted onto sheets of Whatman No. 41 filter paper using an Eppendorf pipet. After the sheets were dried at room temperature inside a desiccator, they were placed into clean polyethylene bags and irradiated with the samples. In these standards, the quantities of each element were: As (1.5 µg), Ba (50), Br (5.2), Ca (800), Ce (4), Cl (200), Cr (1.5), Co (0.10), Fe (271), K (1000), La (0.50), Mn (1.5), Na (130), Rb (4), Sb (0.6), Sc (0.06), Sr (512), Th (1), and Zn (35).

### Neutron Activation Analysis

First, the fine powder of *T. pallida* leaves was obtained by manual grinding in an agate mortar. Approximately 150 mg of sample weighed in clean polyethylene bags were irradiated at the IEA-R1 research nuclear reactor together with the standards. Short irradiations of 5 min for the determination of Cl, K, Mn, Na, and Sr were carried out using a pneumatic transfer system facility under a thermal neutron of  $1.4 \times 10^{12}$  n/cm<sup>2</sup>/sec. Longer irradiations of 16 hr under a thermal neutron flux of approximately  $10^{12}$  n/cm<sup>2</sup>/sec were performed for As, Ba, Br, Ca, Ce, Cr, Co, K, La, Mn, Na, Rb, Sb, Sc, Th, and Zn determinations. After adequate decay times, the irradiated samples and standards were measured using a hyperpure Ge detector Model GX2020 coupled to a Model 1510 integrated signal processor, both from Canberra. Each sample and standard were measured at least twice for different decay times. Counting times from 200 to 50,000 sec were used, depending on the half-lives or activities of the radioisotopes considered. The radioisotopes measured were identified according to their half-lives and  $\gamma$ -ray energies. The concentrations of elements were calculated by the comparative method.<sup>15</sup> Certified reference materials International Atomic Energy Agency 336 Lichen, National Institute for Standards and Technology (NIST) 1572a Citrus Leaves, and NIST 1575 Pine Needles were also analyzed to control the quality of the results. The results obtained in these determinations showed good accuracy and precision for most elements, with relative SDs lower than 10%. There was good agreement between our results and the certified values.<sup>10,16</sup>

### Procedure for pH Measurement of the Soil Samples

The larger residues of soil samples were separated using a 2-mm fine-mesh sieve. A plastic tube with a capacity of 5 mL was used to transfer similar portions of soil and deionized water to a test tube. The mixture was stirred for

**Table 2.** Mean values of elemental concentrations obtained in *T. pallida* samples cultivated at three sites, the pH of the soil, and the detection limit values.

	Caucaia do Alto	Cerqueira César	Congonhas	Detection Limit Values
As ( $\mu\text{g kg}^{-1}$ )	50.5 $\pm$ 16.7	64.9 $\pm$ 7.8	95.9 $\pm$ 7.9	38
Ba <sup>a</sup> ( $\mu\text{g g}^{-1}$ )	26.6 $\pm$ 2.4	151.1 $\pm$ 6.4	219.9 $\pm$ 11.1	22
Br ( $\mu\text{g g}^{-1}$ )	60.1 $\pm$ 0.3	28.0 $\pm$ 0.1	24.3 $\pm$ 0.1	3.6
Ca (%)	2.4 $\pm$ 0.1	2.9 $\pm$ 0.1	2.6 $\pm$ 0.1	0.12
Ce <sup>a</sup> ( $\mu\text{g kg}^{-1}$ )	278 $\pm$ 28	1029 $\pm$ 29	1304 $\pm$ 24	63
Cl ( $\mu\text{g g}^{-1}$ )	33,521 $\pm$ 856	6361 $\pm$ 164	8096 $\pm$ 208	261
Cr <sup>a</sup> ( $\mu\text{g kg}^{-1}$ )	31.4 $\pm$ 21.7	396.3 $\pm$ 22.7	931.2 $\pm$ 23.6	21
Co <sup>a</sup> ( $\mu\text{g kg}^{-1}$ )	60.4 $\pm$ 2.1	216.5 $\pm$ 4.6	301.9 $\pm$ 5.4	2.0
Fe <sup>a</sup> ( $\mu\text{g g}^{-1}$ )	65.7 $\pm$ 1.6	128.4 $\pm$ 2.2	213.4 $\pm$ 1.9	3.0
K (%)	6.7 $\pm$ 0.3	3.9 $\pm$ 0.4	3.8 $\pm$ 0.4	0.4
La <sup>a</sup> ( $\mu\text{g kg}^{-1}$ )	97.0 $\pm$ 5.1	577.8 $\pm$ 4.5	709.6 $\pm$ 4.2	18
Mn ( $\mu\text{g g}^{-1}$ )	102.2 $\pm$ 3.2	129.7 $\pm$ 2.9	109.7 $\pm$ 3.8	1.1
Na ( $\mu\text{g g}^{-1}$ )	2818 $\pm$ 75	82.1 $\pm$ 10.1	109.7 $\pm$ 14.1	74
Rb ( $\mu\text{g g}^{-1}$ )	51.2 $\pm$ 1.0	34.9 $\pm$ 0.6	15.9 $\pm$ 0.2	0.16
Sb <sup>a</sup> ( $\mu\text{g kg}^{-1}$ )	6.0 $\pm$ 1.7	115.2 $\pm$ 3.0	163.7 $\pm$ 2.9	3.0
Sc <sup>a</sup> ( $\mu\text{g kg}^{-1}$ )	5.2 $\pm$ 0.2	14.8 $\pm$ 0.3	30.9 $\pm$ 0.3	0.5
Sr ( $\mu\text{g g}^{-1}$ )	232 $\pm$ 44	290 $\pm$ 29	292 $\pm$ 33	144
Th ( $\mu\text{g kg}^{-1}$ )	41.0 $\pm$ 5.3	46.5 $\pm$ 3.1	54.9 $\pm$ 2.1	6.2
Zn ( $\mu\text{g g}^{-1}$ )	124.1 $\pm$ 0.7	175.3 $\pm$ 0.9	121.5 $\pm$ 0.5	0.2
pH	6.3	5.6	5.6	

<sup>a</sup> Showed significantly higher concentrations in two polluted sites compared with the control site ( $p < 0.05$ ).

15 min using a mechanical shaker. The soil solution was decanted, and the pH of the soil paste was measured with an Orion pH meter 30 min later.<sup>17</sup>

### Statistical Analysis

Parametric statistical analysis was applied to the elemental concentrations obtained in the analyses. The concentrations of elements were expressed as arithmetic means and SDs. Analysis of variance was used to verify whether there were significant differences between means (for  $p < 0.05$ ). Discriminant analysis was also applied using SPSS software for Windows, version 9.

### RESULTS

Table 2 presents mean values of elemental concentrations obtained in the analysis of *T. pallida* collected at the three sites, the pH of the soil, and the detection limits values obtained according to Currie criterion.<sup>18</sup> The pH values of the soils from the vases kept in Cerqueira César varied from 5.2 to 5.9, with an arithmetic mean value of 5.6 (moderately acid). Soil samples from Congonhas presented a range from 5 to 6.2, with an arithmetic mean value of 5.6 (moderately acid). Soils from Caucaia do Alto vases showed a pH range from 5.5 to 6.8, with an arithmetic mean value of 6.3 (slightly acid).<sup>19,20</sup> The pH of

**Table 3.** Standardized canonical discriminant function coefficients obtained for the elements selected by stepwise procedures.

	Function 1	Function 2
Ba	-0.77219	0.61355
Ca	0.47826	-0.56608
Fe	-0.29650	0.56360
Na	0.78709	0.58812

soil from Caucaia do Alto was significantly different from that of the two other sites.

A statistical test applied to the results obtained for samples collected at the three sites indicated that the plant samples from Caucaia do Alto presented lower concentrations for most elements analyzed. Caucaia do Alto samples showed significantly lower concentrations ( $p < 0.05$ ) of the elements Ba, Ce, Cr, Co, Fe, La, Sb, and Sc when compared with those from Cerqueira César and Congonhas. Five elements that presented higher concentrations in the samples from Caucaia do Alto were Br, Cl, K, and Na ( $p < 0.05$ ). Samples from Caucaia do Alto presented a significant difference only from those obtained in Congonhas to Rb and in Cerqueira César to Zn ( $p < 0.05$ ).

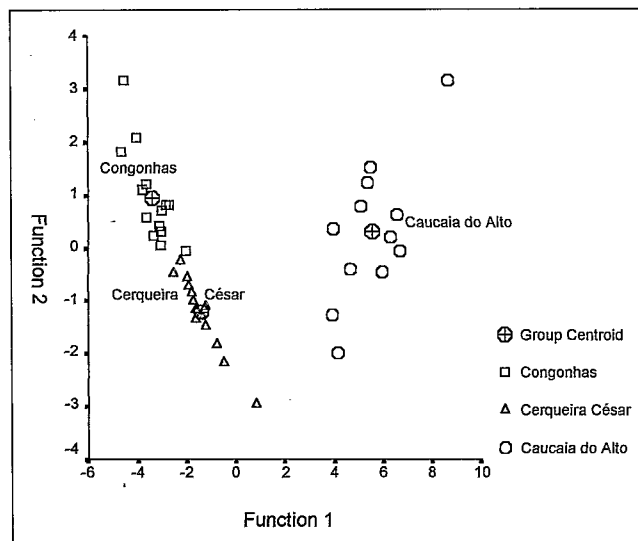
Samples from Congonhas presented higher concentrations of Ba, Ce, Cr, Fe, Sb, and Sc when compared with those from Cerqueira César with a significance level of 0.05. No difference between the control site and the two polluted sites can be seen for the elements As, Ca, Mn, Sr, and Th.

Stepwise discriminant analysis selected Ba, Ca, Fe, and Na to distinguish the groups. Using the discriminant function depicted in Table 3, 97.5% of the cases were properly classified, as shown in Table 4. When the discriminant functions presented in Table 3 were applied, it also was possible to obtain a graphic representation of the spatial distribution of the three groups, as shown in Figure 1, revealing that the distinction among the groups is quite reasonable.

**Table 4.** Classification of the results for the samples taken during the experiment, applying the discriminant function presented in Table 3.

Actual Group	N	Predicted Group Membership		
		Caucaia	Cerqueira César	Congonhas
Caucaia	12	12 (100%)	0 (0%)	0 (0%)
Cerqueira César	14	0 (0%)	14 (100%)	0 (0%)
Congonhas	14	0 (0%)	1 (7.1%)	13 (92.9%)

Note: Percentage of "grouped" cases correctly classified: 97.5%.

**Figure 1.** Individual distribution of the measurements done in the three sites of the study when applying the discriminant canonical functions presented in Table 3. The open dots with a cross inside represent the group centroid, that is, the typical location of each group.

## DISCUSSION

Plants can be used as bioindicators of air pollution, and several studies have been developed to establish the effects of environmental contamination. Because of their capacity to act as efficient interceptors of windborne trace metals, plants are widely used as biomonitors in urban environments.<sup>21</sup> Biological methods may provide data that can be used to estimate the environmental impact and the potential impact on other organisms. Using plants is generally less expensive and particularly suitable for long-term monitoring over large areas, without deploying sophisticated and high-maintenance equipment.<sup>22</sup> In addition, a species with wide geographical distribution allowing comparison of metal concentrations from several regions can draw reliable pollution maps of a large area.

Based on the type and amount of elements accumulated in the leaves, it was possible to identify with great efficiency the regions where plants were exposed to pollutants, as shown in Table 3 and Figure 1. Generally, lichens and mosses are the most common organisms used as biological markers for atmospheric trace elements. Their advantage is that their nutritional needs are provided mainly from aerial sources. In contrast, the higher plants tend to acquire their nutrients from the substratum.<sup>21,22</sup> In our case, it cannot be determined whether pollution accumulation was the result of direct penetration in the leaves through stomata or was dependent on absorption from the soil by the root system. In *T. pallida*, the diameter of the stomata is variable, depending on temperature, relative humidity, hour of the day, and other factors. A series of measurements was made in these

structures, and the maximal lateral opening of stomata was approximately 10  $\mu\text{m}$ . This finding suggests that in the case of penetration of particles into the leaves, we are dealing with the inhalable fraction.

Species and clones of *Tradescantia* are generally used to study the harmful effects of environmental pollution, mostly in terms of genotoxic potential. This property has been evidenced by the micronucleus assay.<sup>11,23-30</sup> This is the first study to explore the capability of one species of *Tradescantia* to accumulate metals in field conditions.

A gradient of accumulation of pollutants was detected in the sites of exposure. Plants maintained in the area of Congonhas tended to present higher levels than did those kept in Cerqueira César, and both polluted sites exhibited much higher levels of most elements than did the control area. These findings were similar to previous measurements of mutagenesis in *Tradescantia* performed in the same locations,<sup>11,12</sup> reinforcing the possible role of this higher plant as a detector of the effects of airborne contaminants.

Based on the elemental composition of leaves taken from areas with different pollution backgrounds, it was possible to distinguish a clean region from two polluted areas with good precision (Table 4, Figure 1). Moreover, based on the amount of pollutant elements measured in the plants, it was possible to distinguish the two polluted areas with good precision (97.5%) (Table 4, Figure 1), the more polluted area presenting higher levels of accumulated pollutants (Table 3).

The plants were cultivated in vases with the same commercial soil sample and then were distributed to the different sites. The sample collection began after six months. The analysis of the soil showed that the pH of Caucaia do Alto soil sample was slightly acidic and significantly different from the other two sites. The moderately acidic pH from the Cerqueira César and Congonhas soil samples was probably sustained by the influence of the polluted atmosphere at these two sites.<sup>31</sup>

Barium is an element that has many applications in the automotive industry, including rubber production and lubricant oil additives, and it has been found in samples of gasoline and diesel oil. It seems to be valuable as a tracer for vehicle emissions, but it also has a close relationship with soil dust and elements of environmental concern (e.g., the manufacture of glass, ceramics, television picture tubes, fireworks).<sup>32,33</sup> This assertion agrees with the data obtained in the present work, because Ba concentrations in samples from polluted sites were higher than those from the clean site.

The use of catalytic converters in motor vehicles and the widespread use of some elements in solid-state laser and superconducting materials are introducing elements that occur in extremely low concentrations in nature. In

particular, lanthanoids are acquiring a greater significance in ecotoxicological research because of their increased emissions.<sup>2,4,16</sup> This situation could explain the high concentration of Ce in both the Congonhas and Cerqueira César districts. This element might be related to emissions from diesel oil, gasoline, alcohol, and kerosene used in aviation. Lanthanum also presented higher concentrations in polluted sites. These data suggest the necessity of detailed studies on lanthanoids in environmental pollution.

A suitable species of plant, analyzed by a standardized method, could improve the sensitivity of biomonitoring for a specific metal of interest. In this context, *T. pallida* seems to be an adequate species for this purpose, because of its capability to accumulate several elements involved in environmental pollution. Our results point toward the potential use of *T. pallida* as an accumulator of air pollution in areas where instrumental methods are not available.

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