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# Exploring the clastogenic effects of air pollutants in São Paulo (Brazil) using the *Tradescantia* micronuclei assay

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**Abstract**

This study was designed to determine the clastogenicity of particulate matter (aerodynamic diameter smaller than 10 μm) in the urban polluted air in the city of São Paulo. The *Tradescantia*-micronucleus (Trad-MCN) assay was used throughout this study to evaluate the clastogenicity of the extracts of the particulate matter. *Tradescantia pallida* (Rose) Hunt. cv. *purpurea*, an indigenous cultivar, was used in the Trad-MCN assay. The efficacy of this plant material for the Trad-MCN assay was validated with dose–response studies using formaldehyde and beta radiation. Dose–response curves were established with these known mutagens. The extracts of the $PM_{10}$ particles at concentrations between 5 and 50 ppm induced a dose-related increase in MCN frequencies. The results indicate that *T. pallida* is equally sensitive to mutagens as the standard *Tradescantia* clone 4430 or 03 and the particulate matter in the urban air are clastogenic to the chromosomes of this plant. Inhalation of these particles by urban dwellers may affect their health by inducing similar genetic damage. 

*Keywords:* Clastogenicity; Micronucleus; Air pollutant; *Tradescantia*

## 1. Introduction

Epidemiological studies have systematically disclosed an increased rate of lung tumors in urban dwellers when compared with individuals living in the countryside [1–4]. Air pollution has been evoked as the possible explanation for this urban–rural difference in lung malignancies [5,6], but the clear demonstration of a causal association is difficult to obtain through the epidemiological survey, mostly because of the confounding variables such as smoking, population density and occupational profile.

In order to explore the possible correlation between urban air pollution and lung tumorigenesis, we

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recently performed a field study in which mice exposed to polluted air in downtown São Paulo, and compared with their controls kept for the same period and in similar conditions in a rural setting [7]. There were marked differences in lung tumor development in the treated and control groups. This result is probably due to the fact that the atmosphere of the city of São Paulo was contaminated with several compounds which were derived from the combustion of fossil fuels, especially diesel exhaust fumes. In this regard, it has been established that polycyclic aromatic hydrocarbons in urban atmosphere are contained mostly in the fine particles that were found in the intrapulmonary deposition [8]. Interestingly enough, most of the studies focusing on the mutagenic effects of hydrocarbons absorbed in the particulate matters of urban air requires the use organic of solvents for their extraction. Since there is no solvent-extraction process existing in the pulmonary environment, such studies would create an artificial situation that, in some circumstances, could mislead the interpretation of the effects of urban particles in enhancing lung carcinogenesis.

Plant bioassays are best suited for addressing these clastogenic effects of 'real world' air pollution, with special emphasis on its soluble fraction. The Trad-MCN (*Tradescantia* clone 4430) bioassay has been extensively used to monitor genotoxicity in the environment [9,10]. In this study, we used pollen mother cells of *Tradescantia pallida* (Rose) Hunt. cv. *purpurea* Boom in the Trad-MCN assay. This cultivar is a popular ornamental plant, which is widely grown in the gardens, roadside and streets of São Paulo.

The Trad-MCN bioassay using *T. pallida* was first standardized with the known mutagens such as X-irradiation and formaldehyde. Particulate matters ($PM_{10}$) of urban air were assayed directly after simple water extraction.

## 2. Materials and methods

The particulate matters of the urban air were collected from downtown São Paulo with a Hi-vol air sampler with a No. 10 filter. Extracts were made with distilled water and used for treatment of the plant cuttings.

### *2.1. Experiments 1 and 2: Testing for the proficiency of T. pallida for the Trad-MCN assay to known carcinogen*

Cuttings of *T. pallida* were obtained from natural populations grown in the gardens of the School of Medicine of the University of São Paulo to conduct the Trad-MCN assay. Cuttings were kept in Hoagland's solution in our laboratory for 24 h, and then submitted to either formaldehyde (Experiment 1) treatment or X-irradiation (Experiment 2). Plant cuttings were grouped at random before treatment. In the formaldehyde experiment, cuttings were immersed in freshly prepared solutions at dosages of 250 and 500 ppm concentrations for 6 h. X-irradiation was given at dosages of 1, 10 and 50 mCy by means of a therapeutic X-ray device (Stabilipan Siemens), operated at 250 kV, 15 mA, with a Thorens 1 filter. For both formaldehyde and X-ray experiments, cuttings were allowed to recover for 24 h in Hoagland's solution and then fixed in 1:3 acetic acid/ethanol solution for 24 h.

### *2.2. Experiment 3: Testing the clastogenic effects of urban air particulate matter, $PM_{10}$*

$PM_{10}$ was collected at the School of Medicine of the University of São Paulo, located in downtown São Paulo. The air sampler was placed at a height of 20 m to minimize the influence of the resuspension of soil dust from the nearby streets. The air sampler was constructed with an inlet that provided a 50% cutoff diameter of 10 μm. The inlet was coupled to an impactor, to collect only particles smaller than 10 μm. The sampling was done during at least 48 h, the particles being trapped in Teflon filters that were weighed before and after collection to determine the dry weight of the particulate matters collected on the filter. Immediately after weighing, the filters were immersed in sufficient distilled water to make concentrations of 15 and 30 mg/l, and then agitated for 30 min. These extractions were used for testing. Treated and control inflorescences were fixed and stored for slide preparation.

### *2.3. Protocol of the Trad-MCN test*

Inflorescences were dissected and young anthers squashed in a solution of acetocarmine stain on a

microslide [9]. Only preparations containing early tetrads were considered. Examination was done at a magnification of 400 ×, and 300 tetrads were examined per slide. The counting of micronuclei was done on coded slides, the key being revealed only after the completion of the experiment.

### 2.4. Statistical analysis

Statistical analysis was done using analysis of variance and post-hoc contrast was performed by the Tukey method. When necessary, logarithmic transformations were employed to stabilize variance among groups. The level of significance was set at 5%.

## 3. Results

Fig. 1 shows the mean and standard errors of the micronuclei counted in the formaldehyde studies. A significant ($p = 0.0005$) difference was observed among groups, being the exposed groups different from the control.

Fig. 2 depicts the micronuclei counted in the radiation experiments. A clear dose-dependent pattern may be identified, with significant differences

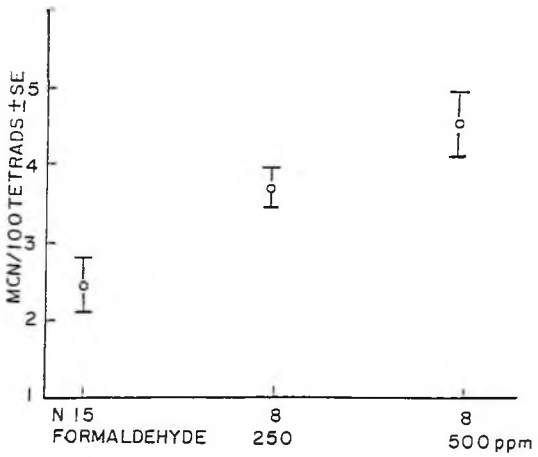


Fig. 1. Mean values and standard errors of the micronuclei obtained in pollen mother cells of *T. pallida* after exposure to different concentrations of formaldehyde. *N* represents the number of cuttings studied for each dose. The control is significantly different from the remaining groups.

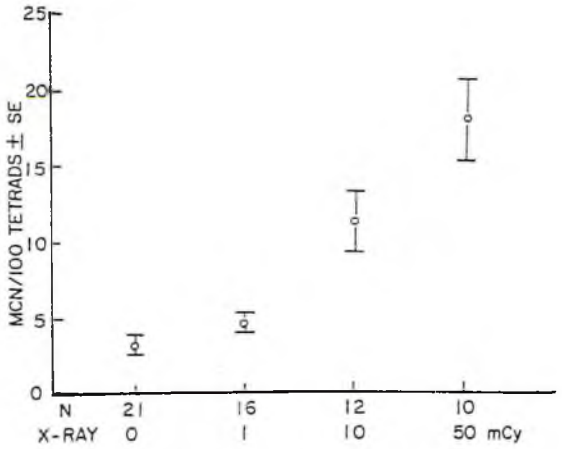


Fig. 2. Mean values and standard errors of the micronuclei obtained in pollen mother cells of *T. pallida* after exposure to different levels of radiation. *N* represents the number of cuttings studied for each dose. Groups exposed to 10 and 50 mCy are significantly different from 1 rad and control.

observed among the experimental groups ($p < 0.0001$). Pollen mother cells exposed to 10 mCy had significantly higher counts of micronuclei than that obtained for 1 rad and the control. Cuttings exposed to 50 mCy were significantly more affected than all the other groups.

Fig. 3 depicts the effects of progressive concentrations of urban $PM_{10}$ on pollen mother cells of *T.*

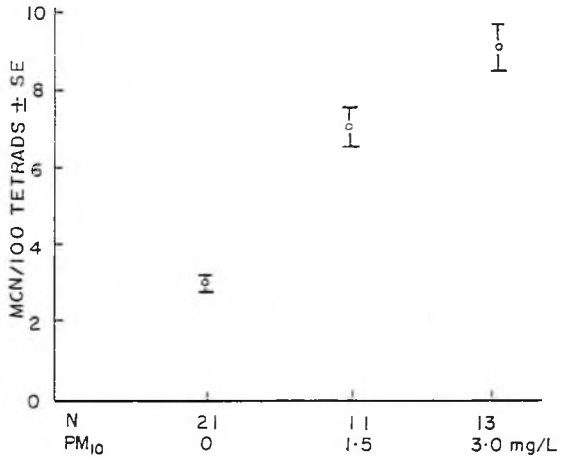


Fig. 3. Mean values and standard errors of the micronuclei obtained in pollen mother cells of *T. pallida* after exposure to different concentrations of $PM_{10}$. *N* represents the number of cuttings studied for each dose. The group exposed to 30 mg/l is significantly higher than that of the others.

*pallida*. A significant difference was observed among groups ($p = 0.008$), the concentration of 30 mg/l exhibiting a higher count of micronuclei than the remaining groups.

## 4. Discussion

The choice of *T. pallida* was based on two criteria generally taken into consideration in biomonitoring, its wide distribution in São Paulo and its easy propagation, even in situations of high levels of contamination, such as the central areas of our jammed avenues. This condition would allow the use of *T. pallida* in field experiments across metropolitan areas of São Paulo, either using specimens prepared in our laboratory or sampling plants already present in the field.

As the first step in our studies, we decided to test the sensitivity of *T. pallida* to known mutagens, testing its capacity to respond in terms of micronuclei formed in pollen mother cells. The results depicted in Figs. 1 and 2 clearly indicate that *T. pallida* responds to mutagens with a dose-dependent pattern. In fact, the response of *T. pallida* to radiation was similar to that of *Tradescantia* clone 4430 [9].

After testing the sensitivity of *T. pallida*, we performed experiments to assess the effects of urban $PM_{10}$ on DNA. Samples of $PM_{10}$ collected from the air in downtown São Paulo, were dissolved in water and incubated with inflorescences of *T. pallida*, providing positive results (Fig. 3). It is important to stress that in this study we employed water solutions of $PM_{10}$, which are not probably rich in polycyclic aromatic hydrocarbons, known to exhibit clear mutagenic capability. The soluble fraction of $PM_{10}$ is also constituted by primary and secondary oxidants, metals and aldehydes that exhibit mutagenic capability [11], and in the conditions of the present study, are the most plausible causative agents for the clastogenic effects as well.

In conclusion, our study indicates that the Trad-MCN test using *T. pallida* is sensitive to clastogens. In addition, the exposure of *T. pallida* to the soluble fraction of $PM_{10}$ is able to induce clastogenesis. Our results support the concept that urban dwellers are chronically exposed to environmental genotoxicants.

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