Evaluation of the toxic effects of urban levels of air pollution using *Tradescantia* bioassays

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Abstract

Medical studies have provided evidences that air pollution causes adverse health outcomes, even when not surpassing the safety levels prescribed by regulatory air standards. Besides, the human activities may deteriorate air quality in areas lacking of pollution measurements, making impossible to establish strategies to preserve public health. Plant bioassays are alternative methods for monitoring ambient air pollution, potentially overcoming the mentioned problem. In the present paper, we present results of a series of biomonitoring studies performed with plants of *Tradescantia* to evaluate the toxicity of urban atmosphere in the city of São Paulo (SE Brazil). We demonstrated that it is possible to detect urban/rural gradients of air pollution by developing the *Tradescantia* micronucleus assay (Trad-MCN) with *Tradescantia pallida* (Rose) Hunt. cv. Purpurea Boom, a garden plant widely used in Brazil. We also revealed the clastogenic effects of aqueous extracts of inhalable particles sampled in São Paulo downtown using the same bioindicator plant species. We investigated the toxic effects of the plume emitted by a solid waste incinerator using the stamen-hair assay with clone 4430 of *Tradescantia* and determined different elemental accumulation in leaves of *T. pallida* exposed to distinct degrees of air contamination, showing that it is possible to distinguish specific sources of air pollution with *Tradescantia*. Finally, we proved that particles significantly contribute to the ambient toxicity by combining selective filtering with *in situ* exposure, employing the stamen-hair assay with clone KU-20 of *Tradescantia*. A preliminary linear relation between the predicted increase in daily children mortality caused by respiratory diseases due to PM_{10} and the concurrent measures of the frequency of pink mutations in stamen hairs of clone KU-20 was obtained, indicating that *Tradescantia* bioassays may be indicators of risks to human health.

Key words: **air pollution, tradescantia, bioassay, plant, biomonitoring**

Introduction

Air pollution and human health – sizing the problem

The knowledge that air pollution promotes adverse health effects is not new. In the first half of the 20th century the episodes of severe air pollution in the Meuse Valley in Belgium [1] and London [2] were so clear in demonstrating acute health effects of air pollution that promoted public awareness and the promulgation of laws regulating emissions from stationary sources. In consequence of su measures, air pollution decreased in most of large western urban centres attaining levels below the air quality standards. The problem of urban air pollution and health was considered solved by most of those involved in regulatory agencies. However, studies made in the late 1980s provided evidence that air pollution causes adverse health outcomes, even when not surpassing the safety levels prescribed by regulatory air standards. A study done by POPE in Utah Valley [3] demonstrated that pollution is still a topic of major concern. In this work, POPE [3] followed the ambient levels of atmospheric particles small enough to penetrate into the lungs (aerodynamic diameter below 10 µm in Utah Valley before and after the closure of a steel mill (1985 and 1986, respectively). A significant decrease of ambient particles was observed during the closure, indicating that this source of combustion particles contributed significantly to ambient pollution in the area. After the reopening of the steel plant, particle concentrations returned to their historical levels. Respiratory admissions followed with a surprising coherence the levels of particles, now in a range of concentration within an order of magnitude smaller that those of London in 1952. The paper of POPE [3] took advantage of another natural experiment – the closure of a significant source of air pollution – to show that even low levels of particles affect adversely human health. Since then, a solid amount of knowledge has been produced on the relationship between air pollution and health, which can be summarised as follows [4–27]:

- There is no significant safety threshold in the relationship relating air pollution to mortality.
- The time lag between increase in air pollution and increased mortality is very short, within a range of few days.
- Elderly people, children and those with previous respiratory and cardiovascular diseases are the most affected in polluted days.
- Chronic effects of air pollution were also detected in the exposed population, leading to decreased life expectancy and higher rate of respiratory malignancies.
- Experimental studies provide support to epidemiological data, reporting significant respiratory and cardiovascular abnormalities in animals exposed to urban levels of air pollution, as well as greater susceptibility to develop lung neoplasms.

The aforementioned data clearly disclose a clear picture of the burden imposed by air pollution to the exposed population, justifying the necessity of studies aiming to define the impacts as well as the mechanisms responsible for the damage mediated by air contaminants on biological systems. Such information is mandatory to establish rational regulatory policies.

The potential role of plant bioassays in monitoring ambient air pollution

In areas where air pollution distribution changes rapidly, such as developing countries, the availability of air pollution monitoring networks is frequently surpassed by the availability of adequate air pollution measurements. In other words, traffic and industrial activities of growing economies may deteriorate air quality in areas lacking of pollution measurements, making impossible to establish strategies to preserve public health. In such context, biomonitoring approaches may be employed for environmental screening and determination of pollution sources.

Our group performed a series of studies to evaluate the toxicity of the urban atmosphere of the city of São Paulo – SE Brazil, employing *Tradescantia* and comparing the results with data of physicalchemical measurements of air pollution. In the present paper, we will present the basic results of this initiative, showing that *Tradescantia* bioassays can be used to evaluate air contamination.

Experimental description

1st Study: Detection of urban-rural gradients of air pollution using the *Tradescantia* micronucleus test

One possibility of assessing the potential of *Tradescantia* bioassays to characterise differences in air pollution is to determine their responses when facing urban-rural gradients of air pollution. In fact, the Metropolitan Area of São Paulo is well suited for this purpose, since marked differences of air pollution can be found within a limited distance. In our first approach [28] we measured the frequency of micronuclei in pollen mother cells (Trad–MCN) of *Tradescantia pallida* (Rose) Hunt. 'Purpurea' Boom, a garden plant widely used in Brazil. Potted plants were exposed in an area devoid of primary pollutants (Caucaia, CO levels under 0.2 ppm) and compared to those grown in two polluted sites of São Paulo downtown: School of Medicine (CO levels about 5 ppm) and Congonhas (CO levels above 8 ppm). Briefly, 15 to 20 young inflorescences from each experimental site were dissected and early tetrads of the meiotic microspore mother cells were squashed in aceto-carmine stain on a microslide [29]. Significant differences among the groups were detected, the higher frequency of micronuclei following the higher levels of air pollution (Figure 1).

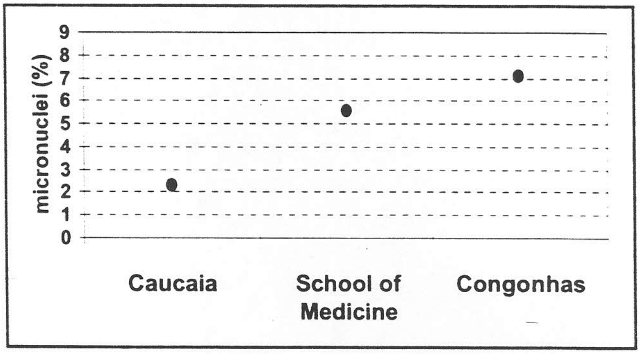

Fig. 1. Mean frequency of micronuclei (and corresponding standard errors) measured in pollen mother cells of *Tradescantia pallida* grown in areas with low (Caucaia), intermediate (School of Medicine) and high levels (Congonhas) levels of traffic pollution.

2nd Study: Exploring the clastogenic effects of aqueous extracts of inhalable particles sampled in São Paulo downtown

When selecting a plant bioassay to characterise the toxic effects of air pollution on human health, it would be wise to know the response of the system to the pollutants more directly related to adverse health effects. The majority of published studies identify small particles as the pollutant that exhibits the most robust associations with adverse health outcomes. Urban air contains solid and liquid particles that vary in size, composition, and origin. Depending on their size, particles may be classified in three categories, namely coarse particles, fine particles, and ultrafine particles. Coarse particles (aerodynamic diameter > 2.5 μm) are derived primarily from soil and other crustal materials. Fine particles ($PM_{2.5}$) are derived mainly from combustion processes. Sulphate and nitrate particles are commonly generated by conversion from primary sulphur and nitrogen oxide emissions, and a varying portion of sulphate and nitrate particles may be acidic. Thus, urban particles are composed by primarily emitted particles due combustion and those produced by gas-particle conversion including sulphates and nitrates. Ultrafine particles (< 0.1 μm) have relatively short residence times in the atmosphere because they accumulate or coagulate to form larger fine particles. We collected $PM_{2.5}$ particles in São Paulo downtown, using Harvard impactors coupled to cellulose filters. Cuttings of *Tradescantia pallida* were immersed during 8 hours in solutions with increasing concentrations of particle extracts and let to recover for 24 hours. After this, micronuclei were scored in pollen mother cells, as described above. A clear dose-response pattern was observed [30], showing that ambient urban particles contain clastogenic substances, as shown in Figure 2.

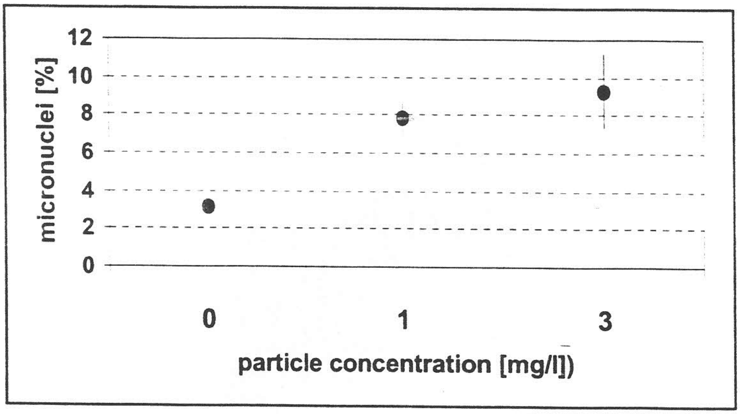

Fig. 2. Mean frequency of micronuclei (and corresponding standard errors) observed in pollen mother cells of *Tradescantia pallida* exposed to different aqueous extracts of urban particles during 8 hours.

3rd Study: Is it possible to distinguish specific sources of air pollution with *Tradescantia?*

We approached this question in two sets of experiments. In the first study [31], we investigated the toxic effects of the plume emitted by a solid waste incinerator located in São Paulo downtown, using the stamen-hair assay in the *Tradescantia* clone 4430 [32]. This test is based on the fact that the stamen-hair cells of this plant are heterozygous for stamen hair colour, allowing the detection of mutations on the basis of their pigmentation change from blue (dominant) to pink (recessive). The incinerator we investigated was built in the 1950s and presented high levels of emission of furans and dioxins, with a quite defined area of dispersion. On the basis of its dispersion model, we maintained our plants during 5 months in 4 points: clean area (Jaguariúna), and areas with low (School), medium (Museum) and high (Incinerator) levels of contamination. A clear increase in mutation rate was detected as incinerator emissions got more important (Figure 3), showing that a single emission source can be tracked using our system even in an area already contaminated by emissions of millions of vehicles. The results of this study contributed to the closure of this incinerator, a measure that had been postponed by the Municipality for several years.

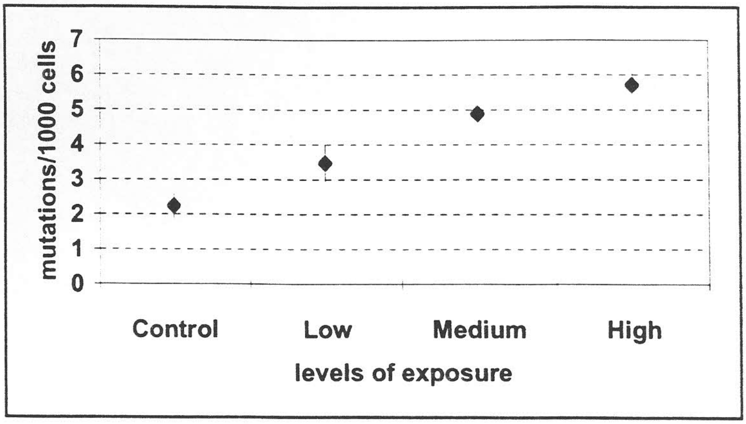

Fig. 3. Mean (and corresponding standard errors) of the frequency of mutations in stamen hair cells of *Tradescantia* clone 4430 kept in areas with different levels of influence of the plume emitted by a solid waste incinerator located in São Paulo downtown.

In the second experiment, we tested the potential of *Tradescantia* as an accumulator plant, allowing the characterisation of not only the amount but also the source of atmospheric pollution in a given spot. In this study [33], we kept pots of *Tradescantia* during one year in the same sites – Caucaia, School of Medicine and Congonhas – referred in the 1st study [28]. Leaves were sampled on a monthly basis, and their elemental composition was determined by neutron activation analysis. Factor analysis provided the identification of three factors, namely soil, Otto and Diesel emissions. Discriminant analysis revealed that it was possible to distinguish, in 100 % of the cases, the samples collected in Caucaia from those obtained from plants kept in São Paulo. In addition, it was possible to distinguish the two polluted areas with a precision of 92.9 % based on the elemental composition of the leaves. These results open the possibility of using *Tradescantia* assays not only to measure biological effects but also to characterise source and levels of air pollution.

4th Study: Is it possible to identify specific components responsible for the toxic effects of air pollution using *Tradescantia* assays?

The problem of identifying the mechanism by which air pollution damages people's health is not trivial. First, we have to consider that there is not a single pollutant in the atmosphere of polluted cities. The exhaust pipe of a truck or a chimney of an industry blows several gases and particles together. It is quite plausible that air pollution toxicity is not dependent on a single component, but of the sum of individual components present in the air. In addition, these primary compounds react among themselves in an extent dependent upon dispersion, humidity and solar irradiation. Thus, the process of evaluating the isolated and/or combined effects of constituents of air pollution implies in the many experiments either concentrating or subtracting components of the complex mixture of atmospheric pollutants. Due to their simplicity, low cost and high efficiency, plant bioassays may play a relevant role in this process.

We moved further on the analysis of the contribution of particles to ambient toxicity by combining selective filtering with *in situ* exposures, employing the *Tradescantia* stamen-hair assay. In these experiments, we used *Tradescantia* clone KU-20, a quite robust and sensitive species. During the winter of 2002, we compared the frequency of spontaneous pink mutations in KU-20 plants in Caucaia, (ambient levels of particles below 10 μm of aerodynamic diameter – PM_{10} – of 14 $\mu g/m^3$ in the period of study) with that of plants kept in the School of Medicine (ambient PM_{10} levels of 64 $\mu g/m^3$). In the same study we brought *Tradescantia* cuttings from Caucaia to São Paulo, keeping them either in open air or in a chamber with a filter with pores of 0.8 μm. Plants growing in São Paulo presented a higher frequency of spontaneous mutations than that of Caucaia. Cuttings brought from Caucaia to São Paulo exhibited a frequency of mutations similar to that presented by the plants originally kept in São Paulo. The time needed to the cuttings brought from Caucaia to reach the mutation frequency of São Paulo was 5 days. Filtering particulate material reduced significantly but not abolished the toxic effects of São Paulo's atmosphere (Figure 4).

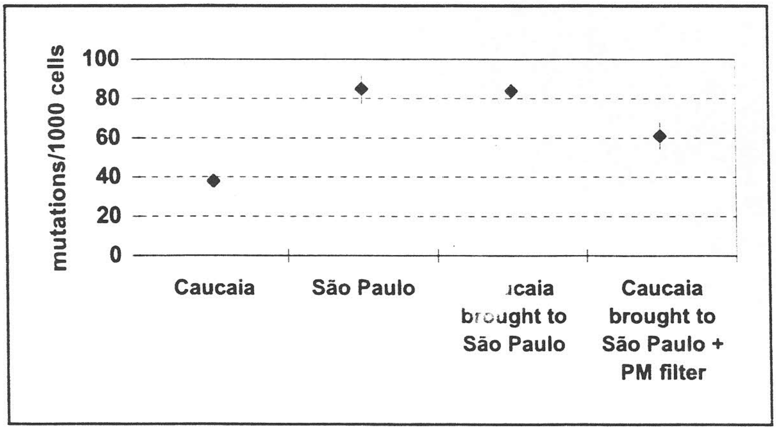

Fig. 4. Mean (and corresponding standard errors) of the frequency of mutations of stamen hair cells of *Tradescantia* clone KU-20 submitted to different exposure regimes.

The above presented results suggest that PM is responsible for a substantial fraction of the toxic effects of air pollution in São Paulo, but that gaseous pollutants (not retained in our filtering system) also play a significant role in environmental toxicity in our town. Presently, we are "peeling out" specific particle components, such as metals or organic compounds, to evaluate the comparative toxicity of particle constituents, as well as employing gas filters to better characterise the toxicity of trace gases of our atmosphere. The measures are of paramount importance to guide controlling strategies aiming air pollution control and, again, our plant system is providing results in a timely and efficient way.

Final remarks: Is it possible to extrapolate findings obtained with *Tradescantia* systems to human health effects?

The finding of positive and robust associations between changes in *Tradescantia* and measures of air pollution evokes our medical bias, which makes us prone to establish relationships relating health effects and alterations in our plants. For instance, in the studies reported in the 4th study, a significant association between PM_{10} levels of the 5th day before flower opening and the frequency of pink mutations stamen hair cells in *Tradescantia* clone KU-20 (r = 0,5, p = 0.025). If one computes the increase in mortality attributed to PM_{10} from previous epidemiological studies done in São Paulo, it is possible to compute possible relationships between changes in mutation rate in *Tradescantia* and increases in health outcomes. We plotted the predicted increase in children mortality due to respiratory diseases due to PM_{10} for the days we measured the frequency of pink mutations in study 4 and obtained the relationship depicted in Figure 5.

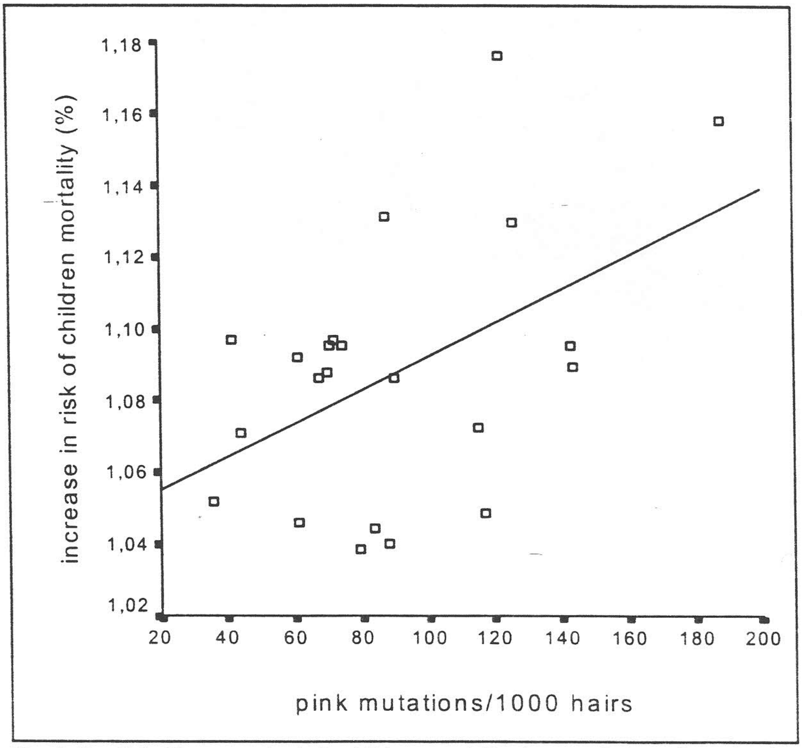

Fig. 5. Predicted increase in daily children mortality due respiratory diseases due to PM_{10} and the concurrent measures of the frequency of pink mutations measured in *Tradescantia* clone KU-20.

The question that is necessary at this point is the above relationship true? Of course, it is too preliminary to reach a definite position, but we sincerely think that further studies should be made to verify whether it is possible to close the gap between human toxicology and plant bioassays.

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