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In situ biomonitoring of the genotoxic effects of vehicular pollution in Uberlândia, Brazil, using a *Tradescantia* micronucleus assay

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ABSTRACT

The growing number of cars in large cities is directly linked to changes in the chemical composition of urban air, which has increasingly high concentrations of potentially genotoxic chemicals. Therefore, discovering and monitoring the risks associated with exposure to atmospheric pollutants is indispensible for preventing environmental and health problems. Because of the lack of reliable data regarding the air quality in the city of Uberlândia, the present study sought to test whether the genotoxic risks in areas with different levels of vehicular traffic can be measured using the *Tradescantia* micronucleus assay (Trad-MN). Therefore, more than twenty inflorescences were exposed to locations with different amounts of vehicular traffic twice per year from the winter of 2006 to the summer of 2011. The inflorescences were then analysed to determine the micronucleus (MN) frequency. In addition, we sought to determine the influence of factors linked to city climate on the MN frequencies obtained at each monitored location. Our results show that, although low relative humidity positively influenced MN formation in *Tradescantia pallida* tetrads, the major determining factor for clastogenic events was the level of vehicular traffic at the locations monitored over the five-year study.

1. Introduction

The current dilemma between economic growth and environmental quality in developing countries is exemplified by the growth of vehicular traffic in Brazil.

According to the Brazilian Department of Transportation DENATRAN, (2011), the country has approximately 70 million automobiles. The country's southeastern region accounts for 50.9 percent of this total, and the state of Minas Gerais alone has 7.6 million vehicles. Uberlândia has the second highest vehicular fleet in the state of Minas Gerais. However, no studies have investigated the potential biological risks caused by the increase in air pollution in the city.

The air in large cities is usually contaminated by pollutants generated from the fuel burning that occurs in automobile engines. Among these contaminants, heavy metals, organic compounds, particulates and sulphur oxides all have serious environmental (Isidori et al., 2003; Umbuzeiro et al., 2008) and health (Mariani et al., 2009) risks.

Due to the growing need to monitor air quality, numerous studies have used plants as biomonitors (Misik et al., 2011). Factors such as simplicity, low cost and high sensitivity make the *Tradescantia* micronucleus assay (Trad-MN) a key tool for detecting the clastogenic effects caused by vehicle emissions (Batalha et al., 1999; Carreras et al., 2006; Carvalho-Oliveira et al., 2005; Guimarães et al., 2000; Klumpp et al., 2006; Meireles et al., 2009; Misik et al., 2006; Prajapati and Tripathi, 2008; Savóia et al., 2009; Umbuzeiro et al., 2008; Villarini et al., 2009). The assay consists of detecting the micronuclei arising from breaks that occur in the chromosomes of pollen grains during meiosis.

The present study used the Trad-MN assay as part of an air quality biomonitoring programme in the city of Uberlândia. The relationship between micronucleus (MN) formation in *Tradescantia pallida* (purple queen) tetrads and vehicular traffic was examined over five years, with consideration given to the effects that climatic factors had on the assays at each monitoring location.

2. Materials and methods

2.1. Monitored locations

According to the Brazilian Institute of Geography and Statistics (IBGE, 2011), the city of Uberlândia is located in southeastern Brazil, covers 4115 km^2 and has an estimated population of 611,903 inhabitants. Uberlândia has a high-altitude tropical (Cwa) climate (Peel, 2007) with dry winters and rainy summers. The average temperature does not change greatly throughout the year, averaging 22.3 °C (INPE/CPTEC, 2011).

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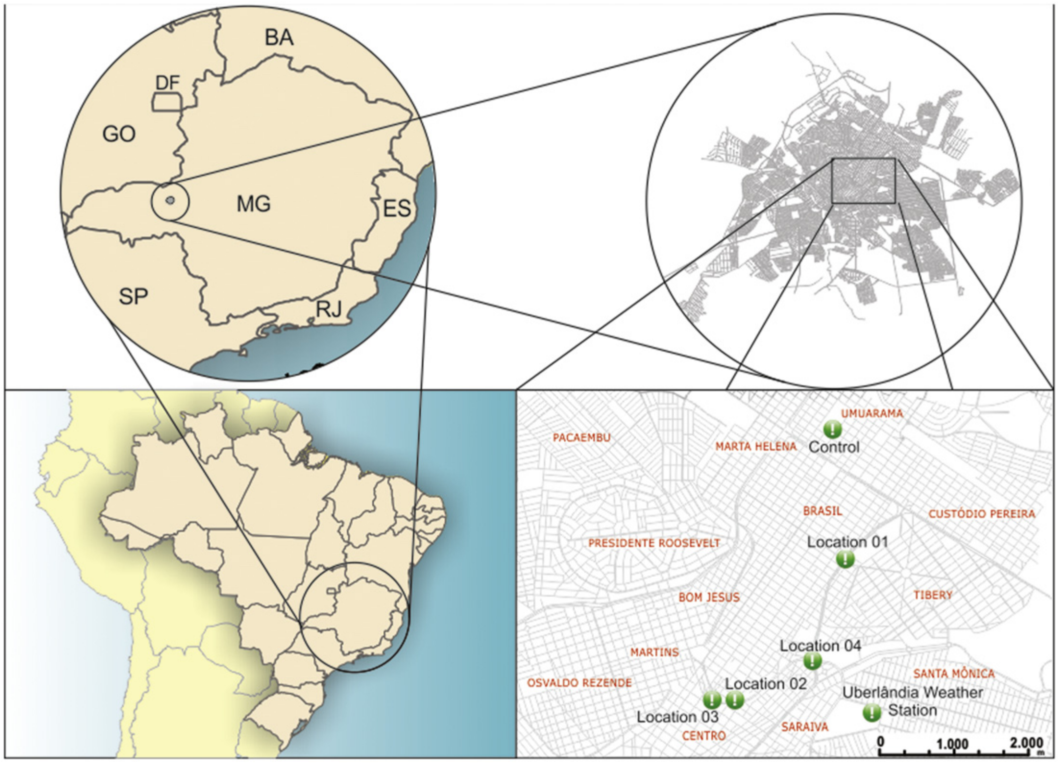

Fig. 1. Map of Uberlândia City showing the monitored locations, control and the Uberlândia weather station.

The monitored locations (Fig. 1) were required to meet four criteria: different rates of vehicular traffic, similar proportions of bus and truck traffic, the daily monitoring of traffic flow by the Uberlândia City Secretary of Transit and Transportation (Secretaria Municipal de Trânsito e Transportes de Uberlândia, SETTRAN) and close proximity to the Uberlândia weather station (18°55′01.63″S, 48°15′20.60W). Four locations were chosen for biomonitoring based on these criteria: (Location 1) the intersection of Rondon Pacheco and Paraná Avenues (18°53′58.42″S, 48°15′31.76″W), (Location 2) the intersection of João Naves de Ávila and Cesário Alvin Avenues (18°54′58.42″S, 48°16′21.37″W), (Location 3) the intersection of Floriano Peixoto and João Naves de Ávila Avenues (18°54′57.59″S, 48°16′27.70″W) and (Location 4) the intersection of Rondon Pacheco and João Naves de Ávila Avenues (18°54′40.92″S, 48°15′45.49″W). The garden at the Federal University of Uberlândia, Institute of Biology (18° 53′08.85″S, 48°15′34.64″W) was used as a control, as it does not have any vehicular traffic.

The Climatology and Water Resources Laboratory at the Federal University of Uberlândia is responsible for the city's weather station, and collected all of the data indicating the meteorological conditions (air temperature, relative humidity, insolation and rainfall) that the plants were exposed to during each biomonitoring period. Locations close to the weather station were chosen to ensure that the weather conditions were similar and known. The number of vehicles that drove through the monitored locations during the plant exposure times was estimated and given to us by SETTRAN.

2.2. Plant growth and exposure

Plants from the species *T. pallida* (Rose) Hunt. cv. *purpurea* Boom were grown in the Federal University of Uberlândia greenhouse. The plants were cultivated in flowerpots using standardised commercial soil ED73, weekly irrigated and fertilised with a commercial liquid NPK (10:10:10) fertiliser. The temperature and relative humidity inside the greenhouse were controlled. The cultivation conditions consisted of day/night temperatures of 26/19, day/night relative humidity of 65%/70% and a day light cycle of 16 h to induce flowering. All of the plants were derived from the same individual by vegetative propagation. The spontaneous mutation rates for the plants in the greenhouse were regularly tested and did not exceed 2 MN/100 tetrads. These data, however, were not included in the between-exposure comparisons at the different monitored locations.

Twenty inflorescences were exposed to each monitored location during winter (dry season) and summer (rainy season), for a total of 10 exposures per location over the course of five years (winter 2006–summer 2011). The inflorescences were exposed to the monitored locations on workdays between 6:00 am and 11:59 pm, for a total of 18 h of exposure.

For exposure, exposure boxes (25 cm $\times$ 25 cm $\times$ 35 cm) with a hole to accommodate the plastic container with the plants, and a shading fabric (50%) to cover the upper part of the boxes were used to protect the plants from excessive heat and insolation, according to Klumpp et al. (2006). To avoid contamination by soil pollutants, the boxes containing the plants should be kept on stands at a height of 2 m above the ground.

2.3. Trad-MN assay

The micronucleus frequency was measured for a minimum of five inflorescences from each monitoring location. After fixation in a 1:3 acetic acid to 70% ethanol solution for 24 h and preservation in 70% ethanol, the Trad-MN assay protocol developed by Ma et al. (1994) was used. Five slides were prepared for each monitoring session at each location to detect the presence of micronuclei. The analyses were performed using an optical microscope at 400 $\times$ magnification. 300 tetrads on each slide were analysed. Fig. 2 shows a photomicrography of early pollen tetrads of *Tradescantia* with (Fig. 2a) and without MN (Fig. 2b).

2.4. Statistical analysis

The differences between the MN frequencies measured during the experiments were tested using one way Analysis of Variance (ANOVA) and then compared using the Dunnett test at a 1% significance level.

A multiple regression analysis was performed to test for a relationship between MN frequency, vehicular traffic and weather conditions (air temperature, relative humidity, insolation and rainfall) at the monitoring locations. The regression curve was successively adjusted so that only the factors that significantly contributed to the MN variances remained in the model.

3. Results and discussion

The number of vehicles being used in the city increased from 205,510 in 2006 to 338,328 in 2011—a significant increase of nearly 65%, according to *t*-test ($p < 0.01$). These data obtained from SETTRAN showed that the number of vehicles at the monitoring locations increased during every period evaluated (Fig. 3). The control location is not shown in the graph, as it represents no vehicles.

Weather conditions typical for the Cwa climate were found in the monitored region during the plant exposure times (Table 1).