SERVIÇO PÚBLICO FEDERAL
UNIVERSIDADE FEDERAL DE UBERLÂNDIA
INSTITUTO DE BIOTECNOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM GENÉTICA E BIOQUÍMICA

BIOMONITORAMENTO DOS EFEITOS GENOTÓXICOS RELACIONADOS À POLUIÇÃO ATMOSFÉRICA EM AMBIENTES DE INTENSO TRÁFEGO DE VEÍCULOS: CONTRIBUIÇÕES PARA A VIGILÂNCIA EM SAÚDE AMBIENTAL DE POPULAÇÕES EXPOSTAS

Aluno: Carlos Fernando Campos

Orientador: Prof. Dr. Boscolli Barbosa Pereira

UBERLÂNDIA - MG
2023

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Tese apresentada à Universidade Federal de Uberlândia como parte dos requisitos para obtenção do Título de Doutor em Genética e Bioquímica (Área Genética)

UBERLÂNDIA - MG
2023

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ATA DE DEFESA PÓS-GRADUAÇÃO

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Aos trinta dias do mês de maio de dois mil e vinte e três, às 13:30 horas, reuniu-se via web conferência pela Plataforma Google Meet, em conformidade com a Portaria nº 36, de 19 de março de2020 da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES, Resolução de nº06/2020 e Resolução nº 19/2022 do Conselho de Pesquisa e Pós-graduação pela Universidade Federal de Uberlândia, a Banca Examinadora, designada pelo Colegiado do Programa de Pós-graduação em Genética e Bioquímica, assim composta: Drª. Juliane Silberschmidt Freitas, Dr. Dieferson da Costa Estrela, Dr. Luis Paulo Pires, Dr. João Vitor Meza Bravo e Dr. Boscolli Barbosa Pereira, orientador do candidato e demais convidados presentes conforme lista de presença. Iniciando os trabalhos o presidente da mesa, Dr. Boscolli Barbosa Pereira apresentou a Comissão Examinadora e o candidato, agradeceu a presença do público, e concedeu ao discente a palavra para a exposição do seu trabalho. A duração da apresentação do discente e o tempo de arguição e resposta foram conforme as normas do Programa de Pós-graduação em Genética e Bioquímica. A seguir o senhor presidente concedeu a palavra, pela ordem sucessivamente, aos examinadores, que passaram a arguir o candidato . Ultimada a arguição, que se desenvolveu dentro dos termos regimentais, a Banca, em sessão secreta, atribuiu os conceitos finais. Em face do resultado obtido, a Banca Examinadora considerou o candidato:

APROVADO.

Esta defesa de Tese de Doutorado é parte dos requisitos necessários à obtenção do título de Doutor. O competente diploma será expedido após cumprimento dos demais requisitos, conforme as normas do Programa, a legislação pertinente e a regulamentação interna da UFU. Nada mais havendo a tratar foram encerrados os trabalhos. Foi lavrada a presente ata que após lida e achada conforme foi assinada pela Banca Examinadora.

Documento assinado eletronicamente por **Boscolli Barbosa Pereira**, **Professor(a) do Magistério Superior**, em 30/05/2023, às 16:59, conforme horário oficial de Brasília, com fundamento no art. 6º, § 1º, do Decreto nº 8.539, de 8 de outubro de 2015.

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ALUNO: Carlos Fernando Campos

COMISSÃO EXAMINADORA

Presidente: Prof. Dr. Boscolli Barbosa Pereira (Orientador)

Examinadores:

Prof. Dr. Luis Paulo Pires (UFU)
Prof. Dr. João Vitor Meza Bravo (UFU)
Profa. Dra. Juliane Silberschmidt Freitas (UEMG)
Prof. Dr. Dieferson da Costa Estrela (IFTM)

Data da Defesa: 30/05/2023

As sugestões da Comissão Examinadora e as Normas do PGGB para o formato da Dissertação/Tese foram contempladas

Boscolli Barbosa Pereira

Prof. Dr. Boscolli Barbosa Pereira

Dedico este trabalho a meu amigo, professor e orientador Boscolli Barbosa Pereira, à minha irmã, Fabiana Batista Campos e à minha mãe, Maria da Glória Batista Campos.

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Agradeço a todos que contribuíram de forma direta ou indireta para minha formação e contribuições neste trabalho.

Você não pode esperar construir um mundo melhor sem melhorar os indivíduos. Para esse fim, cada um de nós deve trabalhar para o seu próprio aperfeiçoamento e, ao mesmo tempo, compartilhar uma responsabilidade geral por toda a humanidade.

(Marie Curie).

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APRESENTAÇÃO

O uso de transporte individual, que no Brasil está representado pela crescente demanda por carros e motocicletas, é insustentável. Do ponto de vista econômico, os custos de aquisição, manutenção e abastecimento são cada vez maiores para os consumidores. Em contraste, transporte público e alternativas não motorizadas não recebem a prioridade necessária na agenda das políticas públicas. No que diz respeito ao eixo socioambiental, as emissões veiculares, bem como suas consequências para o ambiente e saúde, afetam principalmente a parcela mais vulnerável das populações urbanas.

Ainda que a poluição atmosférica seja um problema de Saúde Pública globalmente reconhecido, os parâmetros ambientais utilizados para monitorar a qualidade do ar são corriqueiramente incompletos, desatualizados e inadequados para a proteção de populações expostas aos poluentes do ar. Nessa direção, as evidências científicas devem subsidiar as políticas e os processos decisórios, incluindo a revisão de parâmetros ambientais. Esses parâmetros, inclusive, devem considerar as variáveis físicas, químicas e biológicas de forma integrada, a partir de programas de biomonitoramento da qualidade do ambiente.

Na presente tese, o tema biomonitoramento é abordado com ênfase nos efeitos genotóxicos relacionados à poluição atmosférica. O trabalho foi organizado em três capítulos, sendo o primeiro capítulo uma revisão crítica da literatura. Os capítulos II e III foram escritos na língua inglesa, trazem os resultados dos experimentos realizados e foram escritos no formato de artigo científico.

A partir dos pressupostos teórico-práticos apresentados no capítulo inicial, avaliamos, nos capítulos II e III, a sensibilidade e viabilidade do emprego do Teste de Micronúcleo em *Tradescantia pallida* como ferramenta para complementar os parâmetros físico-químicos de qualidade ambiental, considerando diferentes condições de tráfego veicular.

CAPÍTULO I

FUNDAMENTAÇÃO TEÓRICA

1 THEORETICAL FRAMEWORK

1.1 IMPACT OF INDIVIDUAL MOBILITY MODEL ON ECONOMY, HEALTH, AND ENVIRONMENT

Population growth results in an increased need for urban mobility. The mobility of people is largely dependent on road transportation, particularly individual motorized transportation. Data from the Institute of Applied Economic Research (IPEA) released in 2022 indicate that over the past 20 years, there has been a growing use of individual transportation at the expense of public transportation systems in Brazilian cities (CARVALHO, 2022).

During this period, the country's economic policy granted tax exemptions for industrial goods and favored the expansion of consumer credit, thus facilitating the purchase and financing of vehicles (MENDONÇA; SACHSIDA, 2014; LUCINDA; PEREIRA, 2017). There were also policies implemented to reduce taxes on automobiles and fuels (VASCONCELLOS, 2018). Despite the economic recession experienced from 2015 onwards, in previous years, an increase in the purchasing power of Brazilian families was observed, especially among middle and low-income families in the North and Northeast regions (PEREIRA et al., 2021). Additionally, there has been an increase in public transportation fares due to inflation. All these factors, combined with longer travel times, reduced comfort, and slower speeds of public transportation, favor the choice of private transportation (LIAO et al., 2020).

On one hand, the inclusion of middle and low-income families in the consumer market for durable goods, including cars and motorcycles, and the increase in purchasing power, along with the growth of the automotive sector, are positive factors for the macroeconomy. However, the increase in individual motorized transportation and the reduction in the use of public transportation have negative consequences for public health and the environment. One of the most immediate effects of the expansion of the car fleet is the deterioration of urban mobility conditions, with worsened traffic congestion, increased travel time, a higher number of accidents, reduction of green areas, and increased levels of air pollution (PEREIRA et al., 2021).

Air pollution is the result of the alteration of the normal physical, chemical, or biological characteristics of the atmosphere, causing harm to humans, flora, fauna, and materials (MILARÉ, 2021). Enhanced by anthropogenic factors, atmospheric alteration caused by pollutants originates from various sources that can be classified as stationary and mobile. Stationary sources mainly result from human activities, such as activities in refineries and petrochemical industries. In turn, mobile sources include motor vehicles, which have the highest pollution potential in this category, producing diffuse emissions that rapidly spread through the atmosphere (DERÍSIO, 2017).

Individual urban mobility is responsible for high levels of air pollutant emissions, mainly due to the use of vehicles powered by fossil fuels, which emit compounds such as carbon dioxide (CO_2), nitrogen dioxide (NO_2), particulate matter (PM), and other pollutants harmful to human health and the environment (FERNANDO; HOR, 2017).

The Global Air Quality Guidelines published by the World Health Organization (WHO) recommend air quality levels for six pollutants internationally recognized as significant contributors for a wide range of air-related diseases: particulate matter, sulfur dioxide (SO_2), carbon monoxide (CO), nitrogen oxides (NO_x), hydrocarbons (HC), and other sulfur oxides (SO_x) (WHO, 2021). Their function is to assess the extent to which the composition of contaminated air deviates from its ideal purity, providing data on the severity levels of air pollution (WHO, 2021).

In Brazil, the Air Quality Index, monitored by CETESB (2021), follows the parameters recommended by CONAMA Resolution No. 491, dated November 19, 2018, which establishes air quality standards in the country. Particulate matter with an aerodynamic diameter of up to 10 μm (PM_{10}) is considered inhalable particulate matter, which is further subdivided into fine fraction PM_{10}, with a size of up to 2.5 μm (known as $PM_{2.5}$), and coarse fraction PM10, with particles ranging in size from 2.5 to 10 μm (or $PM_{2.5\text{-}10}$).

Constituted by particles suspended in the air, particulate matter (PM) is one of the main agents causing negative effects on the body (GBD, 2018; VANDERLEI et al., 2009) and the environment (CETESB, 2020). Table 1 depicts the harmful effects caused by the major pollutants released into the atmosphere.

Table 1 - Sources and harmful effects of the main vehicular pollutants on the environment and health.

Pollutant	Main sources	Effects on human health	Effects on the environment
$MP_{2,5}$	Combustion (motor vehicles, industrial), secondary aerosol (formed in the atmosphere) such as sulfate and nitrate, among others.	Pathophysiological changes and effect on the body's defense system; $MP_{2,5}$ reaches the pulmonary alveoli and causes irritation, asthma, bronchitis, and lung cancer.	Damage to vegetation, visibility deterioration and soil and water contamination.
MP_{10}	Combustion reactions (industry and motor vehicles), resuspended dust, secondary aerosol (formed in the atmosphere).	Accumulation in the upper airways, aggravating respiratory problems, such as asthma.	Damage to vegetation, visibility deterioration and soil and water contamination.
Sulfur dioxide (SO_2)	Fuel oil burning processes, oil refineries, diesel vehicles, pulp and paper production, fertilizers.	Breathing difficulty, changes in the defense system of the lungs, worsening of respiratory and cardiovascular diseases.	Formation of acid rain, corrosion of materials, and damage to vegetation and crops.
Nitrogen dioxide (NO_2)	Combustion in motor vehicles, industrial processes, thermal plants that use oil or gas, incinerations.	Causes and/or worsens airway problems.	Formation of acid rain, damage to vegetation and crops.
Carbon monoxide (CO)	Incomplete combustion in motor vehicles.	CO acts on the blood, reducing its oxygenation and causing tissue hypoxia. CO can lead to death after a certain period of exposure and concentration.	Increased heat retention in the atmosphere, greenhouse effect.

Source: Adapted from IPEA (2011) and CETESB (2020).

Exposure to high levels of pollutants can cause respiratory, cardiovascular, and cancer-related diseases, and also increase mortality rates. Previous study indicates that air pollution is one of the main causes of increased risk for cardiovascular diseases, ranking as the tenth leading risk factor for global mortality (GBD, 2018).

According to Felin (2018), living in a city with polluted air increases the risk of a heart attack by 75% compared to cities with clean air. Colombini (2008), in a study conducted in the city of São Paulo, identified a significant number of deaths among individuals aged 65 and older related to exposure to PM_{10}. Furthermore, the findings revealed that for every 100 µg/m^3 increase in pollutant concentration, there was a 13% increase in overall mortality. Thus, levels of vehicular-related air pollution directly influence air quality, determining the degree and extent of effects on the environment and human health (SAN MARTIN; SAN MARTIN, 2020).

1.2 COVID-19 PANDEMIC AND SOCIAL DISTANCING MEASURES

On December 31, 2019, China alerted the WHO about several cases of unusual pneumonia in Wuhan, a city with 11 million inhabitants located in the central province of Hubei. On January 7, 2020, the identification of a new virus, SARS-CoV-2, was announced (WHO, 2020a). Within a few weeks, the virus spread to several other Asian countries. On January 20, the first case of coronavirus was reported by the United States, and on January 24, the first cases were reported in Europe (HOPKINS, 2020). In Brazil, the first confirmed case of the disease was reported by the Ministry of Health on February 25, 2020 (RODRIGUEZ-MORALES et al., 2020).

On March 11, 2020, after more than 118,000 cases registered in 114 countries, the WHO characterized COVID-19 as a pandemic (WHO, 2020b). Due to the high risk of virus transmission, it became urgent to implement measures and actions to contain the increasing number of COVID-19 cases. European countries, such as Italy, Spain, and the United Kingdom, diverged in their views on the need for social distancing and when to implement it. The main reason for this divergence was associated with the impact of the pandemic on the economy (FERGUSON et al., 2020; XIMENES et al., 2021).

However, the health crisis worsened, and projections generated by mathematical models led to an increasing consensus that social distancing measures would be necessary as the only alternative capable of containing the spread of the pandemic and reducing its effects, particularly severe cases, deaths, and the overload on the healthcare system (FERGUSON et al., 2020; XIMENES et al., 2021).

Importantly, as the cases continued to spread, most countries began implementing restrictions on trade, transportation, and cultural activities. Schools, universities, workplaces, and commercial or religious establishments were closed (DANTAS et al., 2020). As a result, the coronavirus outbreak led to a drastic reduction in vehicular traffic and, consequently, had a positive impact on air quality (SAADAT; RAWTANI; HUSSAIN, 2020).

1.3 IMPACTS OF REDUCED VEHICULAR TRAFFIC ON AIR QUALITY

In several cities, especially in large urban centers, levels of air pollutants such as nitrogen dioxide (NO_2) and fine particles dropped drastically during periods of social isolation and lockdown, emergency protocols implemented to prevent the circulation of people for non-essential activities. In terms of the impact on greenhouse gas emissions generated by the combustion of fossil fuels, projections estimated a significant reduction due to the contraction of economic activity and the decrease in car traffic, which represent one of the main sources of air pollution in urban areas, mainly during periods of greater restrictions on the movement of people (SAN MARTIN; SAN MARTIN, 2020).

Indeed, in 2020, air monitoring studies conducted by NASA revealed a significant reduction in nitrogen dioxide (NO_2) levels, particularly in China, as a result of social distancing measures implemented to contain the virus spread (NASA, 2020a,b). On a global scale, data from the International Energy Agency (IEA) indicate a decline of one million tons of CO_2 per day during this period, mainly due to reduced coal and oil consumption (IEA, 2020).

According to the World Meteorological Organization, Southeast Asia recorded a 40% decrease in harmful airborne particulate matter caused by traffic and energy production in 2020 (WMO, 2020). In Brazil, satellite images taken in March and April 2020 showed a significant reduction in particulate matter in the metropolitan regions of São Paulo and Rio de Janeiro. Similar results were found in countries such as China, Italy, Spain, and France (MUHAMMAD et al., 2020; NASA, 2020a,b).

Notably, the decrease in air pollution levels and improvement in air quality had a positive impact on population health. Studies have reported changes in hospitalization patterns for certain conditions, including cancer, cardiovascular diseases, and respiratory disorders, with a reduction in hospitalizations for asthma and chronic obstructive pulmonary disease (COPD) (ARAÚJO-FILHO et al., 2020; FONSECA et al., 2021; NORMANDO et al., 2021).

In Brazil, a national observational study reported a decline in hospitalization rates and in-hospital mortality due to respiratory diseases - except for COVID-19 - in the first eight months of the pandemic in the country as a result of measures to contain the virus spread (ALBUQUERQUE et al., 2023).

Thus, it is crucial to monitor air quality to identify changes in air pollution resulting from the implemented restriction measures to contain the COVID-19 pandemic, especially regarding the effects of anthropogenic pollutants.

1.4 MONITORING THE EFFECTS OF AIR POLLUTION CHANGES IN THE URBAN ENVIRONMENT

The increase in population in urban areas, coupled with industrial activity and fossil fuel combustion caused by vehicles, has led to higher doses of complex mixtures of pollutants in the atmosphere, including mutagenic and carcinogenic compounds. Therefore, the use of environmental quality monitoring techniques is required (WHO, 2021).

Despite significant advances in strategies to reduce air pollutant emissions in recent years, air pollution in urban areas remains a serious environmental and health concern. In this regard, the air quality index and ambient concentrations of major air pollutants can be measured through physical-chemical methods and mathematical models (KLUMPP et al., 2006). The data obtained indicate whether the limit values established by global organizations or recommended by local environmental laws are being respected (CAMARA, 2020).

However, the results of these assessments do not guarantee conclusions about the impact of air pollutants on living organisms. As a consequence, biomonitoring techniques are used to understand the effects of pollutants on the environment, aiming to identify environmental risks that threaten the balance and health of organisms (KOCH et al., 2016; SILVEIRA et al., 2021).

Biomonitors are organisms that accumulate contaminants in their tissues, thereby providing information about the quantitative aspects of environmental quality (HATJE, 2015). Living organisms, or communities of living organisms, can be classified as monitors because they accumulate one or more elements or compounds from the environment, responding simultaneously to different stressors and showing effects on morphological, histological, cellular structures, metabolic processes, behavior, and/or population structure. Moreover, they can reflect stress or the conservation status of a particular environment (KOCH et al., 2016).

In this sense, organisms such as plants, fish, insects, amphibians, birds, mammals, and invertebrates can be used as bioindicators, and their responses can be measured as biomarkers (ARZUMANYAN et al., 2022; GALLITELLI et al., 2022; MURTHY et al., 2022; PARRA-LUNA et al., 2020).

Certain plant species, such as *Tradescantia* sp., *Vicia faba*, and *Allium cepa*, have been employed in biomonitoring research of atmospheric quality. Besides their rapid development and easy propagation, factors such as sensitivity, high efficiency, and low operational cost make these plants ideal bioindicators for monitoring and laboratory or *in situ* investigations (RODRÍGUEZ et al., 2015).

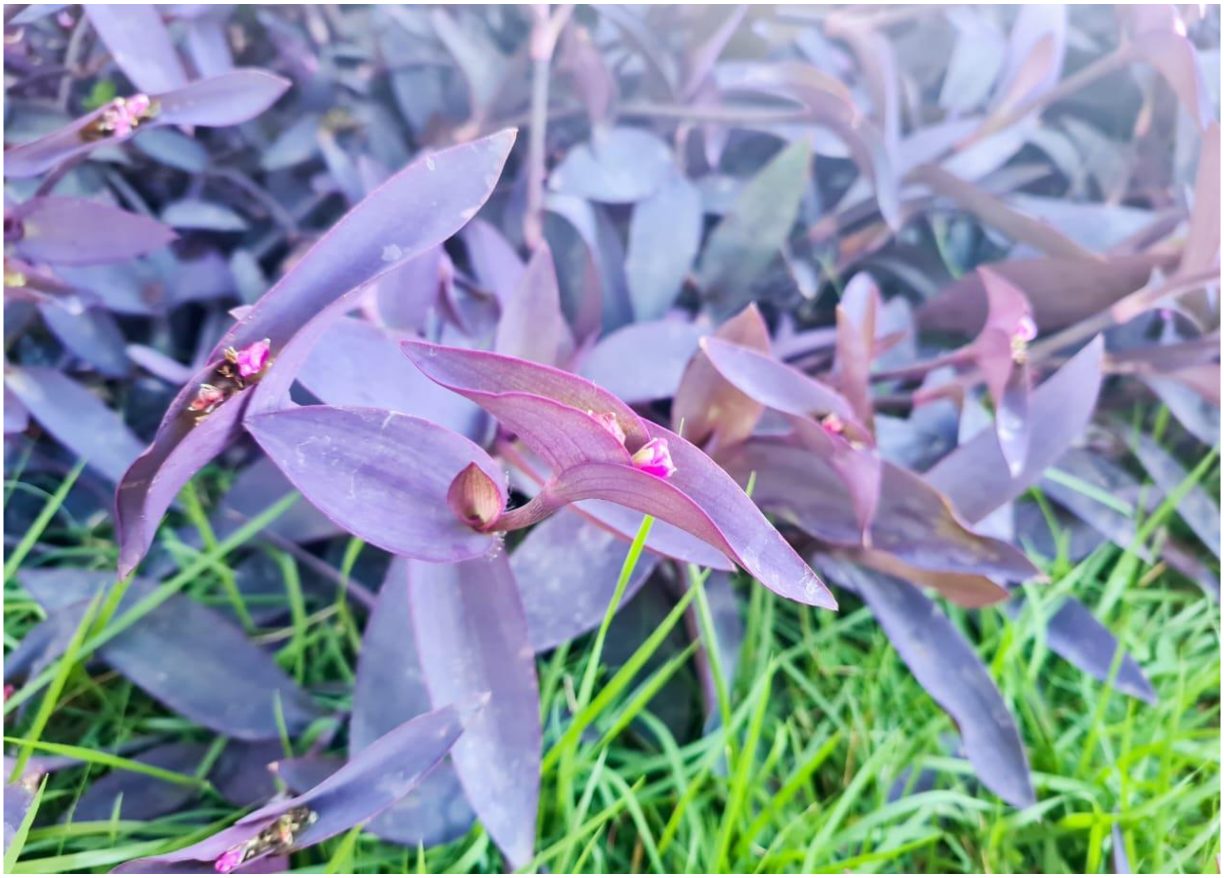
1.5 *Tradescantia* GENUS IN ENVIRONMENTAL BIOMONITORING

Belonging to the family Commelinaceae, the genus *Tradescantia* comprises over 500 species primarily found in tropical and subtropical regions. Some of these species and their clones are used as bioindicators of genotoxicity, for example, *Tradescantia pallida* (FADIC et al., 2016).

Tradescantia pallida (Rose) D.R. Hunt is a small herbaceous plant, measuring 15 to 25 cm in length (Figure 1). Its leaves are fleshy and glabrous, with an epidermis rich in anthocyanin pigments, giving the species a pink or purple coloration. *T. pallida* produces typically solitary flowers throughout the year, in pots, gardens, flowerbeds, and fields, showing easy adaptation and multiplication in any environment (LORENZI, 2015).

T. pallida has six pairs of large and easily observable chromosomes, and cells from almost all parts of the plant - from the root tip to the pollen tube - are continuously developing, favoring its use in cytogenetic and environmental biomonitoring studies (MA; GRANT, 1982). In this regard, the most prominent technique performed with plants of the *Tradescantia* genus is the micronucleus assay (Trad-MCN) (MA et al., 1994).

Figure 1 – *Tradescantia pallida* (Rose) D.R. Hunt



Source: The author (2023).

1.5.1 Trad-MCN as a sensitive biomarker

The micronucleus test in *Tradescantia* was first developed by Ma et al. (1978). This cytogenetic assay is based on a series of procedures for exposing (in the laboratory or *in situ*) *Tradescantia* plants to contaminants, followed by the analysis of micronucleus frequency resulting from chromosomal breakage in meiotic pollen mother cells at the tetrad stage. The tetrad-stage cells are obtained from young inflorescences, which is the ideal period for micronucleus observation, as the cells are in interphase, which in the Tradescantia genus lasts for 36 to 48 hours (RODRIGUES; PIMENTEL; WEINSTEIN et al., 1998; GERAS'KIN, S.; EVSEEVA, T.; OUDALOVA, 2011; PEREIRA; CAMPOS JÚNIOR; MORELLI, 2013).

Micronuclei (MN) are structures that result from whole chromosomes or chromosomal fragments that are lost during cell division, remaining in the cytoplasm of interphase cells. Therefore, they result from structural damage and aneuploidy, allowing the detection of clastogenic and aneugenic agents, respectively (RODRÍGUEZ et al., 2015).

The Trad-MCN test is considered a valuable tool by several researchers due to the simplicity of the methodology and the sensitivity of the Tradescantia sp. genus to genotoxic agents. In fact, these species have demonstrated precision and efficacy in analyzing the genotoxic potential of air pollutants through the bioassay (MA et al., 1994; GUIMARÃES et al., 2000).

1.5.2 Cytogenetic analyses for micronucleus observation

The steps of selection, fixation, and preservation of young inflorescences used in the micronucleus test with Tradescantia are carried out according to the protocol proposed by Ma (1981). In atmospheric biomonitoring studies, this protocol establishes that the inflorescences should be collected and fixed in a solution of acetic acid and ethanol (1:3) for 24 hours (PEREIRA; MORELLI, 2013). Subsequently, the inflorescences should be transferred to 70% ethanol and kept at 6 °C until the cytogenetic analysis.

The preparation of slides is performed by selecting and dissecting the inflorescences, followed by the isolation of floral buds. Initially, intermediate-sized buds from each dissected inflorescence are used to increase the chances of visualizing floral buds with meiotic pollen mother cells at the tetrad stage. The selected floral bud is transferred to a slide and dissected with a histological scalpel to expose the anthers. After the resulting cellular fragments from the dissection of the floral bud are removed and discarded, the anthers are macerated by adding a drop of 2% acetic carmine and covering the slide with a coverslip, gently pressing the anthers to release the cells. Heating for 5 seconds at a temperature of 60 °C is performed to fix the stain in the cells.

Before analyzing the slide, it is recommended that the researcher verify the presence of an adequate number of meiotic pollen mother cells at the tetrad stage. Ma

(1981) establishes the analysis of at least 300 tetrads per inflorescence. The number of micronuclei should be estimated in at least five inflorescences per monitored location or situation. The analyzed slides are observed under an optical microscope with a magnification of 400 times.

The slides should be coded, and the analyses should be performed in a blind study. Structures that measure approximately 1/3 to 1/5 of the main nucleus, exhibit similar chromatin staining and distribution to the nucleus, and are disconnected from it are considered micronuclei.

1.6 HYPOTHESIS AND AIM OF THE STUDY

In this study, we aimed to evaluate the plausibility of employing a biomonitoring program using *T. pallida* in urban places of intense traffic, for periodic assessments of environmental quality. For this purpose, as biomarkers of genotoxicity in response to exposure to an environment of intense vehicular traffic, the frequency of micronuclei and pollen abortivity in inflorescences collected at different intersections with gradual levels of traffic volume were evaluated. The concentrations of bioaccumulated heavy metals in the leaves of the collected plants were also investigated. Furthermore, to test the sensitivity of the proposed biological assessment model, these biological responses were correlated to the environmental variables (i) traffic volume and (ii) concentration of particulate material.

Posteriorly, we focused on the use of *T. pallida* as a biomonitor in situations where traffic varied in the same location, due to business closure measures implemented as a strategy to face the COVID-19 pandemic. We tested the hypothesis that indicators of genotoxicity in *T. pallida* respond significantly to changes in vehicular traffic in the same location. To test our formulated hypothesis, we collected data on vehicular traffic, particulate matter, heavy metals in the soil and plant, besides the genotoxicity analysis using the micronucleus test.

REFERENCES

ALBUQUERQUE, D. de A. R.; MELO, M. D. T.; SOUSA, T. L. F.; NORMANDO, P. G.; FAGUNDES, J. G. M.; ARAÚJO-FILHO, J. A. B. Internações hospitalares e taxas de mortalidade por doenças respiratórias não COVID-19 no sistema público de saúde do Brasil durante a pandemia de COVID-19: um estudo observacional nacional. **Jornal Brasileiro de Pneumologia**, v. 49, 2023. https://doi.org/10.36416/1806-3756/e20220093.

ARAÚJO-FILHO, J. A. B.; NORMANDO, P. G.; MELO, M. D. T.; COSTA, A. N.; TERRA, R. M. Lung cancer in the era of COVID-19: what can we expect? Jornal Brasileiro de Pneumologia, v. 46, n. 6, p. e20200398, 2020. https://doi.org/10.36416/1806-3756/e20200398.

ARZUMANYAN, M.; GHRMAJYAN, A.; MURADYAN, V.; TAMMARU, T.; ARAKELYAN, M. Molluscs as bioindicators of tourism pressure on ecosystems of Dilijan National Park, Armenia. **Journal of Ecotourism**, p. 1-12, 2022. https://doi.org/10.1080/14724049.2022.2100894

CAMARA, S. B.; MARTINS, S. P.; COSTA, N. L.; OLIVEIRA, S. V. Análise das emissões globais de CO_2 a partir da regressão linear múltipla. **Desafios - Revista Interdisciplinar da Universidade Federal do Tocantins**, v. 7, n. 3, p. 198-208, 2020. https://doi.org/10.20873/uftv7-10049.

CARVALHO, C. H. R. de. **Avaliação de políticas de redução tarifária dos sistemas de transporte público coletivo no Brasil**: evidências empíricas. IPEA: Instituto de Pesquisa Econômica Aplicada, 2022. Disponível em: https://repositorio.ipea.gov.br/bitstream/11058/11237/1/td_2770.pdf. Acesso em: 02 fev. 2023.

CETESB. Companhia Ambiental do Estado de São Paulo. **Operação Inverno Qualidade do Ar**. São Paulo: CETESB, 2020. Disponível em: https://cetesb.sp.gov.br/ar/wp-content/uploads/sites/28/2020/03/Relat%C3%B3rio-Opera%C3%A7%C3%A3o-Inverno-2019.pdf. Acesso em: 17 fev. 2023.

CETESB. Companhia Ambiental do Estado de São Paulo. **Padrões de Qualidade do Ar**. São Paulo: CETESB, 2021. Disponível em: https://cetesb.sp.gov.br/ar/padroes-de-qualidade-doar/. Acesso em: 17 fev. 2023.

COLOMBINI, M. P. Poluição atmosférica e seu impacto no sistema cardiovascular. **Einstein**, v. 6, p. 221-226, 2008.

CONAMA. Conselho Nacional do Meio Ambiente. **Resolução nº 491**, de 19 de novembro de 2018. Dispõe sobre padrões de qualidade do ar. Diário Oficial da União, 2018.

DANTAS, G.; SICILIANO, B.; FRANÇA, B. B.; SILVA, C. M.; ARBILLA, G. The impact of COVID-19 partial lockdown on the air quality of the city of Rio de Janeiro, Brazil. **Science of the Total Environment**, v. 729, n. 139085 p. 1-10, 2020. https://doi.org/10.1016/j.scitotenv.2020.139085.

DERÍSIO, J. C. **Introdução ao controle de poluição ambiental**. São Paulo: Oficina de textos, 2017.

FADIC, X.; PLACENCIA, F.; DOMÍNGUEZ, A. M.; CERECEDA-BALIC, F. *Tradescantia* as a biomonitor for pesticide genotoxicity evaluation of iprodione, carbaryl, dimethoate and 4,4′-DDE. **Science of the Total Environment**, v. 575, p. 146–151, 2016. https://doi.org/10.1016/j.scitotenv.2016.09.198.

FELIN, B. World Resources Institute. **Qual o impacto da poluição do ar na**

saúde? 2018. Disponível em: https://wribrasil.org.br/pt/blog/2018/07/qual-oimpacto-da-poluicao-do-ar-na-saude. Acesso em: 24 jan. 2023.

FERGUSON, N. M.; *et al*. Impact of non-pharmaceutical interventions (NPIs) to reduce COVID-19 mortality and healthcare demand. **Imperial College COVID-19 Response Team**, v. 82, n. 4, p. 1-20, 2020. https://doi.org/10.25561/77482.

FERNANDO, Y.; HOR, W. L. Impacts of energy management practices on energy efficiency and carbon emissions reduction: a survey of Malaysian manufacturing firms. **Resources, Conservation and Recycling**, v. 126, p. 62-73, 2017. https://doi.org/10.1016/j.resconrec.2017.07.023.

FONSECA, G. A.; NORMANDO, P. G.; LOUREIRO, L. V. M.; RODRIGUES, R. E. F.; OLIVEIRA, V. A.; MELO, M. D. T.; SANTANA, I. A. Reduction in the Number of Procedures and Hospitalizations and Increase in Cancer Mortality During the COVID-19 Pandemic in Brazil. **JCO Global Oncology**, v. 7, p. 4–9, 2021. https://doi.org/10.1200/GO.20.00471.

GALLITELLI, L.; BATTISTI, C.; PIETRELLI, L.; SCALICI, M. Anthropogenic particles in coypu (Myocastor coypus; Mammalia, Rodentia)'faeces: first evidence and considerations about their use as track for detecting microplastic pollution. **Environmental Science and Pollution Research**, v. 29, n. 36, p. 55293-55301, 2022. https://doi.org/10.1007/s11356-022-21032-0

GBD 2017 Risk Factor Collaborators. Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. **Lancet**, v. 392, n. 10159, p. 1923-1994, 2018. https://doi.org/10.1016/S0140-6736(18)32225-6.

GERAS'KIN, S.; EVSEEVA, T.; OUDALOVA, A. Plants as a tool for the environmental health assessment. *In*: NRIAGU, J. O. **Encyclopedia of Environmental Health**. San Diego, CA, USA: Elsevier Science, 2011. pp. 571-579.

GUIMARÃES, E.; DOMINGOS, M.; ALVES, E.; CALDINI, N.; LOBO, D.; LICHTENFELS, A.; SALDIVA, P. Detection of the genotoxicity of air pollutants in and around the city of São Paulo (Brazil) with the *Tradescantia*-micronucleus (Trad-MCN) assay. **Environmental and Experimental Botany**, v. 44, p. 1-8, 2000. https://doi.org/10.1016/S0098-8472(00)00050-2.

HATJE, V. **Biomonitors**. Encyclopedia of Estuaries. Springer Netherlands: Dordrecht, 2016. p. 83-84.

HOPKINS, J. John Hopkins University of Medicine. **Coronavirus Research Center**, 2020. Disponível em: https://coronavirus.jhu.edu/map.html. Acesso em: 25 fev. 2023.

IEA. Agência Internacional de Energia. **Monthly OECD oil price statistics**. IEA, 2020. Disponível em: www.iea.org/reports/monthly-oecd-oil-price-statistics. Acesso em: 24 mar. 2023.

IPEA. Instituto de Pesquisa Econômica Aplicada. Comunicados do IPEA. **Comunicado nº 103**. Poluição veicular atmosférica. 2011. Disponível em: http://www.ipea.gov.br. Acesso em: 22 fev. 2023.

KLUMPP, A.; ANSEL, W.; KLUMPP, G.; CALATAYUD, V.; GARREC, J. P.; HE, S.; PEÑUELAS, J.; RIBAS, A.; RO-POULSEN, H.; RASMUSSEN, S.; SANZ, M. J.; VERGNE, P. *Tradescantia* micronucleus test indicates genotoxic potential of traffic emissions in European cities. **Environmental Pollution**, v. 139, n. 3, p. 515-522, 2006.

KOCH, N. M.; BRANQUINHO, C.; MATOS, P.; PINHO, P.; LUCHETA, F.; MARTINS, S. M. A.; VARGAS, V. M. F. The application of lichens as ecological surrogates of air pollution in the subtropics: a case study in South Brazil. **Environmental Science and**

Pollution Research, v. 23, n. 20, p. 20819–20834, 2016. https://doi.org/10.1007/s11356-016-7256-2.

LIAO, Y.; GIL, J.; PEREIRA, R. H. M.; YEH, S.; VERENDEL, V. Disparities in travel times between car and transit: spatiotemporal patterns in cities. **Scientific Reports**, v. 10, n. 1, p. 1-12, 2020. https://doi.org/10.1038/s41598-020-61077-0.

LORENZI, H. **Plantas para jardim no Brasil**: herbáceas, arbustivas e trepadeiras. 2. ed. Nova Odessa, SP: Instituto Plantarum de Estudos da Flora, 2015.

LUCINDA, C. R. DE; PEREIRA, L. M. S. **Efeitos da política de redução do IPI sobre o mercado de automóveis novos**. Niterói: ANPEC, 2017.

Ma, T. H. *Tradescantia* micronucleus bioassay and pollen tube chromatid aberration test for in situ monitoring and mutagen screening. **Environmental Health Perspective**, v. 37, p. 85–90, 1981.

MA, T.; SPARROW, A.; SCHAIRER, L.; NAUMAN, A. Effect of 1,2-dibromoethane (DBE) on meiotic chromosomes of *Tradescantia*. **Mutation Research**, v. 58, p. 251-258, 1978.

MA, T, H.; GRANT, W. F. The Tradescantias-adventurous plants. **The Herbarist**, v. 48, p. 36-44, 1982.

MA, T. H.; CABRERA, G. L.; CHEN, R.; GILL, B.S.; SANDHU, S. S.; VANDENBERG, A. L.; SALAMONE, M. F. *Tradescantia* micronucleus bioassay. **Mutation Research**, v. 310, p. 221-230, 1994.

MENDONÇA, M. J.; SACHSIDA, A. **Modelando a demanda de crédito para veículos no Brasil**: uma abordagem com mudança de regime. Rio de Janeiro: Ipea, 2014.

MILARÉ, É. **Direito do ambiente**. São Paulo: Editora Revista dos Tribunais, 2021.

MUHAMMAD, S.; LONG, X.; SALMAN, M. COVID-19 pandemic and environmental pollution: A blessing in disguise? **Science of the Total Environment**, 728, 138820, 2020. https://doi.org/10.1016/j.scitotenv.2020.138820.

MURTHY, M. K.; MOHANTY, C. S.; SWAIN, P.; PATTANAYAK, R. Assessment of toxicity in the freshwater tadpole *Polypedates maculatus* exposed to silver and zinc oxide nanoparticles: A multi-biomarker approach. **Chemosphere**, v. 293, p. 133511, 2022. https://doi.org/10.1016/j.chemosphere.2021.133511

NASA. Administração Nacional do Espaço e da Aeronáutica, 2020a. **Airborne Nitrogen Dioxide Plummets Over China**. Disponível em: https://earthobservatory.nasa.gov/images/146362/airborne-nitrogen-dioxide-plummets-over-china. Acesso em: 13 abr. 2023.

NASA. Administração Nacional do Espaço e da Aeronáutica, 2020b. **Nitrogen Dioxide Levels Rebound in China**. Disponível em: https://earthobservatory.nasa.gov/images/146741/nitrogen-dioxide-levels-rebound-in-china. Acesso em: 13 abr. 2023.

NORMANDO, P. G.; ARAUJO-FILHO, J. A.; FONSECA, G. A.; RODRIGUES, R. E. F.; OLIVEIRA, V. A.; HAJJAR, L. A.; ALMEIDA, A. L. C.; BOCCHI, E. A.; SALEMI, V. M. C.; MELO, M. Reduction in hospitalization and increase in mortality due to cardiovascular diseases during the covid-19 pandemic in Brazil. **Arquivos Brasileiros de Cardiologia**, v. 116, n. 3, p. 371–380, 2021. https://doi.org/10.36660/abc.20200821.

PARRA-LUNA, M.; MARTÍN-POZO, L.; HIDALGO, F.; ZAFRA-GÓMEZ, A. Common sea urchin (*Paracentrotus lividus*) and sea cucumber of the genus *Holothuria* as bioindicators of pollution in the study of chemical contaminants in aquatic media. A revision. **Ecological Indicators**, v. 113, p. 106185, 2020. https://doi.org/10.1016/j.ecolind.2020.106185

PEREIRA, B. B.; CAMPOS JÚNIOR, E. O.; MORELLI, S. *In situ* biomonitoring of the genotoxic effects of vehicular pollution in Uberlândia, Brazil, using a *Tradescantia* micronucleus assay. **Ecotoxicology and Environmental Safety**, v. 87, p. 17–22, 2013. https://doi.org/10.1016/j.ecoenv.2012.10.003.

PEREIRA, B. B.; MORELLI, S. **Teste de micronúcleos com *Tradescantia***: fundamentos, aplicações e orientações técnicas para ensaios de biomonitoramento da qualidade ambiental. Editora FUCAMP, 2013.

PEREIRA, R. H. M.; WARWAR, L.; PARGA, J.; BAZZO, J.; BRAGA, C. K.; HERSZENHUT, D.; SARAIVA, M. **Tendências e desigualdades da mobilidade urbana no Brasil I**: o uso do transporte coletivo e individual. IPEA, 2021. Disponível em: https://repositorio.ipea.gov.br/handle/11058/10713. Acesso em: 22 jan. 2023.

RODRIGUES, G. S.; PIMENTEL, D.; WEINSTEIN, L. H. *In situ* assessment of pesticide genotoxicity in an integrated pest management program I-*Tradescantia* micronucleus assay. **Mutation Research/Genetic Toxicology and Environmental Mutagenesis**, v. 412, p. 235–244, 1998. https://doi.org/10.1016/S1383-5718(97)00193-9.

RODRIGUEZ-MORALES, A. J.; GALLEGO, V.; ESCALERA-ANTEZANA, J. P.; MÉNDEZ, C. A.; ZAMBRANO, L. I.; FRANCO-PAREDES, C.; SUÁREZ, J. A.; RODRIGUEZ-ENCISO, H. D.; BALBIN-RAMON, G. J.; SAVIO-LARRIERA, E.; RISQUEZ, A.; CIMERMAN, S. COVID-19 in Latin America. The implications of the first confirmed case in Brazil. **Travel Medicine and Infectious Disease**, v. 35, n. 101613, 2020. https://doi.org/10.1016/j.tmaid.2020.101613

RODRÍGUEZ, Y. A.; CHRISTOFOLETTI, C. A.; PEDRO, J.; BUENO, O. C.; MALASPINA, O.; FERREIRA, R. A. C.; FONTANETTI, C. S. *Allium cepa* and

Tradescantia pallida bioassays to evaluate effects of the insecticide imidacloprid. **Chemosphere**, v. 120, p. 438-442, 2015. https://doi.org/10.1016/j.chemosphere.2014.08.022.

SAADAT, S.; RAWTANI, D.; HUSSAIN, C. M. Environmental perspective of COVID-19. **Science of the Total Environment**, v. 728, n. 138870, 2020. https://doi.org/10.1016/j.scitotenv.2020.138870.

SAN MARTIN, M. C.; SAN MARTIN, M. C. Condições atuais das emissões dos poluentes atmosféricos durante a quarentena da Covid-19 e as perspectivas futuras. **Boletim de Conjuntura (BOCA)**, v. 2, n. 5, p. 85-96, 2020.

SILVEIRA, E. D. R.; BENVINDO-SOUZA, M.; ASSIS, R. A.; SANTOS, C. G. A.; AMORIM, N. P. L.; BORGES, R. E.; MELO, C. de; SANTOS, L. R. S. Micronucleus and different nuclear abnormalities in wild birds in the Cerrado, Brazil. **Environmental Science and Pollution Research**, 2021. https://doi.org/10.1007/s11356-021-16845-4

VANDERLEI, L. C. M.; PASTRE, C. M.; HOSHI, R. A.; CARVALHO, T. D. D.; Godoy, M. F. D. Basic notions of heart rate variability and its clinical applicability. **Brazilian Journal of Cardiovascular Surgery**, v. 24, p. 205-217, 2009. https://doi.org/10.1590/S0102-76382009000200018.

VASCONCELLOS, E. A. Urban transport policies in Brazil: the creation of a discriminatory mobility system. **Journal of Transport Geography**, v. 67, p. 85-91, 2018. https://doi.org/10.1016/j.jtrangeo.2017.08.014.

XIMENES, R. A. de A. *et al*. Covid-19 no nordeste do Brasil: entre o lockdown e o relaxamento das medidas de distanciamento social. **Ciência & Saúde Coletiva**, v. 26, p. 1441-1456, 2021. https://doi.org/10.1590/1413-81232021264.39422020.

WHO. World Health Organization. **Novel coronavirus (2019-nCoV)**. 2020a. Disponível em: http://www.euro.who.int/en/health-topics/health-emergencies/novel-coronavirus-2019-ncov_old. Acesso em: 23 fev. 2023.

WHO. World Health Organization. **WHO director-general's opening remarks at the media briefing on COVID-19** - 11 March 2020. 2020b. Disponível em: https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-March-2020. Acesso em: 23 fev. 2023.

WHO. World Health Organization. **WHO global air quality guidelines: particulate matter ($PM_{2.5}$ and PM_{10}), ozone, nitrogen dioxide, sulfur dioxide and carbon monoxide**. 2021. Disponível em: https://apps.who.int/iris/bitstream/handle/10665/345329/9789240034228-eng.pdf?sequence=1&isAllowed=y Acesso em: 02 fev. 2023.

WMO. World Meteorological Organization. **WMO air quality and climate bulletin**. 2021. Disponível em: https://library.wmo.int/doc_num.php?explnum_id=10887. Acesso em 24 fev. 2023.

CAPÍTULO II

ARTIGO CIENTÍFICO EXPERIMENTAL

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Analysis of genotoxic effects on plants exposed to high traffic volume in urban crossing intersections

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Analysis of genotoxic effects on plants exposed to high traffic volume in urban crossing intersections

Running title: **Genotoxic effects on plants exposed to high traffic**

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Abstract: A biological assessment of environmental quality was performed using the tropical plant species *Tradescantia pallida* (Rose) D.R. Hunt. var. *purpurea* exposed to different levels of air contamination in urban intersections with high volume of vehicle traffic. Air quality (average daily levels of particulate material in the $PM_{1, 2.5, 10}$ fractions) and traffic volume in crossing intersections were monitored for 30 days before the collection of plants. Frequency of micronuclei and pollen abortivity in inflorescences collected at different intersections with gradual levels of traffic volume were evaluated as biomarkers of genotoxicity. In addition, the concentrations of bioaccumulated heavy metals in the leaves of the collected plants were also investigated. The proposed biological assessment model found a positive association between the environmental variables (traffic volume; concentration of particulate material) and biological effects (leaf concentration of Cr and Cd; micronucleus frequencies and pollen abortivity).

Keywords: Urban gardens; Biomonitoring; Genotoxicity; Heavy metals; Micronuclei; Particulate material.

Introduction

The continuous growth of the motor vehicle fleet has remarkable impacts on the urban environment and health of citizens (Van Veldhoven et al. 2019; Oliveira et al. 2019). In addition to the traffic accidents, congestion and the resulting increase in the daily travel time of people, the increase in the fleet generates emissions of pollutant compounds in the urban atmosphere (Amato-Lourenco et al. 2016; Pereira et al., 2019).

Physical-chemical analysis associated with mathematical models allow measurements of environmental concentrations of the major atmospheric pollutants to verify whether limit values established by local environmental laws or recommended by world organizations have been respected (Montoya et al. 2020). Notwithstanding, although air quality is assessed through physical-chemical parameters, which accurately estimate the concentration of pollutants, in fact the obtained results do not guarantee conclusions regarding the impact of these contaminants on living organisms (AL-Alam et al., 2019).

In this perspective, the biological assessment of environmental quality has been investigated using plant species that accumulate contaminants (Mahapatra et al. 2019). Bioindicator organisms are important tools in environmental monitoring studies (Ramić et al. 2019). Remarkably, they are able to exhibit alterations in biomarkers, even when exposed to low levels of contamination of the environment (Sinha et al., 2014; Qarri et al. 2019).

Thus, the biological assessment of exposure to contaminated air using sensitive plant species to environmental changes is appropriate to detect and early monitor effects that can extend to human health (Placencia et al., 2019; Mišík et al 2019).

Several studies carried out with plants of the genus *Tradescantia* have elucidated genotoxic effects of exposure to air pollution in urban environments of intense traffic (Sposito et al. 2017; Rocha et al. 2018). In these assays, researchers generally cultivate clones of *Tradescantia* species that occur naturally in temperate regions and then expose the plants for specific periods to the environment to be investigated. This evaluation model ensures isogenicity, hence avoiding the bias that genetic variability could induce, but the stress caused by displacement and change of environment also interferes on the observed responses (Pereira; Campos Júnior; Morelli, 2013).

Alternatively, in the present study, a biological assessment model was employed using the species *Tradescantia pallida*, which has been used successfully in assessing environmental quality in tropical regions (Pereira et al. 2014; 2017; Nakazato et al. 2018). In addition, the evaluations were performed on plants that were already present in public flowerbeds of urban intersections.

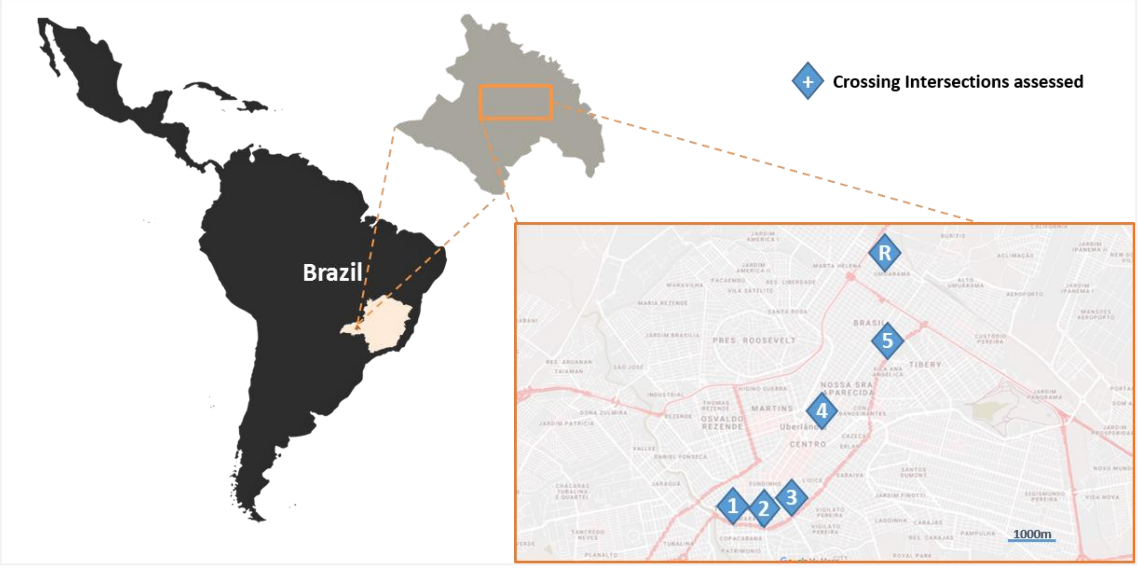
In this sense, the aim of this research was to evaluate the plausibility of employing a biomonitoring program using plants of the species *T. pallida* in urban places of intense traffic, for periodic assessments of environmental quality. For this purpose, as biomarkers of genotoxicity in response to exposure to an environment of intense vehicular traffic, the frequency of micronuclei and pollen abortivity in inflorescences collected at different intersections with gradual levels of traffic volume were evaluated. Furthermore, the concentrations of bioaccumulated heavy metals in the leaves of the collected plants were also investigated. Additionally, in order to test the sensitivity of the proposed biological assessment model, these biological responses were correlated to the environmental variables (i) traffic volume and (ii) concentration of particulate material.

MATERIAL AND METHODS

Collection sites

Plants of the species *Tradescantia pallida* (Rose) Hunt. cv. *purpurea* Boom which were present in the flowerbeds of different intersections in the city of Uberlândia, Minas Gerais, were collected. The collection sites were chosen based on crossings with different volumes of daily traffic, but also with an equivalent proportion of light and heavy fleet of motor vehicles. According this criterion, 40 cm stems with young fluorescences of *T. pallida* were collected in 5 intersections of intense traffic along the major avenues of the city. In addition, *T. pallida* stems found in the garden of Federal University of Uberlândia were also collected and this site was selected as a reference due to the low volume of vehicular traffic. Figure 1 shows the location of the intersections and the reference site.

Figure 1. Location of the crossing intersections and the reference site assessed.



Note: Intersection 1 (-18.93113, -48.28966); Intersection 2 (-18.93196, -48.28358); Intersection 3 (-18.92986, -48.27775); Intersection 4 (-18.89958, -48.2586); Intersection 5 (18.9132, -48.27214) and Reference site (-18.88295, -48.25907).

Street-level traffic data and particulate material sampling

To assess the exposure conditions of plants to vehicle traffic and the emissions of pollutants, the crossing intersections and the reference site were monitored for 30 days before the collection of plants. The traffic volume was estimated based on video monitoring data provided by the Traffic Department of the city of Uberlândia. The traffic volume (vehicles/day) was estimated by the weighted average of vehicles that passed through the roads (in all directions) considering the proportion of working days and weekends. The average daily levels of particulate material in the $PM_{1,\ 2.5,\ 10}$ fractions at each evaluated site was obtained using a portable sampling device (Dusttrak DRX Aerosol Monitor), calibrated for detection of aerosol in sampling volume fixed at 3.0L/min.

Trad-MN assay and Pollen abortivity test

To perform the Trad-MN assay, 5 to 10 young inflorescences collected from each collection site were used. According recommendations by Ma et al. (1994), the biological material was fixed in 1: 3 acetic acid to 70% ethanol solution for 24 h and preserved in 70% ethanol. Considering the same inflorescences used for the Trad-MN assay, flower buds were fixed in a glacial acetic acid and ethanol (96%) solution (1: 3) for 24 hours and preserved in 75% ethanol. The procedures of excision, staining and analysis were performed according to the protocol of Solenská, Micieta, Misík (2006). For each evaluated site, 10 slides were prepared for counting micronuclei and pollen abortion events. For Trad-MN assay, 300 tetrads per slide were examined; for the Pollen abortivity test, 3000 pollen grains per slide were assessed. All analyzes were conducted using an optical microscope at 400 x magnification.

Bioaccumulation

The average leaf concentrations of heavy metals in *T. pallida* cv. *purpurea* collected from different study sites (Kruskal Wallis and Dunn) were obtained according to methodology already described by Campos et al. (2016). The analysis of the heavy metals Lead (Pb), Chromium (Cr), Nickel (Ni) and Cadmium (Cd) in the plant leaves was carried out by using atomic absorption spectrometry in a graphite oven (GFAA), according to the method 7010-USEPA. For the analysis of Cobalt (Co), Barium (Ba), Copper, (Cu), Iron (Fe), Sodium (Na) and Zinc (Zn), the analytical method applied was optical emission spectrometry with argon plasma (ICP-OES), according to protocol stablished in USEPA method 6010C (USEPA, 2007).

Statistical analysis

Initially, the Shapiro-Wilk test was applied to assess the normality of all variables of the study. The Particulate Matter (PM) concentrations and the frequencies of micronuclei and abortions in inflorescences of *T. pallida* were compared between the different sites and statistically evaluated using the Kruskal-Wallis and Dunn tests. The differences between the average concentrations of bioaccumulated heavy metals in the leaves of the plants collected at each crossing intersection were subjected to one-way Analysis of Variance (ANOVA) and then compared to reference site using the Tukey test. Additionally, in order to determine correlation patterns between all studied variables that reported a significant difference between the evaluated sites, a multivariate analysis was performed by application of canonical correlation analysis. The statistical model was adjusted to analyze/compare two blocks of variables in an integrated way, as following: environmental ($PM_{1,\ 2.5,\ 10}$ fractions; traffic volume) and biological (micronuclei and pollen abortion frequencies; bioaccumulation of metal). P values <0.05 were considered statistically significant for all analyzes.

Results and discussion

Figure 2 highlights the gradient in the volume of motor-vehicle traffic that passes through the evaluated intersections on a daily basis, revealing the increase of vehicle traffic flow from the peripheral neighborhoods to the center. In addition, it is noteworthy that the levels of particulate material in all assessed fractions ($PM_{1;\ 2.5;\ 10}$) were significantly higher at the intersections in relation to the reference site.

Micronucleus frequencies (Figure 3A) and the percentage of pollen abortivity (Figure 3B) in the inflorescences of *T. pallida* collected at the intersections were also significantly higher in comparison to those obtained at the reference site.

Also, according to the results of the chemical digestion of the leaves collected at the different crossing intersections, the Cd and Cr concentrations were significantly higher in the samples of the intersections in comparison to the reference site, hence indicating the sensitivity of the species in bioaccumulate these heavy metals. The concentrations of Ni, Pb, Co, Ba, Cu and Zn in leaf samples collected at the intersections and reference site did not differ from each other (Table 1).

Table 1. Bioaccumulation of metals in *T. pallida* leaf samples collected at the studied intersections and Reference site.

SITES	**[Metals (mg.kg^{-1})]**							
	Cd	**Ni**	**Cr**	**Pb**	**Co**	**Ba**	**Cu**	**Zn**
Intersection 1	0.62 ± 0.34b	3.07 ± 2.40a	1.99 ± 1.01b	1.26 ± 0.83a	0.32 ± 0.13a	82.30 ± 62.9a	14.22 ± 8.34a	79.54 ± 19.63a
Intersection 2	0.60 ± 0.24b	3.01 ± 2.22a	2.22 ± 0.80b	1.15 ± 0.65a	0.30 ± 0.12a	77.33 ± 59.8a	13.71 ± 5.85a	91.34 ± 21.33a
Intersection 3	0.55 ± 0.31b	3.03 ± 1.64a	2.01 ± 0.69b	1.23 ± 0.59a	0.28 ± 0.10a	69.71 ± 72.2a	12.21 ± 5.77a	69.83 ± 23.72a
Intersection 4	0.47 ± 0.27b	3.19 ± 2.11a	1.44 ± 0.68b	1.30 ± 0.88a	N.D.	85.08 ± 48.5a	13.84 ± 6.26a	89.12 ± 25.98a
Intersection 5	0.45 ± 0.18b	2.58 ± 2.30a	1.41 ± 0.73b	1.11 ± 0.70a	0.43 ± 0.25a	69.98 ± 66.3a	11.02 ± 4.90a	77.63 ± 23.73a
Reference site	0.12 ± 0.09a	2.04 ± 1.87a	0.35 ± 0.21a	0.81 ± 0.49a	N.D.	71.10 ± 56.4a	10.13 ± 6.11a	80.67 ± 30.41a

Asterisk indicates significant difference from Reference site (ANOVA; Tukey; $p < 0.05$). N.D. Not detected.

Table 2. Results of Canonical Correlation Analysis (CCA) between environmental and biological variables.

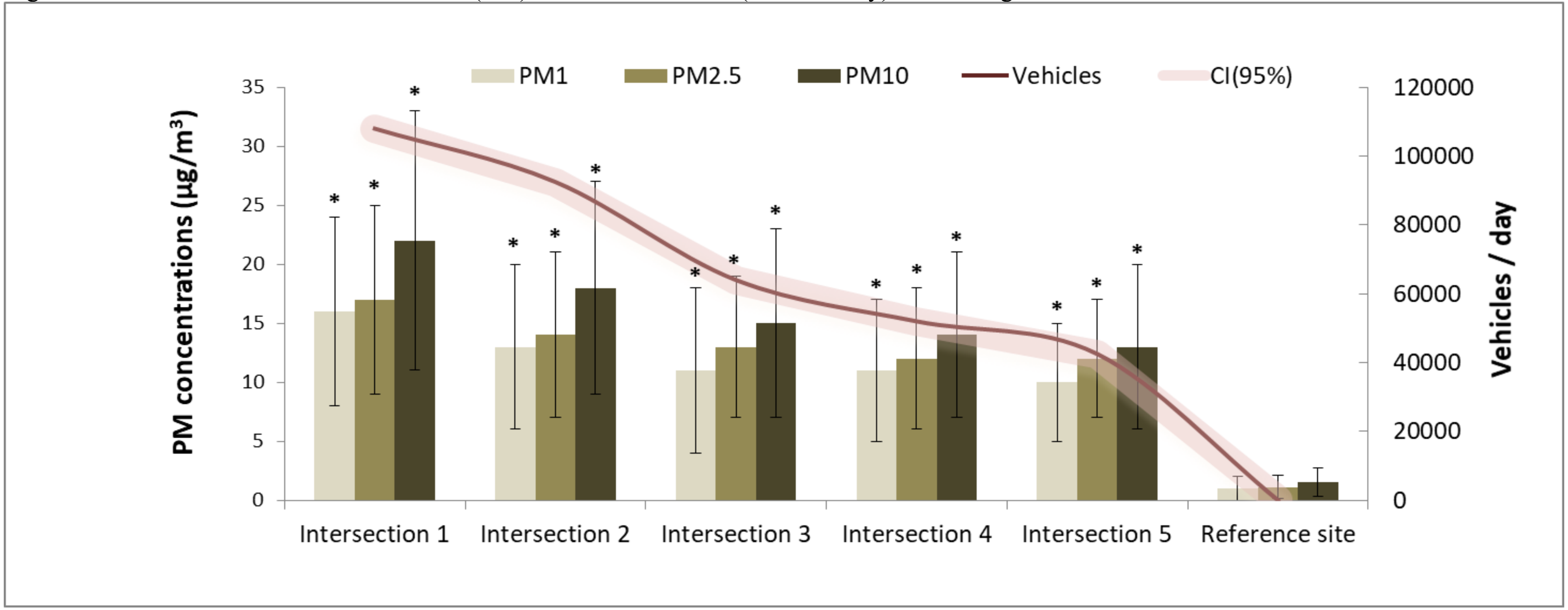
Covariates (environmental variables - *Xi*)	Canonical Variables		Dependent variables (biological variables - *Yi*)	Canonical Variables	
	χ_1	χ_2		η_1	η_2
Traffic (X1)	2.2892	-0.3875	Cr (Y1)	1.0896	-0.4242
PM_1(X2)	2.2953	7.1199	Cd (Y2)	-1.2198	-1.3695
$PM_{2.5}$(X3)	-2.7789	-5.8082	MN (Y3)	1.8741	3.7913
PM_{10}(X4)	-1.0581	-1.4405	Abbort (Y4)	-0.9114	-2.5543
Canonical R	**0.9968**	**0.9220**			
p-value	**0.0001**	**0.0318**			

Table 3. Matrix of correlations (R) between Xi and Yi variables.

	Cr	**Cd**	**MN**	**Ab**
Frota	0.929*	0.910*	0.953*	0.923*
PM1	0.850*	0.860*	0.911*	0.884*
PM2.5	0.871*	0.893*	0.916*	0.920*
PM10	0.747**	0.801*	0.810*	0.782*

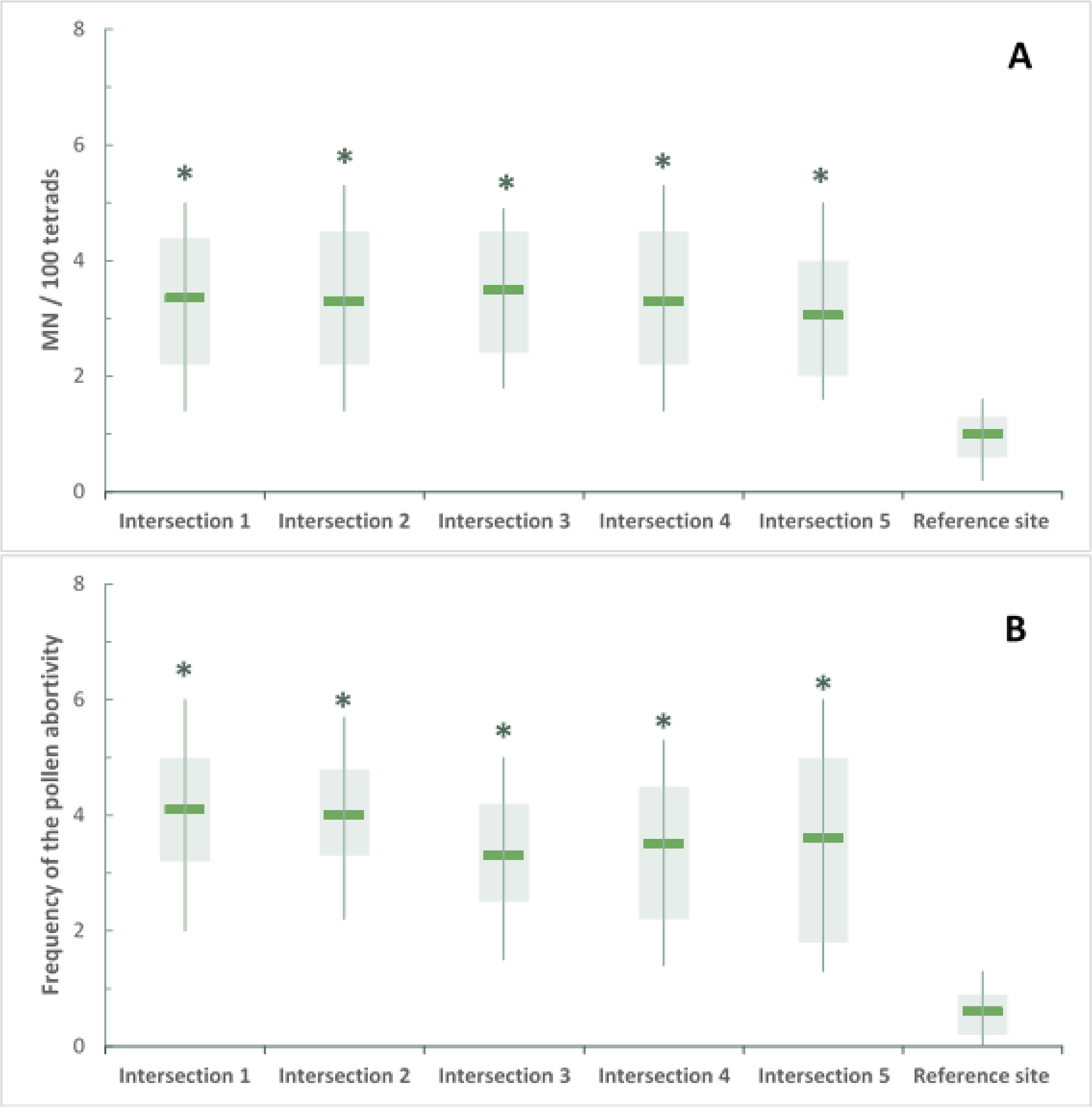
Significant p value ($p<0.05$*; $p<0.05$**), according to Pearson's correlation test.

Figure 2. Environmental Particulate Matter (PM) and traffic volume (vehicles/day) in crossing intersections studied and reference site.



Asterisk indicates significant difference from Reference site (Kruskal-Wallis; Dunn; $p < 0.05$).

Figure 3. Frequency of the micronuclei (A) and pollen abortivity (B) in *Tradescantia pallida* collected in crossing intersections studied and reference site.



Asterisk indicates significant difference from Reference site (Kruskal-Wallis; Dunn; $p < 0.05$).

Table 2 depicts the results of the multivariate test, which was performed to provide a comprehensive understanding on the dependency relationships between exposure variables and biological response markers. The canonical correlation analysis revealed the existence of a significantly positive association ($R = 0.9968$; $p < 0.001$) between the set of environmental data [*Xi:* Traffic (X1); and particulate matter fractions of PM_1(X2); $PM_{2.5}$(X3); PM_{10}(X4)] and the set of biological data [Yi: leaf concentration of Cr (Y1) and Cd (Y2); micronucleus frequencies (Y3) and pollen abortion (Y4)]. In a complementary way, a matrix containing the results of Pearson's correlation tests between all variables used in the multivariate analysis is shown in Table 3. Accordingly, there was significance between all combinations of correlations considering environmental exposure variables and biological effects.

The present investigation was undertaken to continue a biomonitoring program of air quality in areas of intense vehicular traffic, in the city of Uberlândia, Brazil, performed since 2007 (Pereira; Campos Júnior; Morelli, 2013). As an alternative to the assessment models previously applied (Pereira et al., 2014; 2017), characterized by the *ex situ* exposure of plants of the species *T. pallida* (previously cultivated in a reference site) to areas with high volume of vehicle traffic, in this experiment, we investigated the relationship between environmental variables (daily volume of vehicular traffic and concentration of particulate matter in the atmosphere in different aerodynamic fractions) and biological biomarkers (metal bioaccumulation, micronuclei formation and occurrence of pollen abortion) in response to plant samples naturally exposed to air pollutants in permanent flowerbeds at the evaluated sites.

As evidenced by the results, the environmental concentrations of particulate matter detected in this study do not exceed the average daily limits established by WHO guidelines (2005) for preservation of the environmental quality and health of the exposed populations. WHO parameters limited coarse particulate matter (PM_{10}) concentrations to 20 $\mu g/m^3$ based on an annual mean, and 50 $\mu g/m^3$ based on a 24-hour mean; and also limited fine particulate matter ($PM_{2.5}$) concentrations to 10 $\mu g/m^3$ based on an annual mean, and 25 $\mu g/m^3$ based on a 24-hour mean.

Moreover, when there are differences in the concentration of Cd and Cr metals between the leaf samples collected at the intersections and the reference site (approximately 4 x greater in high-traffic locations), (i) the ability of *T. pallida* in bioaccumulating these contaminants and (ii) the hypothesis that the particulate material from vehicle emissions carries metals are confirmed. Furthermore, considering that metals adhered to particulate material may contaminate plant species by direct entry via stomata or / and through the root system when sedimented in the substrate (Campos et al., 2016; Kardel et al. 2018; Alatou, & Sahli 2019), the observed genotoxic effects confirm that, even in concentrations below the standard limits established by environmental legislation, the particulate material causes negative impacts on biological systems, as verified by Domingues et al. (2018).

Interestingly, early detection of a hazardous exposure situation can significantly prevent the occurrence of negative biological effects (Mukhopadhyay et al. 2020). In this sense, biomarkers of response have been intensively applied in biomonitoring programs of environmental quality, with the aim of providing subsidies for the implementation of preventive measures and control of exposure to contaminants in the environment, including the review of legal parameters (Rai 2016; Gillooly et al. 2019). The findings of this study highlight the sensitivity of the genotoxicity biomarkers observed, which revealed a high frequency of MN and pollen abortivity in *T. pallida* inflorescences exposed to contaminated air in areas of intense vehicular traffic, establishing a qualitative and quantitative association between the exposure variables and the detected biological effects.

Indeed, the combination of Trad-MCN test and pollen grain abortivity test, which therein indicates genotoxic and mutagenic effects, respectively, emphasize the potential application of *T. pallida* in environmental biomonitoring programs. Specifically, the associations between the variables considered in this research also point to the perspective of *in situ* monitoring from the planting of sensitive species in strategic locations for assessing environmental quality.

References

AL-Alam, J., Chbani, A., Faljoun, Z., & Millet, M. (2019). The use of vegetation, bees, and snails as important tools for the biomonitoring of atmospheric pollution—a review. Environmental Science and Pollution Research. doi:10.1007/s11356-019-04388-8

Alatou, H., & Sahli, L. (2019). Using tree leaves and barks collected from contaminated and uncontaminated areas as indicators of air metallic pollution. International Journal of Phytoremediation, 1–13. doi:10.1080/15226514.2019.1583723

Amato-Lourenco, L. F., Lobo, D. J. A., Guimarães, E. T., Moreira, T. C. L., Carvalho-Oliveira, R., Saiki, M., … Mauad, T. (2017). Biomonitoring of genotoxic effects and elemental accumulation derived from air pollution in community urban gardens. Science of The Total Environment, 575, 1438–1444. doi:10.1016/j.scitotenv.2016.09.221

Domingues, É. P., Silva, G. G., Oliveira, A. B., Mota, L. M., Santos, V. S. V., de Campos, E. O., & Pereira, B. B. (2018). Genotoxic effects following exposure to air pollution in street vendors from a high-traffic urban area. Environmental Monitoring and Assessment, 190(4). doi:10.1007/s10661-018-6598-2

Gillooly, S. E., Michanowicz, D. R., Jackson, M., Cambal, L. K., Shmool, J. L. C., Tunno, B. J., … Clougherty, J. E. (2019). Evaluating deciduous tree leaves as biomonitors for ambient particulate matter pollution in Pittsburgh, PA, USA. Environmental Monitoring and Assessment, 191(12). doi:10.1007/s10661-019-7857-6

Kardel, F., Wuyts, K., De Wael, K., & Samson, R. (2018). Biomonitoring of atmospheric particulate pollution via chemical composition and magnetic properties of roadside tree leaves. Environmental Science and Pollution Research, 25(26), 25994–26004. doi:10.1007/s11356-018-2592-z

Ma, T. H., Cabrera, G. L., Chen, R., Gill, B. S., Sandhu, S. S., Vandenberg, A. L., Salamone, M. F. 1994. *Tradescantia* micronucleus bioassay. *Mutation Research* 310 : 221-230. doi:10.1016/0027-5107(94)90115-5.

Mahapatra, B., Dhal, N. K., Dash, A. K., Panda, B. P., Panigrahi, K. C. S., & Pradhan, A. (2019). Perspective of mitigating atmospheric heavy metal pollution: using mosses as biomonitoring and indicator organism. Environmental Science and Pollution Research. doi:10.1007/s11356-019-06270-z

Mišík M., Pichler C., Rainer B., Nersesyan A., Mišíková K., Knasmueller S. (2019) Micronucleus Assay with Tetrad Cells of *Tradescantia*. In: Dhawan A., Bajpayee M. (eds) Genotoxicity Assessment. Methods in Molecular Biology, vol 2031. Humana, New York, NY

Montoya, O. L. Q., Niño-Ruiz, E. D., & Pinel, N. (2020). On the mathematical modelling and data assimilation for air pollution assessment in the Tropical Andes. Environmental Science and Pollution Research. doi:10.1007/s11356-020-08268-4

Mukhopadhyay, S., Dutta, R., & Das, P. (2020). A critical review on plant biomonitors for determination of polycyclic aromatic hydrocarbons (PAHs) in air through solvent extraction techniques. Chemosphere, 126441. doi:10.1016/j.chemosphere.2020.126441

Nakazato, R. K., Esposito, M. P., Cardoso-Gustavson, P., Bulbovas, P., Pedroso, A. N. V., de Assis, P. I. L. S., & Domingos, M. (2018). Efficiency of biomonitoring methods applying tropical bioindicator plants for assessing the phytoxicity of the air pollutants in SE, Brazil. Environmental Science and Pollution Research, 25(20), 19323–19337. doi:10.1007/s11356-018-2294-6

Oliveira, M., Slezakova, K., Delerue-Matos, C., Pereira, M. C., & Morais, S. (2019). Children environmental exposure to particulate matter and polycyclic aromatic hydrocarbons and biomonitoring in school environments: A review on indoor and outdoor exposure levels, major sources and health impacts. Environment International, 124, 180–204. doi:10.1016/j.envint.2018.12.052

Pereira, B. B., Campos Junior, E. O., Morelli, S. 2013. *In situ* biomonitoring of the genotoxic effects of vehicular pollution in Uberlândia, Brazil, using *Tradescantia* micronucleus assay. *Ecotoxicology and Environmental Safety* 87: 17-22. doi:10.1016/j.ecoenv.2012.10.003.

Pereira BB, Campos Junior EO, Lima EAP, Barrozo MA, Morelli S (2014). Biomonitoring air quality during and after a public transportation strike in the center of Uberlândia, Minas Gerais, Brazil by *Tradescantia* micronucleus bioassay. Environmental Science and Poluttion Research International 21: 3680–3685. doi: 10.1007/s11356-013-2335-0

Pereira, B. B., da Cunha, P. B., Silva, G. G., de Campos Júnior, E. O., Morelli, S., Filho, C. A., de Lima, E. A., & Barrozo, M. A. (2017). Integrated monitoring for environmental health impact assessment related to the genotoxic effects of vehicular pollution in Uberlândia. Brazil. Environmental Science and Pollution Research International, 24, 2572–2577. doi: 10.1007/s11356-016-8039-5.

Pereira, B. B., Vieira Santos, V. S., Domingues, É. P., Silva, G. G., Brandão da Cunha, P., Oliveira, A. B., Olegário de Campos Júnior, E. (2019). Assessment of genotoxic effects on elderly populations exposed to high traffic areas: Results for supporting public health surveillance. Environmental Research, 179, 108752. doi:10.1016/j.envres.2019.108752

Placencia, F., Fadic, X., Yáñez, K., & Cereceda-Balic, F. (2019). Tradescantia as a biomonitor for genotoxicity evaluation of diesel and biodiesel exhaust emissions. Science of The Total Environment, 651, 2597–2605. doi:10.1016/j.scitotenv.2018.10.009

Qarri, F., Lazo, P., Allajbeu, S., Bekteshi, L., Kane, S., & Stafilov, T. (2019). The Evaluation of Air Quality in Albania by Moss Biomonitoring and Metals Atmospheric Deposition. Archives of Environmental Contamination and Toxicology. doi:10.1007/s00244-019-00608-x

Rai, P. K. (2016). Impacts of particulate matter pollution on plants: Implications for environmental biomonitoring. Ecotoxicology and Environmental Safety, 129, 120–136. doi:10.1016/j.ecoenv.2016.03.012

Ramić, E., Huremović, J., Muhić-Šarac, T., Đug, S., Žero, S., & Olovčić, A. (2019). Biomonitoring of Air Pollution in Bosnia and Herzegovina Using Epiphytic Lichen Hypogymnia physodes. Bulletin of Environmental Contamination and Toxicology. doi:10.1007/s00128-019-02595-0

Rocha, A. do N., Candido, L. S., Pereira, J. G., Silva, C. A. M., da Silva, S. V., & Mussury, R. M. (2018). Evaluation of vehicular pollution using the TRAD-MCN mutagenic bioassay with

Tradescantia pallida (Commelinaceae). Environmental Pollution, 240, 440–447. doi:10.1016/j.envpol.2018.04.091

Solenská M, Micieta K, Misík M. 2006. Plant bioassays for an in situ monitoring of air near an industrial area and a municipal solid waste: Zilina (Slovakia). Environ Monit Assess. 115(1-3):499-508. doi: 10.1007/s10661-006-7240-2.

Sposito, J. C. V., Crispim, B. D. A., Romãn, A. I., Mussury, R. M., Pereira, J. G., Seno, L. O., Grisolia, A. B. 2017. Evaluation the urban atmospheric conditions in different cities using comet and micronuclei assay in *Tradescantia pallida*. *Chemosphere* 175: 108-113. doi:10.1016/j.chemosphere.2017.01.136.

U.S. Environmental Protection Agency. 2007. Method 7010— Graphite furnace atomic absorption spectrophotometry, rev.00. http://www3.epa.gov/epawaste/hazard/test methods/sw846/pdfs/7010.pdf

Van Veldhoven, K., Kiss, A., Keski-Rahkonen, P., Robinot, N., Scalbert, A., Cullinan, P., Vineis, P. (2019). Impact of short-term traffic-related air pollution on the metabolome – Results from two metabolome-wide experimental studies. Environment International, 123, 124–131. doi:10.1016/j.envint.2018.11.034

WHO-World Health Organization. WHO Air quality guidelines for particulate matter, ozone, nitrogen dioxide and sulfur dioxide. Global updated 2005.

CAPÍTULO III

ARTIGO CIENTÍFICO EXPERIMENTAL

Título:

Integrated biological assessment of air pollution in urban areas using passive biomonitoring

Integrated biological assessment of air pollution in urban areas using passive biomonitoring

Abstract: Atmospheric pollution is generally monitored through physicochemical indicator systems. Variations of these indicators in relation to environmental legislation parameters report on air quality, especially in large cities. However, the interpretation of environmental data on pollutants needs to be complemented, as the impacts of pollution on sensitive organisms serve as a warning to public health authorities. The use of species adapted to the urban environment as sentinels enables continuous and integrated monitoring of the effects of environmental pollution on biological systems. In this study, we used the tropical plant species *Tradescantia pallida*, planted in ornamental beds at the main urban intersections in the city of Uberlândia, Brazil, to monitor the genotoxic effects of atmospheric pollution under different vehicular traffic conditions. In four samples, we compared biological, physical-chemical and traffic indicators at different intersections (in residential and commercial areas) during the period of restriction of local commerce activities, imposed by health agencies, as a measure to face the COVID-19 pandemic. With the decrease in vehicle traffic due to the lockdown, it was found that this species is sensitive to environmental changes of pollutants in the air and soil. *T. pallida* bioaccumulated heavy metals under conditions of exposure to greater vehicular traffic, where higher concentrations of these contaminants were also found in the soil. Furthermore, from the Micronucleus Test, we found that genotoxicity can be estimated from a multiple linear regression model, including (X_1) chromium concentration in the soil and (X_2) particulate matter in the atmosphere as the main independent variables. Our findings are important contributions to the (re)definition of parameters and models of Environmental Health Surveillance.

Keywords: Bioaccumulation; Heavy metals; Genotoxicity; Environmental Health; Ecotoxicology.

Introduction

Biological indicators of air quality have been used as an important complement to physicochemical analysis, required by regulatory and environmental protection agencies (Campos et al. 2019, Ramić et al. 2019).

Although the main atmospheric contaminants, such as particulate matter and polluting gases (nitrogen dioxide, sulfur dioxide, ozone, and carbon monoxid) are monitored, there is scarce knowledge about the effects of the association of these contaminants in biological systems (Kumar et al., 2023).

Biomonitoring offers the possibility of evaluating the responses of exposed organisms, also allowing the prediction of genotoxic effects (Araújo et al. 2021). The use of species that are sensitive to changes in concentrations of pollutants released into the environment is a crucial strategy for establishing environmental health surveillance programs based on scientific evidence (Fang et al. 2022).

Permanent monitoring systems are essential for establishing environmental quality parameters based on biological response thresholds (DeBord et al. 2015, Cofin et al. 2022). In a previous study, the analysis of *Tradescantia pallida* samples, present in public flowerbeds located at different intersections in the city of Uberlândia, Brazil, provided evidence of genotoxicity dependent on the intensity of vehicle traffic (Campos et al., 2020).

In this study, we focused on the use of the *Tradescantia pallida* species as a biomonitor in situations where traffic varied in the same location, due to business closure measures implemented as a strategy to face the COVID-19 pandemic.

More specifically, we tested the hypothesis that indicators of genotoxicity in *T. pallida* respond significantly to changes in vehicular traffic in the same location.

To test the formulated hypothesis, we collected data on vehicular traffic, particulate matter, heavy metals in the soil and plant, in addition to the genotoxicity analysis carried out through the micronucleus test. Our results were evaluated based on multivariate analysis with the objective of proposing a prediction model (multiple linear correlation) representing that plants exposed to conditions of intense emission of pollutants from vehicles accumulate heavy metals and present a significant increase in relation to the basal levels of genotoxicity (expressed as frequency of micronucleated tetrads in pollen grains of young inflorescences).

Material and Methods

Study design

Four sampling campaigns of particulate matter, soil and plants were conducted over a period in which local businesses were closed/reopened due to the Covid-19 pandemic (Figure 1). The collection sites were the same points already monitored in a previous feasibility study (Campos et al., 2020). The collection sites were chosen based on crossings with different vehicular traffic intensity, but with an equivalent number of light/heavy vehicles.

Biological material

Samples of leaves and young inflorescences of *T. pallida* were collected to assess heavy metal concentration and genotoxicity, respectively. We used the mother plants, kept at the Federal University of Uberlândia (area with low vehicular traffic intensity) as a reference group for determining the baseline parameters of bioaccumulation and spontaneous frequency of micronuclei.

Heavy metals in soil and plant

We collected and analyzed surface soil samples (volume = 100 cm^3; depth = 5 cm) and *T. pallida* leaves collected at different intersections (in quintuplicate) to determine the concentration of heavy metals Lead (Pb), Chromium (Cr), Nickel (Ni), Cadmium (Cd), Copper (Cu), and Zinc (Zn). We analyzed the biological samples according to the methodology described in previous works (Campos et al. 2016; 2020), by using atomic absorption spectrometry in a graphite oven (GFAA), according to the U.S. Environmental Protection Agency methods (2007). We submitted the soil samples to the acid digestion procedure (HCl 37% and HNO3 70%, 3:1 v/v) according to the ISO 11466:1995 protocol (ISO, 1995).

Street-level traffic data and particulate material sampling

To monitor the effects of the variation in vehicle flow on the emission of particulate matter, we used daily data on the circulating fleet provided by the Municipal Traffic Department. The data was corrected so that the traffic volume was adjusted to the different directions and lanes of the roads, also considering peak hours, weekends, and holidays. The average daily levels of particulate material in the $PM_{1,\ 2.5,\ 10}$ fractions at each evaluated site and period were obtained using a portable particulate matter sampler (Dusttrak DRX Aerosol Monitor - calibrated for detection of aerosol in sampling volume fixed at 3.0L/min).

Tradescantia micronucleus assay (Trad-MN)

We performed the Trad-MN assay to evaluate the biological response of genotoxicity based on the micronucleus frequency. Following the protocol established by Ma et al. (1994), we used young inflorescences collected from each monitored location in quintuplicate, which were fixed in 1: 3 acetic acid to 70% ethanol solution for 24 h and preserved in 70% ethanol. At least 10 slides (300 tetrads per slide) per collection site were analyzed using optical microscope (400 x magnification).

Statistical analysis

Initially, we applied the Shapiro-Wilk test to assess the normality of the studied variables. We compared heavy metal concentrations (both in plants and soil) between the different sites and between collection periods, using two-way Analysis of Variance (ANOVA). To compare the frequencies of micronuclei in inflorescences of *T. pallida* between the different sites, we used the Kruskal-Wallis, followed by Dunn tests.

To evaluate the relationship between environmental variables (MP fractions, fleet and metal concentration in soil and plant) as predictors of the observed genotoxic effect (MN frequencies), we performed a multiple regression analysis and adjusted the mathematical model to represent only the variables that contributed more significantly to the induction of MN (>75%). P values <0.05 were considered statistically significant for all analyzes.

Results

According to the locations monitored during the four samplings carried out (Figure 1), it was possible to observe variations in the intensity of vehicular traffic, as can be seen in Figure 2. The variations reflected measures to restrict commercial activities, imposed as a result of the need control of the COVID-19 pandemic. In addition, it is possible to notice that the concentration of particulate matter was also higher during the samplings carried out in periods of greater intensity of vehicular traffic (first and fourth sampling).

Figure 1. Sampling design of the study.

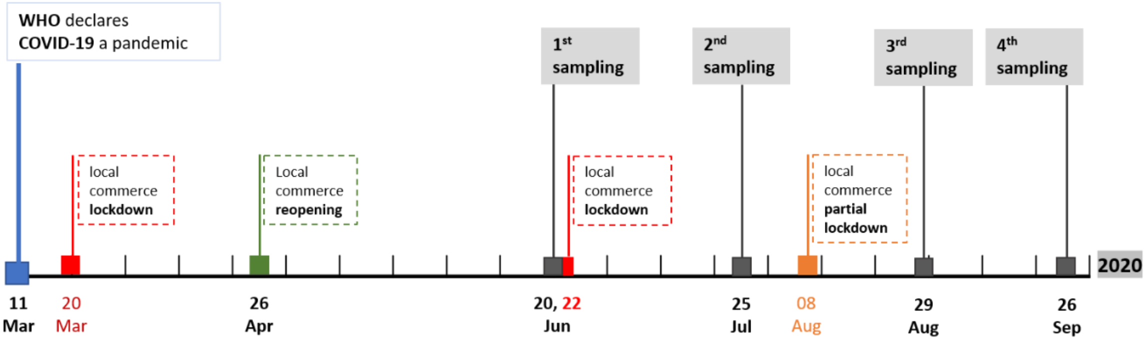
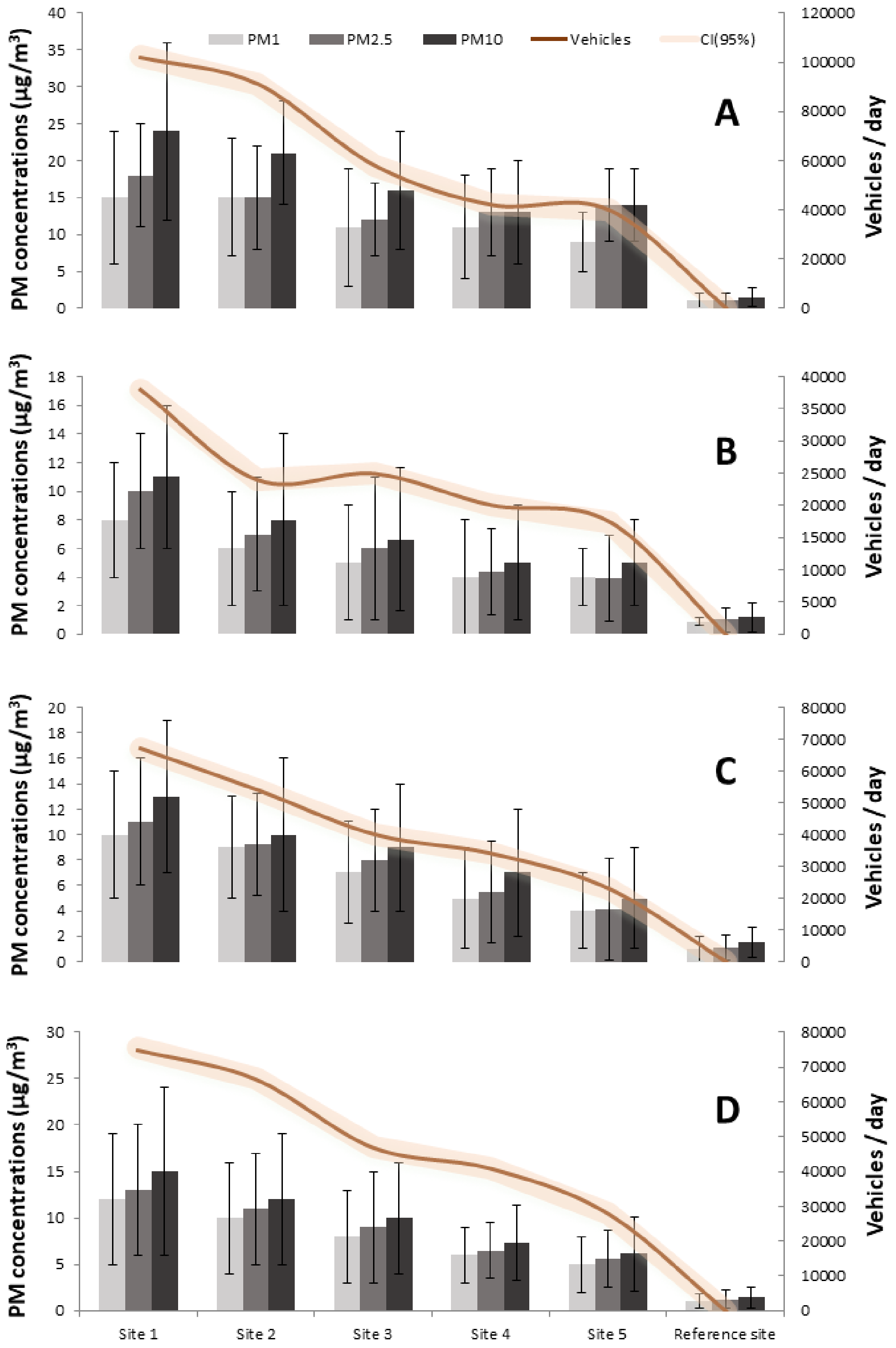


Figure 2. Fig. 2. Particulate Matter fractions ($PM_{1; 2.5; 10}$) concentrations and traffic volume (vehicles/day) in studied sites.



Note: Graphs A-D indicates 1st to 4th sampling according sampling design of the study.

Table 1. Metal analysis in soil and plants.

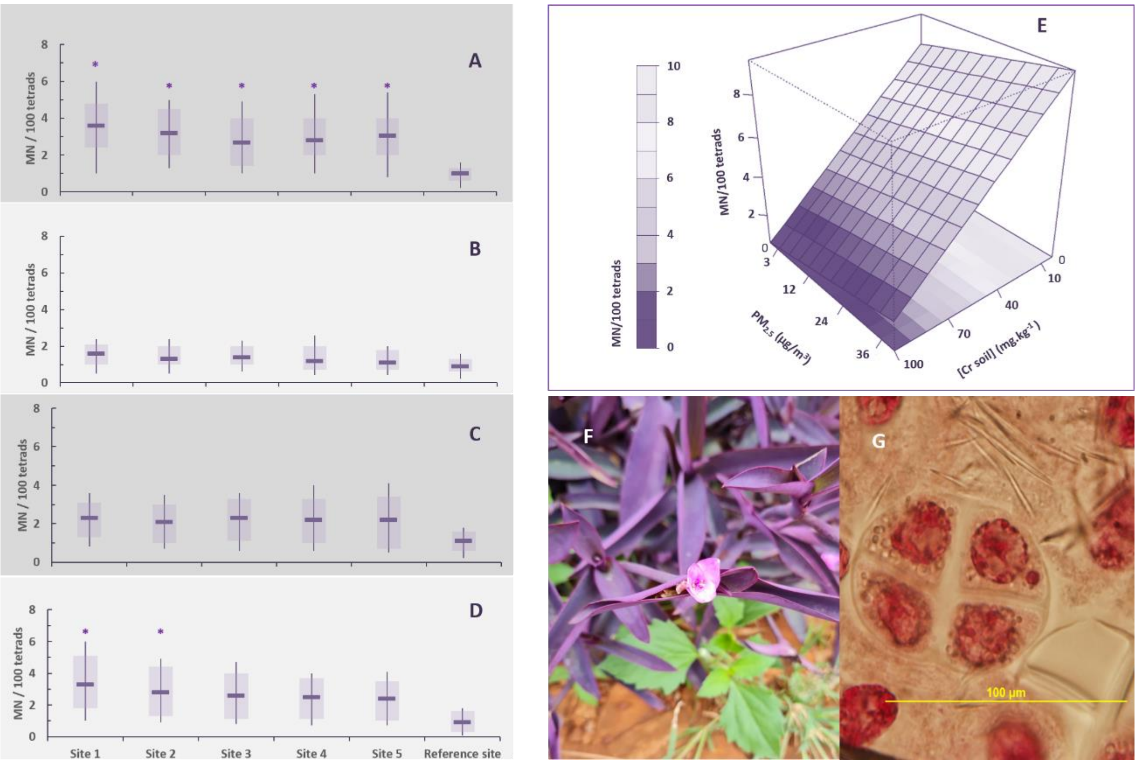
Site		[Metals ($mg.kg^{-1}$)]											
		Cd		**Ni**		**Cr**		**Pb**		**Cu**		**Zn**	
	Sampling	Soil	Plant	Soil	Plant	Soil	Plant	Soil	Plant	Soil	Plant	Soil	Plant
1	1st	0.4 ± 0.2	0.6 ± 0.2*	45.9 ± 31.1*	3.1 ± 2.4	60.2 ± 25.6*	2.3 ± 1.8*	8.5 ± 4.6	1.4 ± 0.9	29.3 ± 14.1	15.3 ± 7.8	96.0 ± 58.1	53.3 ± 15.4
	2nd	0.1 ± 0.1	0.2 ± 0.1	24.7 ± 12.5	2.0 ± 1.0	10.7 ± 5.80	0.6 ± 0.4	5.7 ± 3.7	0.7 ± 0.6	28.2 ± 12.2	12.3 ± 5.4	77.2 ± 44.3	39.2 ± 24.9
	3rd	0.2 ± 0.1	0.2 ± 0.1	18.5 ± 4.8	2.7 ± 1.8	19.5 ±13.4	1.3 ± 0.9	5.6 ± 3.1	1.2 ± 0.7	18.3 ± 15.7	8.1 ± 5.2	82.1 ± 41.4	42.6 ± 31.3
	4th	0.4 ± 0.2	0.5 ± 0.2	37.2 ± 23.6	4.0 ± 3.0	45.2 ± 25.8*	2.4 ± 2.0	10.0 ± 6.6	1.7 ± 1.1	23.5 ± 22.0	18.0 ± 10.1	76.4 ± 55.0	39.1 ± 24.0
2	1st	0.4 ± 0.2	0.6 ± 0.3*	30.1 ± 11.3	2.2 ± 1.5	46.7 ± 16.3*	1.6 ± 0.8*	11.1 ± 5.2*	1.5 ± 1.0	19.5 ± 12.4	10.1 ± 4.9	85.5 ± 38.2	48.6 ± 24.7
	2nd	0.1 ± 0.1	0.3 ± 0.2	14.2 ± 7.3	1.1 ± 0.5	14.4 ± 6.6	0.5 ± 0.3	8.9 ± 5.6	1.5 ± 1.1	16.7 ± 10.1	8.4 ± 4.3	87.2 ± 51.8	50.0 ± 23.9
	3rd	0.1 ± 0.1	0.3 ± 0.2	18.1 ± 7.9	1.7 ± 1.2	13.3 ± 7.2	1.1 ± 0.9	7.5 ± 6.4	1.2 ± 0.9	15.7 ± 11.0	9.3 ± 4.7	72.7 ± 37.4	36.9 ± 19.4
	4th	0.5 ± 0.3	0.7 ± 0.4*	30.0 ± 13.2	2.0 ± 1.7	35.1 ± 15.8*	1.2 ± 1.0	9.4 ± 7.0	1.6 ± 1.1	16.6 ± 12.2	11.6 ± 6.2	89.0 ± 50.2	49.4 ± 22.3
3	1st	0.5 ± 0.4	0.7 ± 0.3*	28.2 ± 10.2	2.4 ± 1.4	35.6 ± 14.1*	1.1 ± 0.4	7.4 ± 3.6	1.1 ± 0.6	15.3 ± 7.2	8.2 ± 3.3	67.1 ± 26.7	37.3 ± 22.0
	2nd	0.3 ± 0.1	0.4 ± 0.3	16.7 ± 9.4	1.5 ± 0.9	11.0 ± 3.4	0.7 ± 0.3	6.0 ± 4.1	1.0 ± 0.5	13.4 ± 5.5	7.6 ± 4.1	57.4 ± 20.8	30.2 ± 15.4
	3rd	0.3 ± 0.2	0.4 ± 0.3	21.3 ± 11.0	1.9 ± 1.2	9.3 ± 4.5	1.0 ± 0.5	8.2 ± 5.1	1.2 ± 0.6	11.9 ± 6.0	6.6 ± 3.7	59.6 ± 19.2	28.8 ± 13.5
	4th	0.6 ± 0.2*	0.8 ± 0.4*	33.2 ± 15.6	2.5 ± 1.6	33.3 ± 16.2*	1.2 ± 0.6	8.2 ± 4.5	1.2 ± 0.6	14.1 ± 6.3	7.6 ± 5.8	69.0 ± 30.0	39.5 ± 25.6
4	1st	0.3 ± 0.1	0.3 ± 0.3	21.1 ± 7.6	2.1 ± 0.7	22.7 ± 10.2*	1.0 ± 0.4	7.5 ± 4.2	0.9 ± 0.5	5.9 ± 3.2	3.2 ± 1.4	60.2 ± 33.2	34.1 ± 17.5
	2nd	0.2 ± 0.1	0.4 ± 0.3	12.3 ± 6.6	1.3 ± 0.8	8.3 ± 4.1	0.5 ± 0.2	7.1 ± 4.3	0.7 ± 0.4	7.2 ± 4.4	3.5 ± 1.3	48.0 ± 24.4	27.3 ± 13.8
	3rd	0.2 ± 0.1	0.3 ± 0.3	10.5 ± 5.8	1.0 ± 0.5	7.6 ± 3.6	0.6 ± 0.3	6.7 ± 4.6	0.4 ± 0.4	5.6 ± 3.1	2.7 ± 0.9	49.7 ± 29.1	31.6 ± 15.7
	4th	0.3 ± 0.2	0.4 ± 0.2	14.6 ± 7.7	1.4 ± 0.7	24.8 ± 9.0*	1.1 ± 0.6	8.4 ± 3.7	0.8 ± 0.4	6.4 ± 3.7	2.8 ± 0.8	59.3 ± 23.6	36.8 ± 22.0
5	1st	0.4 ± 0.2	0.5 ± 0.3	23.7 ± 10.2	2.3 ± 0.9	14.5 ± 6.2*	0.9 ± 0.5	7.7 ± 3.7	1.1 ± 0.5	7.5 ± 4.4	4.0 ± 2.1	77.4 ± 28.6	46.4 ± 19.4
	2nd	0.2 ± 0.1	0.2 ± 0.2	9.8 ± 5.5	0.9 ± 0.5	7.9 ± 5.0	0.4 ± 0.3	4.3 ± 2.8	0.6 ± 0.4	6.6 ± 3.9	4.5 ± 2.2	57.8 ± 32.1	29.9 ± 17.3
	3rd	0.2 ± 0.1	0.3 ± 0.2	10.4 ± 6.1	1.1 ± 0.6	6.6 ± 4.7	0.7 ± 0.3	5.3 ± 3.1	0.6 ± 0.4	6.6 ± 3.1	4.2 ± 2.5	61.6 ± 40.7	38.6 ± 28.0
	4th	0.3 ± 0.1	0.4 ± 0.2	17.1 ± 9.8	1.9 ± 0.8	15.3 ± 6.8*	1.0 ± 0.7	6.2 ± 4.0	0.9 ± 0.4	8.4 ± 3.7	5.6 ± 3.1	67.5 ± 31.4	44.2 ± 31.2
R	1st	0.1 ± 0.1	0.1 ± 0.1	11.6 ± 5.6	1.4 ± 0.7	4.9 ± 2.3	0.3 ± 0.2	3.1 ± 1.1	0.4 ± 0.1	10.2 ± 5.2	5.3 ± 3.4	80.3 ± 34.4	52.3 ± 22.4
	2nd	ND	0.1 ± 0.1	12.3 ± 6.1	0.8 ± 0.6	3.5 ± 3.1	0.3 ± 0.2	1.5 ± 0.9	ND	11.1 ± 6.1	5.6 ± 4.6	62.3 ± 26.6	34.5 ± 21.7
	3rd	0.1 ± 0.1	0.1 ± 0.1	7.9 ± 4.4	0.9 ± 0.5	5.0 ± 2.0	0.4 ± 0.3	2.1 ± 1.2	0.3 ± 0.1	7.8 ± 5.4	5.0 ± 2.7	89.7 ± 33.3	44.7 ± 32.2
	4th	ND	ND	8.8 ± 7.0	1.1 ± 0.8	5.6 ± 2.6	0.2 ± 0.2	2.0 ± 1.3	0.4 ± 0.1	9.6 ± 4.9	4.9 ± 2.9	67.2 ± 36.1	40.1 ± 31.0

Note: Gray collor backgroud indicates difference between sampling in a same site; *(Asterisk) indicates significant difference from reference site. Two-way Analysis of Variance (ANOVA).

According to the values depicted in Table 1, the concentrations of Cd and Cr were significantly higher in soil and plant samples collected in sites of greater commercial activity, during the period after the reopening of trade, indicating an effect of bioconcentration of metals heavy in *T. pallida* - as a result of the transfer of contaminants between the atmosphere, soil and biota compartments.

Figure 3 illustrates the results of the analyzes obtained from the Trad-MN assay. The frequency of micronuclei observed in plants collected in commercial areas during the first and fourth samplings was significantly higher compared to the values observed in the reference site, which corresponds to the basal rate of micronuclei for the biomonitor plant.

Figure 3. Analysis of frequency of the micronuclei in *Tradescantia pallida* collected in monitoring and reference sites.



Note: Micronucleus frequency (MN/100 tetrads) observed from locations 1st to 4th sampling (**A-D**) *(Asterisk) indicates significant difference from reference site according Kruskal-Wallis analysis, followed by Dunn tests. Graphical display of selected variables from a multiple regression analysis (**E**). *Tradescantia pallida* var. purpurea (Rose) D.R.Hunt (**F**). Micronucleus (MN) in early pollen tetrad cell (**G**).

From the mathematical model of multiple linear regression, we verified that genotoxicity can be estimated with 79.72% of response dependent on the variables X1 (particulate matter in the atmosphere – PM2.5) and X2 (concentration of chromium in the soil), which added, respectively 77.50% and 2.22% to the coefficient of determination (R2). Therefore, MN frequency (Y) can be predicted by the linear combination of these two variables (X1 and X2) by the mathematical model: [$\log_{10} MN = 1.1033 + 0.0001X1 + (0.0222X2)$, $R2 = 0.91$, $F=17.07$, $p < 0.0001$].

Discussion

Intense urbanization, accompanied by the maintenance of a vehicular fleet predominantly powered by fossil fuels, results in deterioration of air quality, an important cause of human illness in urban areas with intense vehicular traffic (Gao et al. 2023, Qi et al. 2023).

Vehicle emissions contain contaminants that, isolated or combined, produce genotoxic effects on exposed organisms (Campos et al., 2020, Piccini et al., 2023, Qi et al 2023). Thus, vehicle pollution is directly related to increased genomic instability, contributing to a higher prevalence of chronic diseases.

The analysis of soil and air contamination is relevant and justified, especially as it allows exposure via the food chain (Kumar et al. 2019), since microorganisms and plants accumulate pollutants such as heavy metals (Wai et al. 2017, Sharafi et al. 2022).

Furthermore, the integrated monitoring of pollutants in the air, soil, and sentinel plants allows to assess the persistence of genotoxic agents in the environment (Érseková et al. 2014). Our findings showed how the changes in vehicle traffic promote effects on the atmospheric concentration of particulate matter. We also demonstrate that the suspension of pollutant emission sources alters the concentrations of metals in the environment but does not completely remove them, since chemicals, especially heavy metals, are transferred between different environmental compartments, such as by the air-soil-plant (Chen et al. 2019, Moradi et al. 2021). Our results are compatible with the few studies that evaluated the genotoxicity induced by atmospheric environmental contamination from vehicles in accumulator plants (Badran et al. 2020). Previous reports have shown the accumulating potential of *T. pallida* for Cr (Sinha et al. 2017), Cd and Pb (Campos et al., 2016), and Mn (Amato-Lourenço et al. 2017).

We also observed that the Cr bioconcentration was significantly higher in collection sites and periods with higher traffic intensity and particulate matter emission - the participation of Cr in the oxidative processes related to genotoxicity effects has already been reported in the literature (Kapoor et al. 2022). The genotoxic effect can be monitored from the visualization of micronuclei, which result from chromosomal losses or breaks, indicating, respectively, aneugenic and clastogenic responses (Sharma et al. 2012).

Our results demonstrate the concentration-dependent relationship between particulate matter and micronucleus frequency in *Tradescantia pallida*. Here, it is important to highlight that the maintenance of *T. pallida* matrices to recompose the beds (monitoring sites) was an important factor to guarantee isogenicity in evaluating genotoxic effects, so that the responses can be attributed to environmental variations.

Thus, our results not only validate the sensitivity of *T. pallida*, already evaluated in other studies (Santos et al., 2015, Campos et al. 2020, Khosrovyan et al., 2022), but also confirm the hypothesis that the plant responds significantly to changes in vehicular traffic in the same environment, based on indicators of genotoxicity.

Our data reinforce the recent understanding that air quality assessments are incomplete when the focus is on the physicochemical analysis of contaminants. It is important to evaluate the effects of complex mixtures that occur in the environment and cause impacts on exposed organisms, such as the genotoxic effects evidenced in our study.

Based on the mathematical model presented, our findings report that continuous monitoring of environmental exposure indicators (atmospheric and soil contamination) and effects (bioconcentration of metals and genotoxicity) can be used as a surveillance and warning system about the risks of atmospheric pollution. Additionally, the results obtained are scientific evidence, supporting decision-makers about investments, control, inspection, and other measures that improve the quality of life of urban populations.

References

Amato-Lourenço, L. F., Lobo, D. J. A., Guimarães, E. T., Moreira, T. C. L., Carvalho-Oliveira, R., Saiki, M., Saldiva, P. H. N., Mauad, T., 2017. Biomonitoring of genotoxic effects and elemental accumulation derived from air pollution in community urban gardens. Sci Total Environ. 575, 1438–1444. https://doi.org/10.1016/j.scitotenv.2016.09.221

Araújo, R., Ramalhete, L., Paz, H., Ladeira, C., Calado, C. R. C., 2021. A new method to predict genotoxic effects based on serum molecular profile. Spectrochim. Acta A Mol. Biomol. Spectrosc. 255, 119680. https://doi.org/10.1016/j.saa.2021.119680

Badran, G., Ledoux, F., Verdin, A., Abbas, I., Roumie, M., Genevray, P., Landkocz, Y., Guidice, J. M., Garçon, G., Courcot, D., 2020. Toxicity of fine and quasi-ultrafine particles: Focus on the effects of organic extractable and non-extractable matter fractions. Chemosphere. 243, 125440. https://doi.org/10.1016/j.chemosphere.2019.125440

Campos, C.F., Campos Júnior, E. O., Souto, H. N., Sousa, E. F., Pereira, B. B., 2016. Biomonitoring of the environmental genotoxic potential of emissions from a complex of ceramic industries in Monte Carmelo, Minas Gerais, Brazil, using *Tradescantia pallida.* J. Toxicol. Environ. Health, Part A. 79, 123–128. https://doi.org/10.1080/15287394.2015.1118714

Campos, C. F., Cunha, M. C., Santos, V. S. V., Campos Júnior, E. O., Bonetti, A. M., Pereira, B. B., 2020. Analysis of genotoxic effects on plants exposed to high traffic volume in urban crossing intersections. Chemosphere. 259, 127511. https://doi.org/10.1016/j.chemosphere.2020.127511

Campos, C.F., Morelli, S., Campos Júnior, E. O., Santos, V. S. V., Morais, C. R. de, Cunha, M. C., Souto, H. N., Pavanin, L. A., Bonetti, A. M., Pereira, B. B., 2019. Assessment of the genotoxic potential of water courses impacted by wastewater treatment effluents using micronucleus assay in plants from the species *Tradescantia*. J. Toxicol. Environ. Health, Part A. 82, 752–759. https://doi.org/10.1080/15287394.2019.1648345

Chen, J., Xia, X., Wang, H., Zhai, Y., Xi, N., Lin, H., Wen, W., 2019. Uptake pathway and accumulation of polycyclic aromatic hydrocarbons in spinach affected by warming in enclosed soil/water-air-plant microcosms. J. Hazard. Mat. 379, 120831. https://doi.org/10.1016/j.jhazmat.2019.12083

Coffin, S., Weisberg, S. B., Rochman, C., Kooi, M., Koelmans, A. A., 2022. Risk characterization of microplastics in San Francisco Bay, California. Micropl. Nanopl. 2, 19. https://doi.org/10.1186/s43591-022-00037-z

DeBord, D. G., Burgoon, L., Edwards, S. W., Haber, L. T., Kanitz, M. H., Kuempel, E., Thomas, R. S., Yucesoy, B., 2015. Systems Biology and Biomarkers of Early Effects for Occupational Exposure Limit Setting. 12, 41–54. https://doi.org/10.1080/15459624.2015.1060324

Érseková, A., Hilscherová, K., Klánová, J., Giesy, J. P., Novák, J., 2014. Effect-based assessment of passive air samples from four countries in Eastern Europe. Environ. Monit. Assess. 186, 3905–3916. https://doi.org/10.1007/s10661-014-3667-z

Fang, Z., Wang, Q., Zhang, Q., Li, S., Li, S., Wang, X., Cheng, X., He, Z., Li, Z., 2022. Effects of Cr6+ stress on chromium chemical speciation distribution and bacterial community structure in the *Coix lacryma*-jobi L. constructed wetlands. Env. Pollut. Bioaval. 34, 433–445. https://doi.org/10.1080/26395940.2022.2128427

Gao, G., Pueppke, S. G., Tao, Q., Wei, J., Ou, W., Tao, Y., 2023. Effect of urban form on PM2.5 concentrations in urban agglomerations of China: Insights from different urbanization levels and seasons. J. Environ. Manage. 327, 116953. https://doi.org/10.1016/j.jenvman.2022.116953

ISO 11466. International Organization for Standardization; Geneva, Switzerland: 1995. Soil Quality – Extraction of Trace Elements Soluble in Aqua Regia.

Kapoor, R. T., Mfarrej, M. F. B., Alam, P., Rinklebe, J., Ahmad, P. Accumulation of chromium in plants and its repercussion in animals and humans. Environ. Pollut. 301, 119044. https://doi.org/10.1016/j.envpol.2022.119044

Khosrovyan, A., Aghajanyan, E., Avalyan, R., Atoyants, A., Sahakyan, L., Gabrielyan, L., Aroutiounian, R., 2022 Assessment of the mutagenic potential of the water of an urban river by means of two *Tradescantia*-based test systems. Mutat. Res. Genet. Toxicol. Environ. Mutagen. 876-877, 503449. https://doi.org/10.1016/j.mrgentox.2022.503449

Kumar, S., Prasad, S., Yadav, K. K., Shrivastava, M., Gupta, N., Nagar, S., Bach, Q. V., Kamyab, H., Khan, S. A., Yadav, S., Malav, L. C., 2019. Hazardous heavy metals contamination of vegetables and food chain: Role of sustainable remediation approaches - A review. Environ. Res. 179, 108792. https://doi.org/10.1016/j.envres.2019.108792

Kumar, R. P., Perumpully, S. J., Samuel, C., Gautam, S., 2023. Exposure and health: A progress update by evaluation and scientometric analysis. Stoch Environ Res Risk Assess. 37, 453–465. https://doi.org/10.1007/s00477-022-02313-z

Moradi, H., Abbasi, M., Soleimani, M., 2021. Bioaccumulation of heavy metals in *Stachys inflata* and *Scariola orientalis* affected by particulate matters of a cement factory in central Iran. Environ. Sci. Pollut. Res. 28, 44098–44110. https://doi.org/10.1007/s11356-021-13552-y

Pereira, B. B., Campos Júnior, E. O. de, & Morelli, S., 2013. *In situ* biomonitoring of the genotoxic effects of vehicular pollution in Uberlândia, Brazil, using a *Tradescantia* micronucleus assay. Ecotoxicol. Environ. Saf. 87, 17–22. https://doi.org/10.1016/j.ecoenv.2012.10.003

Piccini, I., Macrì, M., Gea, M., Dessì, L., Bonetta, S., Schilirò, T., Santovito, A., Bonelli, S., 2023. Genotoxic effects of particulate matter on larvae of a common and widespread butterfly along an urbanization gradient. Ecotoxicol. Environ. Saf. 252, 114638. https://doi.org/10.1016/j.ecoenv.2023.114638

Qi, G., Che, J., Wang, Z., 2023. Differential effects of urbanization on air pollution: Evidences from six air pollutants in mainland China. Ecol. Indic. 146, 109924. https://doi.org/10.1016/j.ecolind.2023.109924

Ramić, E., Huremović, J., Muhić-Šarac, T., Đug, S., Žero, S., Olovčić, A., 2019. Biomonitoring of air pollution in bosnia and herzegovina using epiphytic lichen *Hypogymnia physodes*. Bull. Environ. Contam. Toxicol. https://doi.org/10.1007/s00128-019-02595-0

Santos, A. P. M., Segura-Muñoz, S. I., Nadal, M., Schuhmacher, M., Domingo, J. L., Martinez, C. A., Takayanagui, A. M. M., 2015. Traffic-related air pollution biomonitoring with *Tradescantia pallida* (Rose) Hunt. cv. purpurea Boom in Brazil. Environ. Monit. Assess. 187, https://doi.org/10.1007/s10661-014-4234-3

Sharafi, K., Mansouri, B., Omer, A. K., Bashardoust, P., Ebrahimzadeh, G., Sharifi, S., Massahi, T., Soleimani, H., 2022. Investigation of health risk assessment and the effect of various irrigation water on the accumulation of toxic metals in the most widely consumed vegetables in Iran. Sci. Rep. 12, 20806. https://doi.org/10.1038/s41598-022-25101-9

Sharma, V., Kumar, A., Dhawan, A., 2012. Nanomaterials: exposure, effects and toxicity assessment. Proc. Natl. Acad. Sci. 82, 3–11. https://doi.org/10.1007/s40011-012-0072-7

Sinha, V., Manikandan, N. A., Pakshirajan, K., Chaturvedi, R. Continuous removal of Cr(VI) from wastewater by phytoextraction using *Tradescantia pallida* plant based vertical subsurface flow constructed wetland system. Int. Biodeterior. Biodegrad. 119, 96–103. https://doi.org/10.1016/j.ibiod.2016.10.003

U.S. Environmental Protection Agency. 2007. Method 7010— Graphite furnace atomic absorption spectrophotometry, rev.00. http://www3.epa.gov/epawaste/hazard/testmethods/sw846/pdfs/7010.pdf

Wai, K.M., Dai, J., Yu, P.K.N., Zhou, X., Wong, C.M.S., 2017. Public health risk of mercury in China through consumption of vegetables, a modelling study. Environ. Res. 159, 152-157. https://doi.org/10.1016/j.envres.2017.08.010