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**Avaliação dos Efeitos da Exposição  
Humana a Contaminantes Atmosféricos  
Associada ao Biomonitoramento da  
Qualidade do Ar.**

**UFCSPA**

Universidade Federal de Ciências da Saúde  
de Porto Alegre

**Porto Alegre**

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Dissertação submetida ao Programa de Pós-Graduação em Ciências da Saúde da Fundação Universidade Federal de Ciências da Saúde de Porto Alegre como requisito para a obtenção do grau de Mestre.

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## RESUMO

A poluição atmosférica é exacerbada nas proximidades de avenidas de intenso tráfego automotor nas cidades. A composição e concentração dos poluentes variam por região e é dependente do gradiente urbano-rural. O objetivo deste estudo foi avaliar a qualidade do ar em áreas com diferentes gradientes populacionais próximas a vias de intenso tráfego automotor por meio de biomonitoramento vegetal e monitoramento passivo, bem como relacionar estes dados com marcadores de exposição humana. As áreas de estudo foram selecionadas em regiões de alta, intermediária e baixa densidade populacional. Para o monitoramento vegetal, foi realizada a medição de elementos acumulados em casca de árvore e o ensaio do aborto polínico em *Bauhinia variegata*. O grau exposição dos estudantes destas áreas foi avaliado pela quantificação de elementos acumulados em unhas e o ensaio do micronúcleo em mucosa bucal. As concentrações de dióxido de nitrogênio e ozônio foram medidas por monitoramento passivo. Nós reportamos aqui que a frequência de aborto polínico e a concentração de dióxido de nitrogênio foram fortemente associadas com o fluxo veicular e a densidade populacional. A frequência de micronúcleo foi maior na área de maior grau de urbanização seguindo o mesmo padrão do ozônio. Da mesma maneira, os elementos Ba, Cd, Co, Cu, Mg, Mn, Ni, Pb, Sb, Sr, V e Zn foram encontrados em maiores concentrações nas cascas de árvore da área de alta densidade populacional, assim como os elementos Cd, Co, Cu, Mg, Mn, Ni e Sr tiveram maiores níveis nas unhas dos estudantes desta região. Em conclusão, o grau de urbanização na cidade de Porto Alegre está associado com a genotoxicidade humana e vegetal, além do maior acúmulo, em ambas as matrizes, de elementos marcadores da poluição veicular.

Palavras- chave: Poluição atmosférica, micronúcleo, metais, biomonitoramento, genotoxicidade, monitoramento passivo.

## ABSTRACT

Air pollution is exacerbated near heavy traffic roads in cities. Air pollution concentration and composition vary by region and depend on urban-rural gradients. The aim of this study was to evaluate the air quality in areas of varying population densities near heavy traffic roads by plant biomonitoring and passive sampling, as well as associate these data with biomarkers of human exposure. Study areas were selected in regions with high, intermediate and low population density in Porto Alegre. For plant biomonitoring assay, elements accumulated in tree barks and the pollen abortion assay was performed on *Bauhinia variegata*. The exposure of students in these areas was evaluated by the quantification of elements accumulated in nails and the micronucleus assay in buccal cells. Concentrations of nitrogen dioxide and ozone were measured by passive sampling. We report here that that the pollen abortion frequency and nitrogen dioxide concentration were strongly associated with vehicular flow and population density in the studied areas. Micronuclei frequency in buccal cells was higher in the regions with more degree of urbanization, following the same pattern of ozone concentrations. In addition, the elements Ba, Cd, Co, Cu, Mg, Mn, Ni, Pb, Sb, Sr, V and Zn showed higher concentrations in tree barks of the area with high population density, while Cd, Co, Cu, Mg, Mn, Ni and Sr were in higher levels of students nails in this area as well. In conclusion, the urbanization gradient in Porto Alegre is associated with human and plant genotoxicity, as well as higher levels of traffic-related trace elements in both matrices.

Keywords: air pollution, micronucleus, metals, biomonitoring, genotoxicity, passive sampling.

## LISTA DE ABREVIATURAS

As - Arsênio

Br - Bromo

Ca – Cálcio

Cd- Cádmio

CO – Monóxido de Carbono

CO<sub>2</sub> – Dióxido de Carbono

Cr - Cromo

Cu - Cobre

FFG - *Feulgen-Fast Green*

HPA – Hidrocarbonetos Policíclicos Aromáticos

H<sub>2</sub>O<sub>2</sub> - peróxido de hidrogênio

IARC – *International Agency for Research on Cancer*

K - Potássio

MP - Material Particulado

MP<sub>0,1</sub> – Material Particulado Ultrafino

MP10 – Material Particulado Grosso

MP<sub>2,5</sub> – Material Particulado Fino

MN - Micronúcleo

NO<sub>2</sub> – Dióxido de Nitrogênio

NO<sub>x</sub> - Óxidos de Nitrogênio

O<sub>2</sub><sup>-</sup> - Ânion Superóxido

O<sub>3</sub> – Ozônio

OH<sup>-</sup> - radical hidroxila

OMS – Organização Mundial da Saúde

S - Enxofre

Sb – Antimônio

Se - Selênio

SO<sub>2</sub> - Dióxido de Enxofre

Trad-MN – Micronúcleo em *Tradescantia pallida*

Zn – Zinco

## **1. Introdução**

### **1.1– Poluição Atmosférica**

A poluição atmosférica é uma mistura complexa e dinâmica de poluentes. Dentre estes poluentes, destacam-se o material particulado (MP), ozônio (O<sub>3</sub>), dióxido de enxofre (SO<sub>2</sub>), monóxido de carbono (CO), óxidos de nitrogênio (NO<sub>x</sub>), hidrocarbonetos policíclicos aromáticos (HPA), compostos orgânicos voláteis e metais em concentrações desproporcionais no ar (AKIMOTO, 2003).

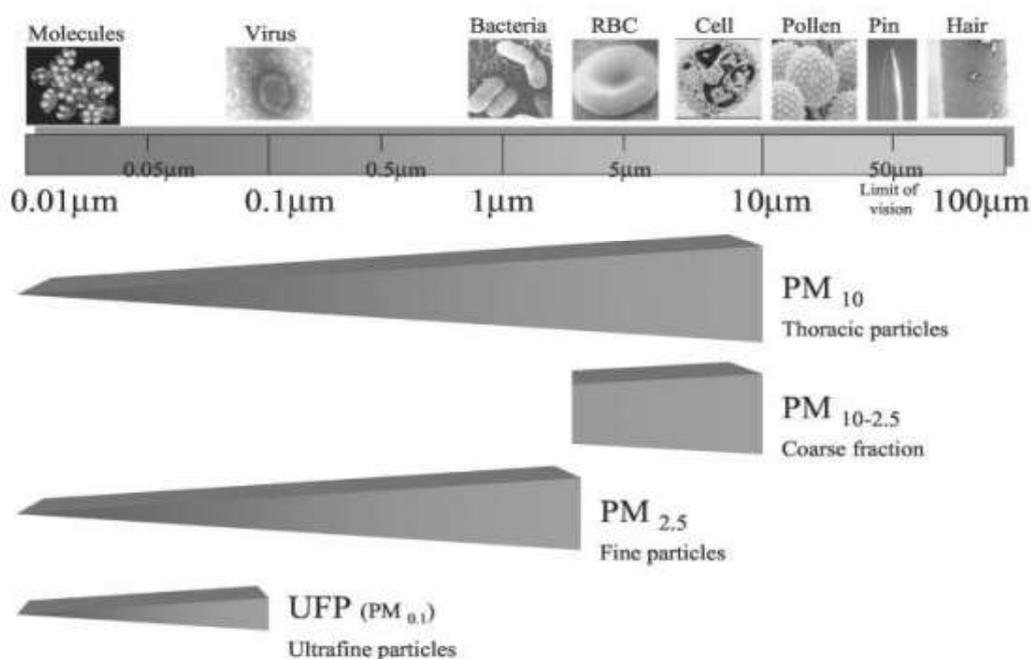
A poluição atmosférica pode ser categorizada de acordo com a origem de sua fonte de emissão. Poluentes de fonte antropogênica são aqueles cuja emissão está relacionada diretamente com a ação humana como, por exemplo, emissões industriais, veículos automotores, atividade mineradora ou na queima intencional de biomassa, como ocorre em regiões canavieiras no Brasil. Em contrapartida, a poluição do ar pode ainda ser classificada como de origem natural quando sua emissão ocorre por fontes não humanas, como em erupções vulcânicas, ressuspensão de poeira do solo, e incêndios florestais (FERREIRA BRAGA *et al.*, 2007; DE MIRANDA *et al.*, 2012).

Os poluentes atmosféricos podem ainda ser classificados em poluentes primários ou secundários. Poluentes primários são aqueles emitidos diretamente da fonte para a atmosfera sem que haja transformações químicas dos mesmos, exemplos de poluentes primários são o MP, dióxido de nitrogênio (NO<sub>2</sub>) e dióxido de carbono (CO<sub>2</sub>). Os poluentes secundários são assim denominados pois são formados na atmosfera através de reações químicas envolvendo os poluentes primários. Por exemplo, o ácido sulfúrico (H<sub>2</sub>SO<sub>4</sub>), agente causador da chuva ácida, é formado na atmosfera através da conversão do SO<sub>2</sub> em trióxido de enxofre (SO<sub>3</sub>) seguido pela reação com a umidade presentes na atmosfera (NARESH, SUNDAR E SHUKLA, 2006).

Diversos estudos têm como objetivo caracterizar os componentes do ar ou ainda descrever a toxicidade relacionada a um poluente específico. Neste contexto, o MP tem sido um dos poluentes ao qual a atenção da comunidade científica tem estado mais focada. O MP consiste em uma complexa mistura de componentes sólidos e líquidos presentes no ar. Em sua estrutura incluem carbono elementar e orgânico, compostos inorgânicos (sulfatos, nitratos, amônia e cloreto), além de compostos orgânicos voláteis, componentes biológicos e metais adsorvidos à superfície (HARRISON E YIN, 2000). O MP é dividido

de acordo com seu tamanho aerodinâmico, sendo sua toxicidade inversamente proporcional ao tamanho da partícula (Figura 1). Partículas grossas, de tamanho aerodinâmico entre  $10\mu\text{m}$  e  $2,5\mu\text{m}$  ( $\text{MP}_{10}$ ), são originárias da poeira de estradas, desgaste de pneus, atividades ligadas à agricultura, construção e na combustão da madeira. As partículas finas, de tamanho aerodinâmico entre  $2,5\mu\text{m}$  e  $0,1\mu\text{m}$  ( $\text{MP}_{2,5}$ ), são formadas durante o processo de combustão ou atividade industrial. Fontes de  $\text{MP}_{2,5}$  incluem escapamento de automóveis, desgaste de freios, refinarias de petróleo e plantas industriais. Por último, as partículas ultrafinas, ou menores do que  $0,1\mu\text{m}$  ( $\text{MP}_{0,1}$ ), são emitidas ao ambiente principalmente através do escapamento de veículos automotores (CRAIG *et al.*, 2008; BLOCK E CALDERON-GARCIDUENAS, 2009). O sistema respiratório é o principal alvo da toxicidade do MP. Seus mecanismos incluem estresse oxidativo e aumento da inflamação pulmonar e das vias aéreas (SCHAUMANN *et al.*, 2004; VALAVANIDIS, FIOTAKIS E VLACHOGIANNI, 2008; ORONA *et al.*, 2014). Contudo, partículas de menor tamanho aerodinâmico têm a capacidade de causar efeitos tóxicos fora do sistema respiratório, o que inclui o sistema nervoso central (LOANE *et al.*, 2013) e sistema cardiovascular (WANG, ELIOT E WELLENIUS, 2014).

Figura 1: Classificação aerodinâmica do MP (BROOK *et al.*, 2004).



Outro poluente com conhecidas implicações à saúde é o O<sub>3</sub>. Este é um poluente secundário formado na atmosfera a partir de reações entre compostos orgânicos voláteis e NO<sub>2</sub> na presença de luz solar, e sua distribuição no ambiente urbano é dependente da direção do vento e características geográficas locais (SILLMAN, 1999). Em razão de sua formação na atmosfera estar diretamente associada à quantidade de luz solar, a concentração de O<sub>3</sub> em ambientes externos é significativamente maior do que em ambientes internos (MONN, 2001). Por ser um gás altamente oxidante, a toxicidade do O<sub>3</sub> concentra-se principalmente no sistema respiratório. Diversos estudos relacionam a exposição a altas concentrações deste gás com a diminuição da função pulmonar, inflamação das vias aéreas e exacerbação de episódios asmáticos (WHITE *et al.*, 1994; SILVERMAN E ITO, 2010; GROVES *et al.*, 2012; MODIG *et al.*, 2014).

O NO<sub>2</sub> é um poluente primário emitido à atmosfera através do escapamento de veículos. Por este motivo, o NO<sub>2</sub> é considerado um marcador da queima de combustíveis automotores. Além disso, o nível atmosférico deste poluente está correlacionado com diversos outros compostos veiculares como MP, *black carbon*, HPAs e SO<sub>2</sub> (BECKERMAN *et al.*, 2008). Os efeitos do NO<sub>2</sub> na saúde estão principalmente focados ao sistema respiratório. A exposição a este poluente está relacionada ao surgimento e exacerbação de episódios asmáticos, especialmente em crianças (TAKENOUE *et al.*, 2012; ESPOSITO *et al.*, 2014).

Além dos efeitos individuais de poluentes à saúde, diversos estudos buscam elucidar os riscos da poluição atmosférica em sua totalidade. Esta complexa abordagem tem como objetivo caracterizar tais efeitos de maneira que a poluição atmosférica seja considerada fator de risco para diversas morbidades, independentemente do local, fonte ou diferenças na composição. Neste contexto, já é bem presente na literatura a associação entre poluição atmosférica e câncer de pulmão (FAJERSZTAJN *et al.*, 2013; RAASCHOU-NIELSEN *et al.*, 2013). Tais evidências levaram, em 2013, a Agência Internacional de Pesquisa sobre Câncer (IARC) a incluir a poluição atmosférica *outdoor* como agente cancerígeno do grupo 1. Apesar de que a composição e os níveis dos poluentes podem variar entre diversas localidades, tal conclusão se aplica a todas as regiões do mundo (LOOMIS *et al.*, 2013).

## 1.2- A Poluição Atmosférica em Centros Urbanos

A emissão de poluentes à atmosfera devido a ações humanas se intensificou a partir da época à qual o ser humano passou a basear sua cultura econômica no desenvolvimento industrial em detrimento da sociedade rural vigente até então. No entanto, foi apenas em meados do século XX que começou a ser estabelecida uma relação causal entre poluição atmosférica e aumento da mortalidade ou morbidade em centros urbanos. O episódio de maior destaque relacionado à poluição atmosférica em uma metrópole, conhecido como “*London Smog*”, ocorreu em Londres no inverno de 1952. Nesta ocasião, houve um acréscimo de cerca de 4.000 mortes devido à alta concentração de poluentes no ar na cidade (LOGAN, 1953). Uma revisão posterior mostra que a taxa de mortalidade entre Dezembro de 1952 e Fevereiro de 1953 pode ter chegado a 12.000 devido a efeitos agudos e persistentes deste episódio (BELL E DAVIS, 2001).

Nos Estados Unidos, um grande estudo epidemiológico demonstrou a relação entre taxa de mortalidade e concentrações atmosféricas de partículas finas em seis cidades. A taxa de mortalidade esteve positivamente associada com morte por câncer de pulmão e doenças cardiovasculares mesmo após correção para o hábito de fumar (DOCKERY *et al.*, 1993).

No Brasil, foram analisadas as concentrações de  $MP_{2,5}$  em seis regiões metropolitanas (Porto Alegre, Rio de Janeiro, São Paulo, Curitiba, Recife e Belo Horizonte) onde foi estimado o custo da morbidade atribuível à fontes veiculares em R\$ 687.691.763,00 ao ano nas cidades São Paulo, Recife, Belo Horizonte e Rio de Janeiro. Neste contexto, Porto Alegre foi considerada a segunda capital mais poluída no inverno, tendo seus índices de MP menores apenas do que São Paulo neste período (MINISTÉRIO DO MEIO AMBIENTE, 2009). Este resultado pode ser explicado também pelas diferenças climáticas de Porto Alegre em relação às outras capitais. Nos meses de Junho, Julho e Agosto há uma queda na temperatura, resultando no resfriamento da atmosfera e uma menor circulação do ar, principalmente no período da manhã. Consequentemente, há uma menor dispersão dos poluentes e maior concentração destes nas camadas mais baixas da atmosfera. Apesar das medições precisas da concentração anual de  $MP_{2,5}$  em Porto Alegre, não foi possível realizar a identificação estatística das fontes poluidoras, incluindo a poluição veicular (ANDRADE *et al.*, 2012).

Diversos outros estudos buscaram demonstrar os efeitos da poluição atmosférica na Região Metropolitana de Porto Alegre com a utilização de modelos experimentais. Estes incluem modelos de biomonitoramento vegetal em *Tradescantia pallida* onde foi observada uma maior taxa de micronúcleo no ambiente urbano quando comparado ao ambiente rural (COSTA E DROSTE, 2012), além da análise de elementos acumulados em líquens, onde foram medidas altas concentrações de S, Cr, Zn, Pb e Hg nestes bioindicadores em três áreas de Porto Alegre com influência de tráfego automotor (KAFFER *et al.*, 2012). Adicionalmente, foi verificada a genotoxicidade de HPAs relacionados a emissões veiculares e industriais na Região Metropolitana de Porto Alegre e capital em modelos *in situ* (DE BRITO *et al.*, 2013) e animal (IANISTCKI *et al.*, 2009). Além de danos genotóxicos, foram verificadas alterações bioquímicas e fisiológicas importantes causadas pela poluição atmosférica de Porto Alegre. Zanchi et al. (2010) demonstrou um aumento no dano lipídico causado pelo aumento de substâncias oxidantes no córtex de ratos expostos cronicamente à poluição da cidade, aliado a um prejuízo na memória discriminativa de curta duração (ZANCHI *et al.*, 2010). Ademais, taxistas ocupacionalmente expostos à HPAs provenientes da poluição veicular apresentaram um alto nível de marcadores inflamatórios e marcadores de eventos cardiovasculares quando comparados a indivíduos não expostos ocupacionalmente a estes compostos (BRUCKER *et al.*, 2013; BRUCKER *et al.*, 2014).

Os estudos citados nos parágrafos acima ganham ainda mais destaque quando considerada a situação atual do monitoramento de poluentes atmosféricos em Porto Alegre. Em 2014, a Organização Mundial da Saúde (OMS) disponibilizou um relatório contendo a média anual de MP<sub>10</sub> e MP<sub>2,5</sub> de 1622 cidades de 91 países ao redor do mundo, sendo estas 40 no Brasil (a maioria no estado de São Paulo) (OMS, 2014). Contudo, apesar do grande número de cidades, inclusive no Brasil, Porto Alegre não é citada neste relatório, visto que a rede de monitoramento automático da cidade não se encontra operacional. A falta de uma rede de monitoramento automático estruturada em Porto Alegre, além de inviabilizar a realização de estudos epidemiológicos relacionando a concentração dos diferentes poluentes com efeitos adversos à saúde do ser humano, ainda causa a privação de informações para a população acerca da qualidade do ar na cidade e os seus riscos relacionados. Infelizmente, a aferição da concentração dos poluentes atmosféricos em Porto

Alegre assim como a avaliação de seus efeitos adversos está limitada a alguns poucos estudos experimentais.

### 1.3 – Mobilidade Urbana

Um problema comum à maioria das grandes cidades é a perda de mobilidade gerada pelos congestionamentos em vias públicas. Existe um padrão na sociedade que leva a população a locomover-se em uma faixa de horário específica. Seja por causa do horário de trabalho ou escolar, esse padrão obriga milhares de pessoas a viajar na parte da manhã, entre 7h00 e 9h00, e na parte da tarde, entre 17h00 e 19h00. Aliado a este padrão de mobilidade conjunta e sincronizada, está a preferência pelo uso de transporte individual em detrimento do transporte coletivo ou não motorizado. Quando os congestionamentos do pico da manhã começam a se prolongar de maneira assustadora, podendo, em alguns anos, se encontrar com os picos da tarde, isso resulta em travamento das principais vias, com graves consequências para a qualidade de vida dos cidadãos, associadas a enormes prejuízos econômicos, sociais, ambientais e de saúde (RESENDE E SOUZA, 2009).

Neste contexto, observamos um aumento contínuo na frota veicular de Porto Alegre. Nos últimos anos, Porto Alegre teve um incremento de 591.598 veículos em 2007 para 780.551 veículos em 2013 (Fonte: Detran/RS). Em termos absolutos isto significa um aumento de 188.953 veículos, enquanto que em termos relativos estes números representam um aumento de 31,9% na frota veicular de Porto Alegre em apenas sete anos (Tabela 1).

Tabela 1. Aumento da frota veicular de Porto Alegre entre 2007 e 2013.

	2007	2008	2009	2010	2011	2012	2013	Total
Frota Veicular	591.598	627.580	659.418	686.142	716.493	748.751	780.551	188,953
Aumento (%)		6,1	5,1	4	4,4	4,5	4,2	31,9

Fonte: Adaptado de Detran/RS.

Porto Alegre é uma importante capital nacional que não apresenta alternativas eficientes ao transporte automotor dentro de seu território. Apesar de planos existentes para a construção do sistema metroviário e ampliação de ciclovias, esta primeira alternativa de transporte está longe de ser viabilizada e disponível para a população, enquanto que as ciclovias, até o presente momento, localizam-se em uma porção restrita na região central da cidade. Além disso, a região central de Porto Alegre é caracterizada como destino do

deslocamento de milhares de trabalhadores e estudantes durante o dia, sendo ainda o ponto de convergência para um número significativo de linhas de ônibus que trafegam na cidade.

Como observado na Tabela 1, o aumento no número de veículos em um período tão curto supera qualquer esforço público voltado à melhoria da mobilidade urbana e, em consequência, aumento dos prejuízos à saúde causada pela poluição atmosférica proveniente de tal fonte. Assim, a ampliação do sistema viário assume grande destaque e as administrações municipais dedicam uma parcela enorme de seus esforços e recursos para a sua expansão. O modelo de urbanização atual gera a fragmentação do espaço urbano, criando bairros residenciais cada vez mais distantes dos locais de trabalho e de lazer, além de deslocar a população mais carente para a periferia dos grandes centros. Esta ocupação gera vazios urbanos e a infraestrutura construída para a circulação de automóveis ou mesmo para o transporte coletivo gera áreas degradadas física e economicamente. A lógica dos investimentos reforça esta forma de ocupação e as cidades menores reproduzem o modelo de desenvolvimento das cidades maiores, apesar da demonstração diária dos problemas resultantes (BOARETO, 2008).

O que se observa como resultado é o prejuízo na mobilidade, aumento do tempo de deslocamento, congestionamento, aumento da frota de veículos particulares e prejuízos na saúde como consequência do aumento dos poluentes atmosféricos. No entanto, a poluição atmosférica não atinge a todos democraticamente, mas tem sua exposição e efeitos mais acentuados na população mais pobre, usuária mais frequente do sistema público de transporte. Os usuários de transporte público presentes ao longo do corredor de ônibus estão expostos a maiores concentrações de poluentes atmosféricos devido à proximidade com os veículos enquanto esperam pelo ônibus (MOORE E FIGLIOZZI, 2013). Neste contexto, a perda de mobilidade gerada pelo aumento do número de veículos nas vias acarreta, também, no aumento do tempo de espera das pessoas no corredor de ônibus, elevando a exposição a níveis críticos de poluentes.

## **1.4 – Monitoramento da Qualidade do Ar**

### **1.4.1 – Monitoramento Ativo x Monitoramento Passivo**

Diversos países possuem redes de monitoramento bem estabelecidas que mensuram de maneira contínua os níveis dos poluentes presentes no ar. O  $MP_{10}$ ,  $MP_{2,5}$ ,  $O_3$ ,  $SO_2$  e  $NO_2$

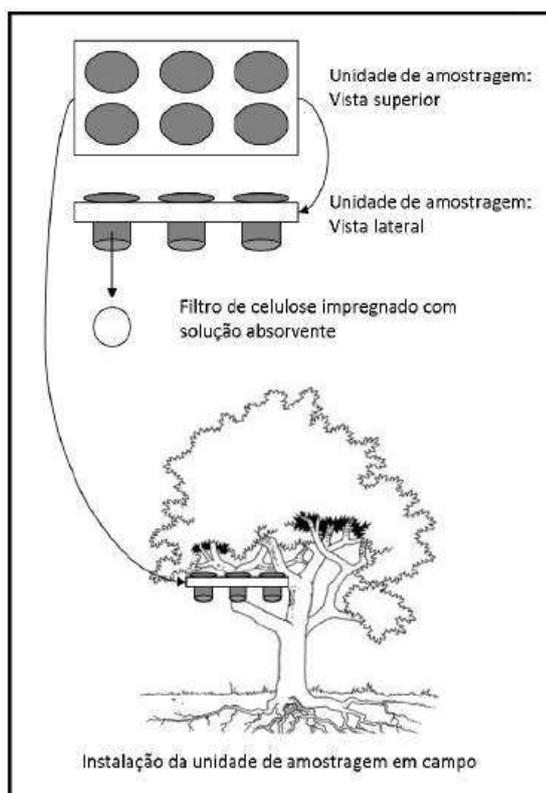
são os poluentes frequentemente avaliados nas redes de monitoramento ativo (LOOMIS *et al.*, 2013). Estas redes de monitoramento possuem papel fundamental em estudos epidemiológicos que buscam elucidar os efeitos da poluição atmosférica em um ambiente urbano, além de servirem de suporte para políticas públicas locais voltadas para o controle de emissões e monitoramento a nível municipal. No entanto, apesar da confiabilidade dos dados e a possibilidade de medição de maneira contínua, algumas limitações estão inerentes às redes de monitoramento ativo. Em razão dos monitores fixos possuírem um alto custo e estarem primariamente objetivando a vigilância, eles estão frequentemente restritos a monitorar emissões de fontes industriais específicas ou de cidades altamente populosas. Isto leva a uma fraca cobertura em regiões rurais de países desenvolvidos, enquanto que países em desenvolvimento possuem pouca ou nenhuma cobertura. Como consequência, redes fixas de monitoramento possuem pouca eficiência em monitorar os efeitos da poluição do ar na saúde em regiões rurais ou em países em desenvolvimento (PRUD'HOMME *et al.*, 2013).

As limitações supracitadas do uso de redes de monitoramento ativo têm estimulado o aperfeiçoamento e emprego de técnicas alternativas para análise da qualidade do ar em locais de interesse que não possuem redes de monitoramento estabelecidas, principalmente em países em desenvolvimento, entre estas se destaca o monitoramento passivo. Esta técnica é baseada na impregnação de um reagente (solução absorvente) específico para determinado poluente gasoso em filtros, que por sua vez são posicionados em tubos de difusão protegidos da ação da luz e chuva. A solução reagente presente no filtro ao entrar em contato com o poluente no ar, por difusão, reage formando um segundo composto que é extraído, quantificado e relacionado com a concentração aproximada deste poluente na atmosfera (COX, 2003). Estes tubos de difusão, os quais possuem a função de albergar os filtros impregnados, são geralmente colocados em suportes e instalados em árvores ou postes no local de interesse. (Figura 2).

As principais vantagens relacionadas à análise de gases poluentes por monitoramento ativo estão relacionadas à não necessidade de energia elétrica, à portabilidade e à análise da concentração absoluta do ar sem a necessidade de calibração, fatores que determinam sua importância para o monitoramento em áreas isoladas. Já o baixo custo do material, além da simplicidade dos procedimento analítico são fatores que

propiciam o controle de emissões em países em desenvolvimento ou que não possuam uma rede de monitoramento estabelecida (COX, 2003; CAPE, 2009). Os poluentes comumente analisados com esta metodologia são o NO<sub>2</sub> (SEKINE et al., 2008), O<sub>3</sub> (ALEJO et al., 2011) e SO<sub>2</sub> (CRUZ et al., 2004).

Figura 2: Unidade de amostragem de monitoramento passivo (CARNEIRO, 2010).



#### 1.4.2 – Biomonitoramento da Qualidade do Ar

Por definição, biomonitoramento é caracterizado como o uso de organismos (bioindicadores) para obter informações sobre certas características da biosfera. A informação gerada no biomonitoramento é comumente deduzida a partir de mudanças no comportamento do organismo ou da mensuração de substâncias específicas no tecido do monitor. As principais vantagens do uso do biomonitoramento na caracterização da poluição atmosférica estão relacionadas com a vastidão de espécies que podem ser utilizadas (líquens, fungos, animais e vegetais), facilidade dos procedimentos operacionais e a não necessidade do uso de tecnologias onerosas (WOLTERBEEK, 2002).

Dentre a variedade de monitores, as plantas têm sido vastamente empregadas em estudos acerca do impacto da poluição atmosférica. Neste contexto, estes bioindicadores podem ainda ser classificados de acordo com a abordagem do monitoramento. A abordagem passiva, também conhecida como sentinela, faz uso da coleta de material de bioindicadores já presentes no local de estudo, esta abordagem é utilizada principalmente em estudos com plantas superiores; em que, devido ao seu tamanho, o transporte do bioindicador ao ponto de monitoramento torna-se inviável. Por outro lado, o monitoramento ativo, ou portátil, pode ser utilizado quando há a disponibilidade operacional de realocar plantas até o local de estudo. As vantagens do bioindicador portátil baseia-se na possibilidade de procedência da planta a partir de um ambiente controlado, além da eliminação de vieses relacionados à composição do solo (uma vez que esta pode ser controlada) e possibilidade de uniformização de cuidados ao biomonitor através da disponibilidade controlada de água e nutrientes (GUIMARAES *et al.*, 2000; SUMITA *et al.*, 2003).

Os bioindicadores podem ainda ser classificados de acordo com sua resposta à exposição ao poluente. Neste contexto, há a divisão entre bioindicadores sensíveis (biosensores) e bioindicadores acumuladores (bioacumuladores) (WOLTERBEEK, 2002). Dentro desta divisão, a resposta do organismos é específica quando somente um fator ambiental determina a resposta ao organismo, ao passo que respostas não específicas ocorrem inúmeros fatores ambientais determinam a resposta do bioindicador.

#### **1.4.2.1 – Bioindicadores Sensíveis**

Bioindicadores sensíveis são aqueles que reagem a um poluente modificando-se fisiologicamente, geneticamente, etologicamente ou morfológicamente, expressando tal modificação de maneira microscópica ou macroscópica, sendo também denominados organismos de reação (KLUMPP *et al.*, 2001). Entre os diversos modelos desta categoria que são empregados em estudos com a poluição atmosférica, destacam-se o ensaio da *Nicotiana tabacum* (específico para O<sub>3</sub>) e os ensaios para avaliação de genotoxicidade, seja por meio da avaliação do micronúcleo em *Tradescantia pallida* (Trad-MN) e/ou por aborto polínico.

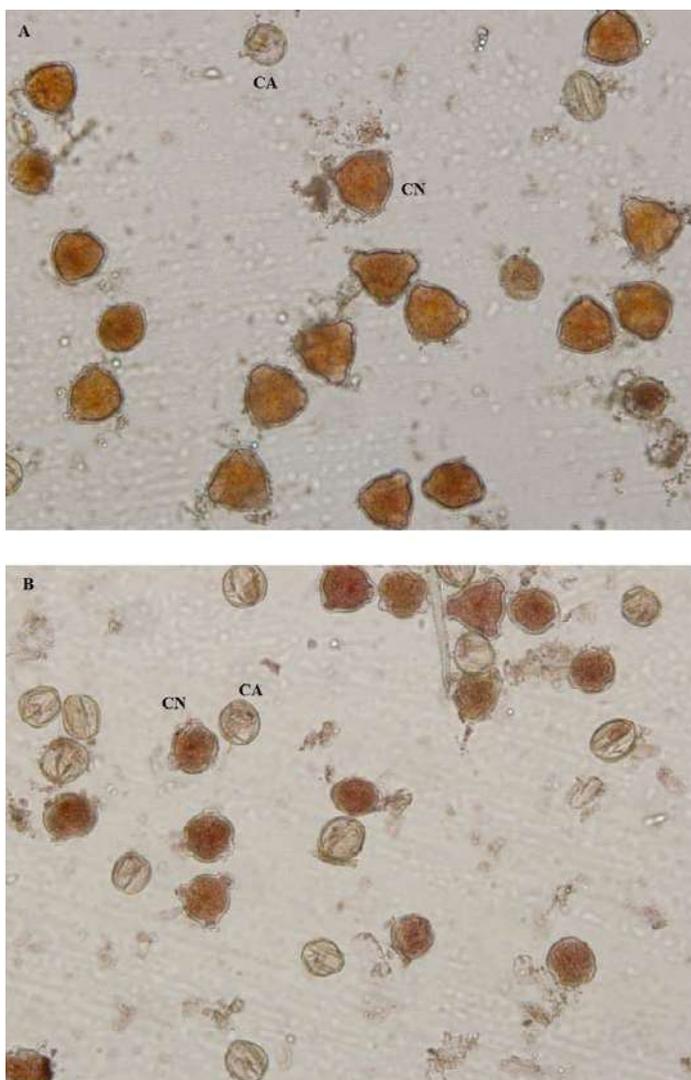
A folha do tabaco (*Nicotiana tabacum*) é um bioindicador sensível específico ao O<sub>3</sub>, sendo empregado em diversos estudos que investigam os danos oxidativos deste poluente em centros urbanos (CRISTOFOLINI *et al.*, 2011; BOROWIAK E WUJESKA, 2012; BOROWIAK, 2013). A injúria foliar induzida pelo ozônio é consequência da produção de espécies reativas de oxigênio como o ânion superóxido (O<sub>2</sub><sup>-</sup>), peróxido de hidrogênio (H<sub>2</sub>O<sub>2</sub>) e radical hidroxila (OH<sup>-</sup>) dentro da folha. As respostas ao estresse oxidativo ocorrem desde o nível celular até o organismo como um todo, ocasionando mudanças na estrutura da membrana celular, alterações na atividade antioxidante, diminuição na fotossíntese e aceleração da senescência celular (SILVA, MEIRELLES E MORAES, 2012).

O Trad-MN é um dos bioensaios mais comumente empregados para detecção de danos genotóxicos em decorrência de poluentes ambientais. Este ensaio é baseado na formação de micronúcleo resultante da quebra cromossômica nas células-mãe de pólen em resposta a um agente estressor (KLUMPP *et al.*, 2006). A *Tradenscatia pallida* possui boa adaptação a climas tropicais e subtropicais, produzindo flores durante todo o ano, independentemente se foram cultivadas em canteiros, vasos ou jardins, o que justifica a sua aplicação em diversos locais no mundo (SAVOIA *et al.*, 2009). Este ensaio foi originalmente desenvolvido para a avaliação dos efeitos mutagênicos do 1,2-Dibromoetano (MA *et al.*, 1978). Posteriormente, diversos pesquisadores empregaram este bioindicador para análise de agentes genotóxicos presentes no ar. Tais estudos incluem investigações de emissões em indústrias petroquímicas, incineradores, queima de biomassa e tráfego automotor (ISIDORI *et al.*, 2003; MISIK *et al.*, 2007; SISENANDO *et al.*, 2011; PEREIRA *et al.*, 2014).

O ensaio do aborto polínico é outra metodologia amplamente utilizada para a detecção de agentes que causam danos ao material genético vegetal. Assim como o ensaio do Trad-MN e ao contrário do ensaio com *Nicotiana tabacum*, este teste reage de maneira inespecífica aos poluentes ambientais, sendo extremamente útil para avaliação geral dos efeitos de agentes genotóxicos em determinado local. A formação do grão de pólen abortado impacta na fertilidade da planta e reflete processos de toxicidade ao material genético aos quais refletem na maturação do grão. Diversos estudos relacionam uma elevada taxa de grãos de pólen em diversas espécies com exposição à radiação (DEMCHIK

e DAY, 1996), poluentes ambientais de diversas fontes como metais pesados (CALZONI *et al.*, 2007), drogas citostáticas (PICHLER *et al.*, 2014), poluição veicular (CARNEIRO *et al.*, 2011), incineradores industriais e emissão de indústria petroquímica (MISIK *et al.*, 2007). A seleção da espécie é dependente de seu período de florescimento e da presença na região de interesse. Os critérios para determinação de um grão abortado são o tamanho significativamente maior do que o normal, presença de forma alterada ou deficiência na coloração (Figura 3) (MIČIETA E MURÍN, 1996).

Figura 3: Células de pólen de um ambiente livre de poluentes atmosféricos (A) e de um ambiente com altas concentrações de poluentes atmosféricos (B). CN: Células normais. CA: Células abortadas.



#### 1.4.2.2 – Bioindicadores Acumuladores

Os bioacumuladores têm a capacidade de reter elementos tóxicos, principalmente metais, refletindo quantitativamente a concentração destas substâncias na atmosfera. A análise de elementos acumulados pode ser realizada em cascas e folhas de árvores e também em líquens (BALABANOVA *et al.*, 2012; ŠKRBIĆ, MILOVAC E MATAVULJ, 2012; BALTRENAITE *et al.*, 2014).

Musgos e líquens estão entre os bioindicadores mais adequados para a análise de metais pesados e outros elementos presentes na poluição atmosférica. Estes indicadores captam seus nutrientes principalmente através da deposição úmida e seca. Como estes organismos não possuem sistemas de raízes, a contribuição do solo para o acúmulo destes elementos é desprezível, exceto pela ressuspensão de material do solo pela ação do vento. Além disso, musgos e líquens possuem boa capacidade de retenção de íons, especialmente os de metais pesados (WOLTERBEEK, SARMENTO E VERBURG, 2010).

A camada externa da casca de árvore, devido a sua estrutura porosa e capacidade de acúmulo e retenção de partículas de aerossóis, é considerada um bioacumulador passivo promissor no monitoramento da poluição atmosférica em longo prazo. As espécies de metais encontradas no exterior da casca são separadas fisicamente dos elementos absorvidos a partir do solo, não havendo, portanto, relação entre os metais encontrados nesta porção com aqueles provenientes a partir do solo (SCHULZ *et al.*, 1999; SCHELLE *et al.*, 2008). Além disso, a contaminação da parte externa da casca de árvore por metais provenientes do solo devido à ressuspensão deste pelo vento é minimizada com a coleta de amostras acima de uma altura de 1,5 m (WOLTERBEEK E BODE, 1995). Além do mais, como as árvores são bem distribuídas na maior parte dos centros urbanos, a coleta das cascas aliada à análise química pode direcionar às principais fontes emissoras de metais pesados e identificar regiões com diferentes graus de deposição destes elementos.

Na análise de elementos acumulados, alguns destes compostos possuem especial relevância ao serem analisados, visto que representam uma forte relação com fontes poluentes antropogênicas. Cobre (Cu) e Antimônio (Sb) estão relacionados ao desgaste de pastilhas de freio. Potássio (K), Cálcio (Ca), Bromo (Br), Cu e Zinco (Zn) são elementos encontrados em lubrificantes e aditivos de motor de veículos leves. O Zn pode também ser encontrado em compostos utilizados como antioxidantes em aditivos de óleo de motor.

Além disso, o enxofre (S), relacionado com a formação do SO<sub>2</sub>, e o Vanádio (V) são emitidos à atmosfera durante a combustão de diesel (BIRMILI *et al.*, 2006; DA SILVA *et al.*, 2010; DE MIRANDA *et al.*, 2012).

Alguns requisitos específicos devem ser cumpridos para a escolha de um bioindicador adequado para análise de elementos acumulados provenientes da atmosfera:

- 1) O organismo deve ser comum na área de interesse;
- 2) Este deve estar disponível para amostragem durante todas as estações;
- 3) O indicador deve ser tolerante a altos níveis de poluentes;
- 4) O organismo deve conter baixa concentração basal dos elementos a serem analisados;
- 5) O método de amostragem e preparação deve ser simples;
- 6) A variação biológica do organismo deve ser limitada (RÜHLING, 1994).

## **1.5 – Biomonitoramento Humano**

### **1.5.1 -Ensaio do Micronúcleo**

Como um dos principais desfechos da poluição atmosférica é o câncer, a avaliação dos efeitos genotóxicos destes poluentes sobre uma população é fundamental. Sendo assim, da mesma forma que os ensaios Trad-MN e do aborto polínico avaliam a capacidade de poluentes causarem danos ao DNA de bioindicadores vegetais, o MN em células humanas é um bioindicador utilizado para avaliação da genotoxicidade em seres humanos.

O MN (figura 4) é originado a partir de fragmentos de cromossomos ou cromossomos inteiros deixados para trás durante a anáfase da divisão nuclear. Instabilidade genômica ou danos genotóxicos são os fatores que levam ao dano/perda cromossomal e à formação do MN (HOLLAND *et al.*, 2008). Os agressores externos que podem causar dano genotóxico a seres humanos são diversos, entre muitos destacam-se a radiação ionizante (SAKLY *et al.*, 2013), o cigarro (CHANDIRASEKAR *et al.*, 2014; MOTGI *et al.*, 2014), os agrotóxicos (TIMOROGLU *et al.*, 2014), os solventes orgânicos (BURGAZ *et al.*, 2002) e a poluição atmosférica (DEMARINI, 2013; SANTEJO SILVEIRA *et al.*, 2013).

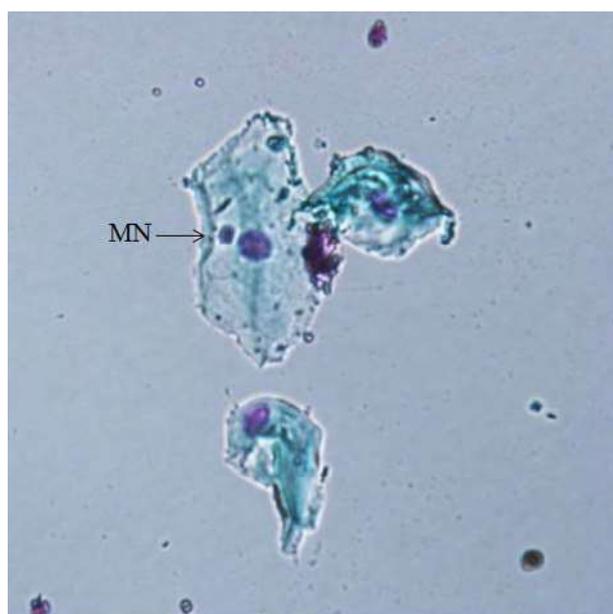
A principal vantagem deste teste é o baixo custo, facilidade de contagem e a precisão dos resultados ao contar um grande número de células. O ensaio do MN é geralmente realizado em linfócitos de sangue periférico e, em menor escala, em células

epiteliais, eritrócitos, fibroblastos e macrófagos alveolares (NERI *et al.*, 2003). A coloração de Feulgen-Fast Green (FFG) é a preferida em diversos estudos por causa de sua especificidade com o DNA e uma aparência limpa e transparente do citoplasma, o que permite fácil identificação de MN. Outra coloração amplamente utilizada é a de Giemsa. No entanto, muitos estudos reportaram uma frequência excessiva de MN após utilização desta coloração o que sugere que estruturas celulares parecidas com MN, como grânulos de querato-hialina e bacterias, podem levar a falsos positivos (HOLLAND *et al.*, 2008). Um estudo comparando quatro técnicas de coloração concluiu que o método de Giemsa é o mais propenso a levar a falsos positivos e recomendou-se FFG como a coloração de escolha (NERSESYAN *et al.*, 2006).

Os critérios sugeridos para identificação de um MN são :

- 1 – Perímetro arredondado sugestivo de uma membrana;
- 2 – Diâmetro de até 1/3 do núcleo, grande o suficiente para identificar cor e forma;
- 3 – Feulgen positivo (coloração rosa em alto brilho de campo);
- 4- Intensidade de cor similar à do núcleo;
- 5- Textura similar à do núcleo;
- 6- Mesmo plano focal do núcleo;
- 7- Ausência de sobreposição ou ponte com o núcleo (TOLBERT, SHY E ALLEN, 1992; HOLLAND *et al.*, 2008).

Figura 4: MN em célula epitelial bucal.



Este marcador de genotoxicidade humana tem sido utilizado progressivamente nos últimos anos em estudos que avaliam a relação entre a poluição atmosférica e danos ao DNA. Tais estudos indicam uma elevada taxa de micronúcleo em trabalhadores ocupacionalmente expostos à poluição veicular em ambientes urbanos, incluindo frentistas (HALLARE *et al.*, 2009), policiais, taxistas (KARAHALIL, KARAKAYA E BURGAZ, 1999) e trabalhadores de túneis (VILLARINI *et al.*, 2008).

Uma das metodologias preferíveis em estudos ambientais tem sido a coleta de células epiteliais bucais. Comparado com outros marcadores de genotoxicidade, o ensaio do MN com estas células é um método útil, não invasivo e simples para o monitoramento genético uma vez que as células epiteliais bucais representam um alvo preferencial para eventos genotóxicos iniciais induzidos por agentes carcinogênicos introduzidos no organismo pela via inalatória (HOLLAND *et al.*, 2008). Neste contexto, Santejo Silveira e colegas (2013) verificaram frequência de MN em células bucais 7 vezes maior em trabalhadores de canaviais expostos à queima de biomassa comparados com indivíduos não expostos (SANTEJO SILVEIRA *et al.*, 2013).

Além de trabalhadores, as crianças também são um grupo de estudos abordado em pesquisas que relacionam os contaminantes do ar com os danos induzidos ao material genético. Como vantagem, estudos de biomonitoramento em crianças não são afetados por uma extensão muito grande de confundidores como hábito de fumar, ingestão de bebidas alcoólicas, exposição ocupacional e dieta. Tais hábitos são fatores confundidores de grande preocupação em adultos (NERI *et al.*, 2003). Diversos estudos demonstraram elevadas taxas de MN em células epiteliais bucais de crianças expostas a altos índices de poluição do ar em áreas urbanas (LAHIRI *et al.*, 2000; CHEN *et al.*, 2006), além de sugerir uma maior suscetibilidade destas a estes poluentes quando comparadas a adultos (NERI *et al.*, 2003; NERI *et al.*, 2006). De maneira confirmatória, Van Leeuwen e colegas (2008) descreveram que rotas específicas relacionadas ao dano em DNA e ao sistema imune, que são responsivas à poluição atmosférica, se encontravam ativas em crianças e não em adultos, reforçando, por conseguinte, a hipótese de maior suscetibilidade das crianças à poluição atmosférica (VAN LEEUWEN *et al.*, 2008).

### 1.5.2- Acúmulo de metais em unhas

Inúmeros processos antropogênicos como industrialização, urbanização, atividades mineradoras, aumento do tráfego veicular e uso de pesticidas têm contribuído com a liberação de metais ao ambiente ao longo dos anos. Além de trabalhadores expostos ocupacionalmente, a populacional também corre riscos devido à exposição crônica a estes elementos. Ao contrário do sangue, que indica uma concentração transitória de metais no organismo, as unhas fornecem um registro contínuo da concentração de elementos traço no corpo (WILHELM *et al.*, 1991; MEHRA E JUNEJA, 2005).

As unhas são matrizes promissoras na avaliação de exposições ambientais a elementos tóxicos uma vez que este método de coleta se caracteriza como não invasivo e as amostras podem ser armazenadas em temperatura ambiente. A incorporação de metais às unhas se dá durante a síntese de queratina pela unha ou ainda pela ligação a grupos sulfidríla (HORNOS CARNEIRO *et al.*, 2011).

Esta matriz biológica tem sido objeto de estudos para a avaliação de contaminantes provenientes de diversas fontes. Nestas se incluem a relação entre a exposição a chumbo (Pb), cádmio (Cd), cromo (Cr) e Cu e o consumo de peixes (OYOO-OKOTH *et al.*, 2010), Arsênio (As) e o consumo de água de poços (NORMANDIN *et al.*, 2014), assim como Cobalto (Co), Pb, Selênio (Se) e Zn em residentes de uma cidade mineradora (NDILILA *et al.*, 2014).

Ainda que de maneira não tão frequente, as unhas têm sido utilizadas no biomonitoramento humano da exposição a metais presentes na atmosfera. Um estudo comparando área urbanizada e área rural no Kenya observou um maior nível de metais nas unhas de estudantes provenientes da área urbana. Os resultados ainda demonstraram que a localização das escolas tem grande influência sobre o aumento desses níveis, além da influência da proximidade de uma via de intenso tráfego automotor com a concentração de Pb (WERE *et al.*, 2008; WERE *et al.*, 2009). Outro estudo, que avaliou a exposição de policiais à poluição veicular, verificou alta concentração de Pb nesses indivíduos ao serem comparados com os controle. Além disso, tal elemento esteve positivamente correlacionado com o tempo de exposição, ao qual foi medido pelo tempo de trabalho, e ainda com marcadores de dano renal (MORTADA *et al.*, 2001).

Além da exposição a elementos tóxicos, o biomonitoramento humano em unhas pode identificar deficiências de elementos essenciais no organismo, podendo, de forma paralela, ser estabelecida uma relação com o desenvolvimento de algumas doenças, como a asma. Um dos mecanismos relacionados à fisiopatologia da asma envolve as espécies reativas de oxigênio (EROs). As concentrações destas espécies no organismo são controladas por antioxidantes enzimáticos como a superóxido dismutase, catalase e a glutathione peroxidase os quais possuem o Zn, Se, Mn e Cu como cofatores (KOCYIGIT, EREL E GUR, 2001; RAHMAN, BISWAS E KODE, 2006). Neste contexto, baixas concentrações de Zn e Se em unhas de crianças foram relacionadas com uma maior prevalência de asma nestes indivíduos (HORNOS CARNEIRO *et al.*, 2011). No entanto, quando comparados com um marcador de inflamação pulmonar, esta relação com elementos traços não foi observada (MORESCO *et al.*, 2014).

## **2 – Justificativa**

A poluição atmosférica é um problema crescente nas grandes cidades e, através de diversos estudos mundiais, tem sido demonstrada a relação destes poluentes com um número cada vez maior de morbidades à saúde. Além disso, a aplicação de métodos alternativos de medições dos impactos dos contaminantes do ar, como o monitoramento passivo e biomonitoramento, são de grande importância, principalmente em cidades que não contam com redes de monitoramento automático operacionais.

A união de ensaios de acumulação e sensibilidade é importante para determinar o perfil dos poluentes que agem sobre o bioindicador e, por conseguinte, na porção urbana analisada. Além disso, a comparação entre ensaios de biomonitoramento vegetal e exposição humana é importante para a determinação do grau de exposição humana aos poluentes atmosféricos locais e seus efeitos, sendo tal abordagem translacional escassa na literatura científica.

### **3. Objetivos**

#### **3.1. Objetivo Geral**

Avaliar a qualidade do ar em áreas de alta, média e baixa densidade populacional - próximas a vias de intenso tráfego automotor - por meio de biomonitoramento vegetal e monitoramento passivo, bem como relacionar estes dados com marcadores de exposição humana

#### **3.2. Objetivos específicos**

- Realizar a medição dos poluentes NO<sub>2</sub> e O<sub>3</sub>, por monitoramento passivo, em cada uma das 3 áreas de investigação;
- Realizar o bioensaio de aborto polínico e análise de elementos acumulados em cascas de árvore de *Bauhinia variegata* distribuídas em cada uma das 3 áreas de investigação;
- Medir a concentração de elementos acumulados em unhas e micronúcleo em células da mucosa bucal em adolescentes estudantes de escolas públicas próximas às áreas de investigação;
- Relacionar as concentrações de elementos acumulados encontrados nas cascas de árvore com aquelas encontradas nas unhas dos adolescentes;
- Relacionar a frequência de grãos de pólen abortados de *Bauhinia variegata* com a frequência de micronúcleo da mucosa bucal dos adolescentes.

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## 5. Artigos Científicos

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## **A Comparison of the Human Buccal Cell Assay and the Pollen Abortion Assay in Assessing Genotoxicity in an Urban-Rural Gradient**

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**Abstract:** Air pollution is exacerbated near heavy traffic roads in cities. Air pollution concentration and composition vary by region and depend on urban-rural gradients. The aim of this study was to evaluate the distribution of air pollution in areas of varying population densities and to compare plant biomonitoring with an established biomarker of human exposure to traffic-related air pollution in children. The areas of study were selected near a major street in 3 different regions. Areas A, B and C represent high, intermediate and low population densities, respectively. Micronucleus assay, an established biomarker of human exposure, was performed in children from these areas. For a plant biomonitoring assay, the pollen abortion assay was performed on *Bauhinia variegata* in these areas. NO<sub>2</sub> and O<sub>3</sub> concentrations were determined by passive sampling. We report here that the pollen abortion frequency in *Bauhinia variegata* is correlated with NO<sub>2</sub> concentration ( $P = 0.004$ ) and is strongly associated with vehicular flow and population density in the studied areas. Micronuclei frequency in buccal cells of children was higher in the regions with more degree of urbanization ( $P < 0.001$ ) following the same pattern of O<sub>3</sub> concentrations ( $P = 0.030$ ). In conclusion, our results demonstrate that high concentrations of air pollutants in Porto Alegre are related to both human and plant genotoxicity. Areas with different concentration of pollutants demonstrated to have an urbanization gradient dependent pattern which also reflected on genotoxic damage among these areas.

**Keywords:** micronucleus; air pollution; biomonitoring; bioindicator; passive sampling; genotoxicity; ozone; nitrogen dioxide

## 1. Introduction

Air pollution is a mixture of gaseous and particulate pollutants at disproportionate concentrations and is constantly modified by sunlight and temperature. Traffic-related pollution consists of multiple toxic moieties, including particulate matter (PM), nitrogen dioxide (NO<sub>2</sub>), ozone (O<sub>3</sub>), polycyclic aromatic hydrocarbons (PAHs), metals and volatile organic compounds (VOCs) [1]. Air pollution is exacerbated near heavily trafficked roads in large cities. However, the concentration and composition of the pollutants has high variability in different regions, and it is dependent on the urban-rural gradient [2]. The variability in air pollution patterns throughout urban areas is strongly associated with traffic emissions and has a direct effect on the health of those who live near heavily trafficked routes. Thus, the density of neighborhood traffic is associated with exposure to higher doses of pollutants and leads to adverse health impacts such as respiratory disease, cardiovascular disease and lung cancer [3–7].

Because one of the main outcomes of air pollution exposure is cancer, evaluating the genotoxic effects of air pollution in an exposure population is critical. Multiple studies have utilized the micronucleus assay to demonstrate a relationship between air pollution and genotoxic effects [8–10]. In addition, research has shown increased micronuclei frequency in workers who are chronically exposed to traffic-related pollution in an urban environment [11], including gas station attendants [10], traffic police [12] and tunnel workers [13]. Compared to other biomarkers of genotoxicity, the micronucleus assay in buccal cells is a useful, noninvasive and simple method for monitoring genetic damage in humans since oral epithelial cells represent a preferred target population for early genotoxic events induced by carcinogenic agents introduced via inhalation [14].

A reliable alternative method for evaluation of air pollution-induced genotoxicity in an urban environment is vegetal biomonitoring. The effects on plants can be used for the qualitative and quantitative evaluation of atmospheric contamination and to delimit risks to biological systems exposed to environmental pollutants [15]. The trees of the genus *Bauhinia* are a good candidate for small-scale air pollution monitoring because they have been used for street ornamentation and have widespread distribution in cities. Carneiro *et al.* have demonstrated that the pollen abortion assay in *Bauhinia blakeana* is effective in determining the area of influence of pollution emissions produced in a traffic corridor [16].

Despite increased numbers of studies using vegetal biomonitoring to assess air pollution effects, only a few studies associated vegetal bioindicators with human health [17] or assessed the relationship between human biomarkers and vegetal bioindicators for genotoxicity [18].

The aim of this study was to evaluate the effects of air pollution in areas with varying population densities via biomonitoring and to compare plant biomonitoring data with genotoxic and mutagenic markers of human exposure to traffic-related air pollution in children.

## **2. Experimental Section**

### *2.1. Study Area*

Porto Alegre is the capital of the state of Rio Grande do Sul, located in southern Brazil. It has 1,467,823 inhabitants distributed over 496,827 km<sup>2</sup>. The climate is humid-subtropical, with consistent and above-average precipitation throughout the year.

Monitoring groups were selected near major streets in three areas with different population profiles. Area A: Protásio Alves Avenue (30°02'22.44"S/51°10'35.13"W)—high population density (11,458 pop/km<sup>2</sup>). Area B: Cavahada Avenue (30°05'49.69"S/51°13'44.39"W)—intermediate population density (5205 pop/km<sup>2</sup>). Area C: Juca Batista Avenue (30°09'38.07"S/51°11'13.14"W)—low population density (2660 pop/km<sup>2</sup>). The distance between each site is approximately 7 km. To avoid differences due to spatial distribution of air pollutants, the NO<sub>2</sub> and O<sub>3</sub> passive sampling data and the pollen abortion assay data were collected at distances of 0, 100 and 200 meters from the main road at each site.

### *2.2. Study Population*

Students at public schools in the three study areas of Porto Alegre were invited to participate in the study. Only children living near their respective monitoring areas were selected to ensure that their living and studying regions were similar and to minimize the chance of cross-contamination of pollutants from non-site areas. Children living more than 3 km from their respective sampling site were excluded from the study. The study was

approved by the Ethics Committee of the Federal University of Health Sciences of Porto Alegre (No. 315.260).

### *2.3. Nitrogen Dioxide Measurement*

NO<sub>2</sub> measurements were collected by passive sampling in winter (June and July 2013) and summer (February and March 2014). Cellulose filters (37 mm, Energética, Rio de Janeiro, Brazil) were impregnated with triethanolamine absorbent solution (2 mL) and dried at 37 °C for 24 hours. Filters were then inserted into open diffusion tubes and placed at monitoring sites for 14 days (n = 18 per group). Blanks were obtained from filters exposed in the same conditions but were sealed from atmosphere contact. Nitrite ions are produced when atmospheric NO<sub>2</sub> reacts with triethanolamine. These ions were extracted with methanol and reacted with sulfanilamide and 8-anilino-1-naphthalenesulfonic acid (ANSA) [19]. Solutions were then analyzed by spectrophotometry at 550 nm (Perkin-Elmer Lambda 35, São Paulo, SP, Brazil).

### *2.4. Ozone Measurement*

O<sub>3</sub> measurement was also conducted by passive sampling during the same period of time as the NO<sub>2</sub> measurements. Filters (Energética) were impregnated with indigotine disulphonate (IDS) solution (400 µL), inserted into open diffusion tubes and placed at monitoring sites for 8 h (n = 18 per group). Blanks were obtained from filters exposed in the same conditions but were sealed from atmosphere contact.

After exposure, the filters were removed from the samplers, placed into glass tubes with distilled water (5 mL) and sonicated in an ultrasonic bath for 5 min. The tubes were then centrifuged for 10 min at 3800 rpm to clear filter debris. Supernatants were then analyzed by spectrophotometry at 610 nm (Perkin-Elmer Lambda 35) [20].

### *2.5. Pollen Abortion Assay*

The pollen abortion test was chosen as the vegetal biomonitoring assay. The analysis was performed in *Bauhinia variegata* flower buds located near Areas A, B and C. Flower buds were collected at the end of passive sampling period in winter of 2013.

Flower buds were collected and fixed in 3:1 v/v ethanol/acetic acid solution and transferred to 70% ethanol solution after 24 h. Pollen grains were extracted from anthers and spotted onto slides, which were stained with 0.5% aceto-carmin for microscopic evaluation. Slides were then photographed with a digital camera (Olympus DP72, Tokyo, Japan) directly attached to the microscope (Olympus BX51, Tokyo, Japan). For each collection area, 300 cells were evaluated per slide, resulting in a total of 9000 cells per area. Pollen abortion criteria consisted of the presence of abnormally large pollen, presence of altered pollen forms, and staining deficiency, as described previously [21].

#### *2.6. Micronucleus Assay*

Buccal epithelial cells were collected with a wooden spatula from 101 students of public schools in the three study areas of Porto Alegre from June 2013 to March 2014. Cells were collected by rotating the spatula 20 times in a spiral motion against the inner surface of the cheek wall. Samples were stored in 3:1 v/v ethanol/acetic acid solution. Afterwards, cells were centrifuged at 1500 rpm for 5 min, and the fixation buffer was changed; this operation was repeated three times. Fixed cells were hydrolyzed in HCl and stained according to the Feulgen method [22,23]. Micronucleus frequency was determined by counting 1000 cells per sample in duplicate using an optical microscope with 1000× magnification (Olympus BX51).

Confounding factors for micronucleus frequency include children distribution, socioeconomic status, age, gender, smoking and drinking habits. These factors were controlled for this study by applying the socioeconomic section of International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire [24].

#### *2.7. Statistical Analysis*

Analysis of the data was performed using the Sigma Plot 12.0 software. The means of the NO<sub>2</sub> and O<sub>3</sub> concentrations, pollen abortion frequency and micronuclei were compared by one-way ANOVA followed by Bonferroni correction. The association between NO<sub>2</sub> and pollen abortion was assessed by Pearson Correlation. The influence of confounding factors was assessed by Chi-Square test and one-way ANOVA. The level of significance for these analyses was set at 5%.

### 3. Results

#### 3.1. Study Area

Figure 1 shows the distribution of the studied subjects and monitoring points. The hourly mean traffic flow was collected from public traffic data (Public Company of Transport and Circulation—EPTC) in a single period in each monitoring area. The streets measured were Protásio Alves Avenue (Area A), Cavallhada Avenue (Area B) and Juca Batista Avenue (Area C). Area A averaged 5060 vehicles per hour, 4054 vehicles per hour were measured in Area B and 1607 vehicles per hour passed through Area C.

**Figure 1.** Subject distribution by monitoring area.

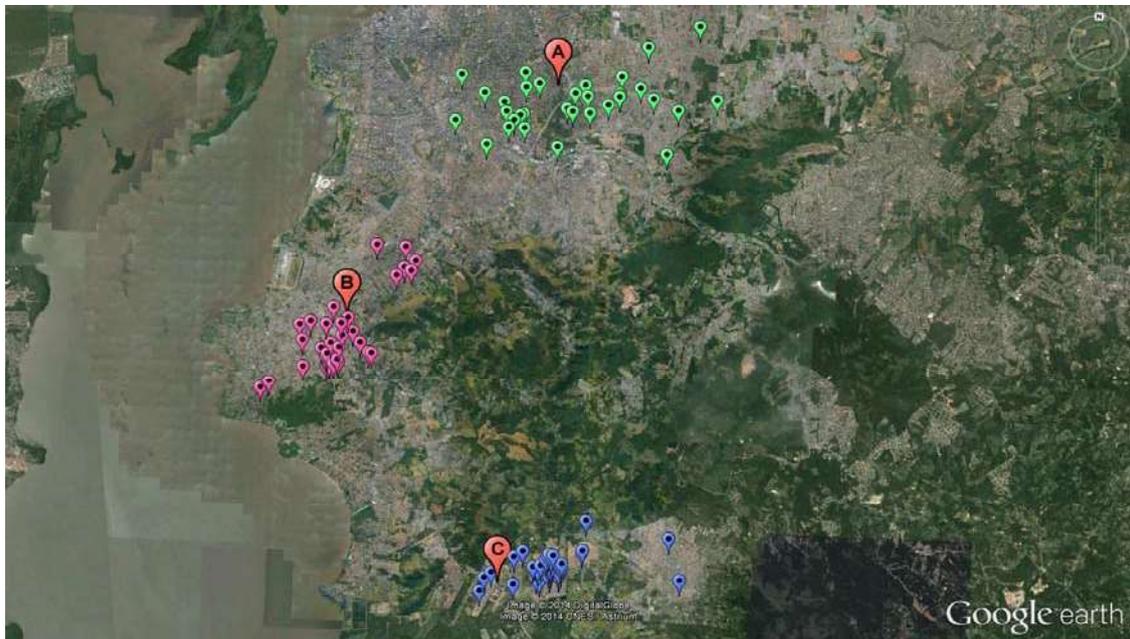


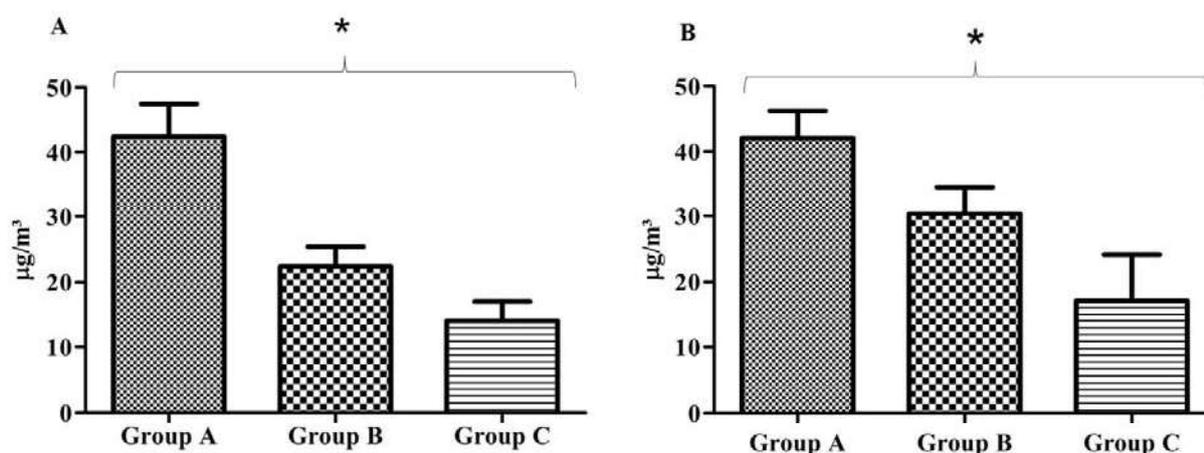
Table 1 presents the environmental conditions in Porto Alegre during the winter and summer sampling periods. The mean summer temperature was 12.2 °C higher than the mean winter temperature, and the rainfall was 39.4 mm higher in summer period than in winter [25].

Season	Mean Temperature (°C)	Mean Maximum Temperature (°C)	Mean Minimum Temperature (°C)	Relative Humidity (%)	Total Rainfall (mm)	Wind Speed (mps)	Wind Direction
Winter	14.1	19.7	10.4	82.7	216.6	1.9	SE
Summer	24.7	30.5	20.63	76.35	274.1	2.51	SE

### 3.2. Nitrogen Dioxide Measurement

Nitrogen dioxide concentrations were related with population density and traffic flow. The high population density area showed the highest concentration of NO<sub>2</sub>, followed by the intermediate and low population density areas. This pattern was maintained in both the summer and winter monitoring periods.

**Figure 2** shows the NO<sub>2</sub> concentrations by monitoring area in summer (A) and winter (B).



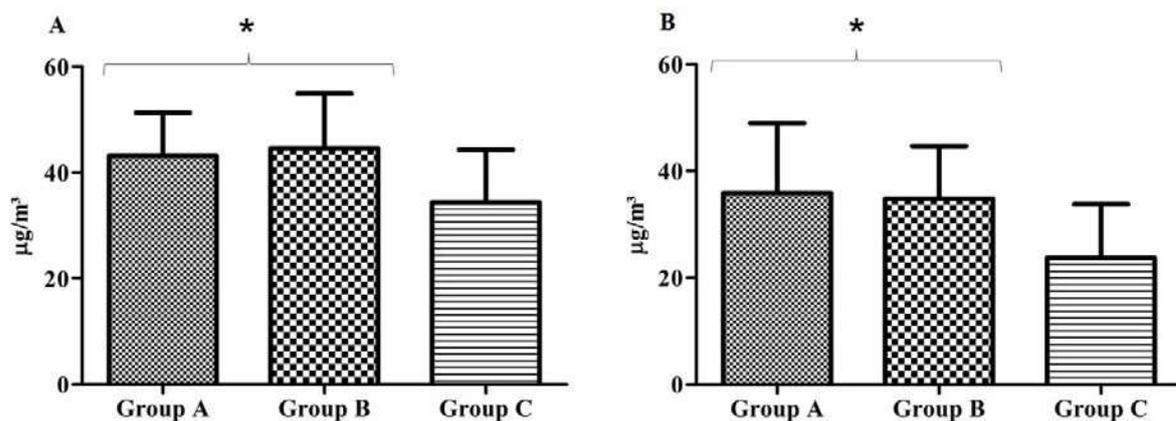
The mean concentrations of NO<sub>2</sub> in summer were  $42.4 \pm 5.0 \mu\text{g}/\text{m}^3$  in Group A,  $22.3 \pm 3.1 \mu\text{g}/\text{m}^3$  in Group B and  $13.9 \pm 3.0 \mu\text{g}/\text{m}^3$  in Group C. All groups were significantly different from each other ( $P < 0.001$ ). In winter, the mean concentrations of NO<sub>2</sub> were  $42.1 \pm 4.1 \mu\text{g}/\text{m}^3$  in Group A,  $30.4 \pm 4.2 \mu\text{g}/\text{m}^3$  in Group B and  $17.1 \pm 7.0 \mu\text{g}/\text{m}^3$  in Group C. All groups were significantly different from each other ( $P < 0.001$ ). The data are expressed as the mean  $\pm$  standard deviation.

### 3.3. Ozone Measurement

Like NO<sub>2</sub>, the O<sub>3</sub> concentration maintained a consistent distribution pattern during the two seasons. However, unlike the NO<sub>2</sub> concentrations across groups, the O<sub>3</sub> concentrations were similar between the high and intermediate population groups, and both were higher than the low population density group.

Figure 3 shows the O<sub>3</sub> concentration in the monitoring areas in summer (A) and winter (B). In summer, the mean concentration of Group A was  $43.2 \pm 8.1 \mu\text{g}/\text{m}^3$ , whereas Group B had a mean concentration of  $44.5 \pm 10.4 \mu\text{g}/\text{m}^3$  and Group C had a mean concentration of  $34.3 \pm 9.6 \mu\text{g}/\text{m}^3$ . Groups A and B were not different ( $P = 1.000$ ), but both were higher than Group C ( $P = 0.030$ ). In winter, the mean concentration of O<sub>3</sub> in Group A was  $35.9 \pm 12.9 \mu\text{g}/\text{m}^3$ , whereas the mean concentration was  $34.9 \pm 9.8 \mu\text{g}/\text{m}^3$  in Group B and  $23.7 \pm 10.1 \mu\text{g}/\text{m}^3$  in Group C. Similar to the data from summer, the O<sub>3</sub> concentrations in Groups A and B were higher than that in Group C ( $P = 0.036$ ), but they were not different between themselves ( $P = 1.000$ ). The data are expressed as the mean  $\pm$  standard deviation.

**Figure 3.** O<sub>3</sub> concentrations in the monitoring areas for summer (A) and winter (B).

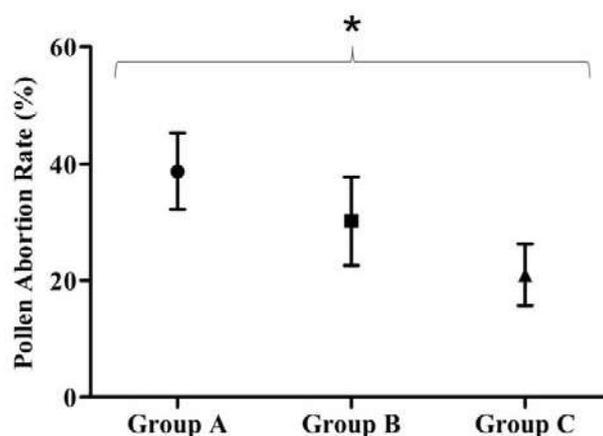


### 3.4. Pollen Abortion Assay

Similar patterns of distribution were found in pollen abortion rates compared with the distributions of NO<sub>2</sub> concentration, population density and traffic flow. Group A showed the highest rate of genetic damage, followed by Groups B and C. The pollen

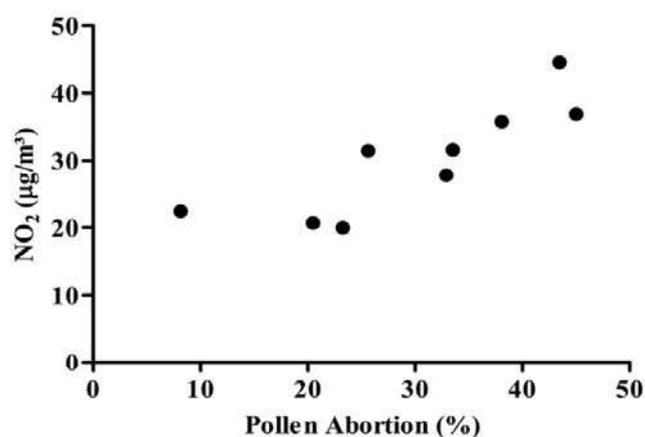
abortion rate by monitored area is presented in Figure 4. The mean concentration of Group A was  $38.8 \pm 6.5 \mu\text{g}/\text{m}^3$ , followed by  $30.2 \pm 7.6 \mu\text{g}/\text{m}^3$  in Group B and  $20.9 \pm 5.2 \mu\text{g}/\text{m}^3$  in Group C. All groups were significantly different from each other ( $P < 0.001$ ). The data are expressed as the mean damaged cell rate  $\pm$  standard deviation.

**Figure 4.** Pollen abortion rate in monitoring areas.



$\text{NO}_2$  and pollen abortion were strongly positively correlated ( $r = 0.842$ ;  $P = 0.004$ ), (Figure 5). This correlation demonstrates that the pollen abortion assay, a genotoxicity biomarker, is a good proxy for the degree of air pollution (and corresponding traffic density) in regions with varying population density.

**Figure 5.** Positive association between  $\text{NO}_2$  and pollen abortion.



### 3.5. Micronucleus Assay

The micronucleus assay results are presented in Table 2. Group A had a mean of  $4.57 \pm 2.05$  micronucleus per 1000 cells, Group B had had a mean of  $4.30 \pm 1.89$  micronucleus per 1000 cells and Group C had a mean of  $2.31 \pm 1.10$  micronucleus per 1000 cells. The data are expressed as the mean  $\pm$  standard deviation. Groups A and B were similar ( $P = 1.000$ ), but both groups presented a higher rate of genotoxic damage when compared with Group C ( $P < 0.001$ ). N = number of children. MCN/1000 cells = Micronucleus per 1000 cells. SD = standard deviation.

**Table 2.** Micronucleus frequency.

<b>Groups</b>	<b>N</b>	<b>MCN/1000 Cells</b>	<b>SD</b>	<b>P</b>
Group A	33	4.57 *	2.05	<0.001
Group B	34	4.30 *	1.89	<0.001
Group C	34	2.31	1.10	<0.001

Note: \*  $P < 0.001$ .

Confounding factors and student profiles are presented in Table 3. There were no significant differences in gender ( $P = 0.317$ ), age ( $P = 0.084$ ), socioeconomic status ( $P = 0.612$ ) or exposure to passive smoking ( $P = 0.815$ ) in the children across the study areas.

**Table 3.** Children profiles by monitoring area.

<b>Children Profile</b>	<b>Group A (n = 33)</b>	<b>Group B (n = 34)</b>	<b>Group C (n = 34)</b>	<b>P</b>
<b>Age (Years)</b>	$13.7 \pm 1.1$	$13.7 \pm 1.2$	$13.1 \pm 0.9$	0.084
<b>Gender</b>				
Male	9	14	15	0.317
Female	24	20	19	
<b>Socioeconomic Status</b>				
$\leq 2$ Minimum Wage	19	22	18	0.612
$> 2$ Minimum Wage	14	12	16	
<b>Passive Smoking</b>				
Yes	19	20	22	0.815
No	14	14	12	

Thus, there were no differences in student profile factors between study areas that could act as confounders of micronucleus rate, such as gender, age and passive smoking.

Children with assumed smoking habits or frequent alcohol consumption were excluded from this study because of known correlations to micronucleus frequency.

#### **4. Discussion**

To our knowledge, this is the first study at this site to demonstrate a relationship between human and plant biomonitoring assays for genotoxicity to urban pollutants. Our data demonstrate that there are differences in air quality and genotoxic effects in plants and humans in regions with varying population densities near heavy traffic roads. We chose to examine the known pollutant NO<sub>2</sub> because its outdoor concentrations are highly related with the degree of urbanization and the frequency of vehicle traffic [26,27]. Our study, in which the NO<sub>2</sub> concentrations are highly related with vehicular flow and population density, is consistent with these previous studies. However, the O<sub>3</sub> and NO<sub>2</sub> concentrations displayed a different distribution pattern. In the present study, intermediate and high population areas displayed similar O<sub>3</sub> concentrations to each other, and both exhibited elevated concentrations compared with the low population area. The fact that O<sub>3</sub> is a secondary pollutant may in part explain the results. The formation of O<sub>3</sub> is caused by reactions of the key precursors volatile organic compounds (VOCs) and nitrogen oxides (NO<sub>x</sub>) in the presence of sunlight, and its distribution in an urban environment is dependent on wind direction and geographic characteristics [28].

The pollen abortion assay was chosen for vegetal biomonitoring. This assay is highly sensitive because the target cells (microspores) are haploid, and it can detect lethal mutations that affect the development of pollen [29]. Several studies have demonstrated the relationship between the pollen abortion assay in wild plants and air pollution from different sources [30,31]. In urban areas, this assay has been shown to be a reliable marker of air pollution that is caused by vehicular emissions near high-traffic streets because pollen cells are sensitive to spatial gradient of air pollutants, including NO<sub>2</sub> [16]. In our study, we found a strong positive correlation between the pollen abortion rate and NO<sub>2</sub> concentrations, thus confirming the relationship of this vegetal biomarker with the spatial dispersion of air pollution and the degree of urbanization. However, the observed genotoxic effects cannot be explained solely by the NO<sub>2</sub> levels. Nitrogen dioxide is a marker of fuel combustion and is correlated with many other compounds in exhaust, including PM, VOCs,

black carbon and sulfur dioxide (SO<sub>2</sub>) [32]. These pollutants have known genotoxic effects and were not measured in this study.

Our data show that the frequency of micronuclei in buccal cells of children is higher in more urbanized regions and suggests that human genotoxicity in children is strongly associated with air pollution levels in Porto Alegre. Several studies have shown increased rates of genetic damage in buccal epithelial cells of children exposed to high levels of air pollution in urban areas [33,34] as well as higher susceptibility to air pollution in children [35,36]. Van Leuween *et al.* found that specific DNA-damage pathways and immune pathways that are responsive to air pollution were active in children but not in adults, demonstrating the increased susceptibility of children to air pollution [37]. Whereas we found a relation between NO<sub>2</sub> and vegetal bioindicator, micronucleus frequency was associated with O<sub>3</sub> concentrations on the three regions of Porto Alegre. In a study made with children and adults in California, Huen *et al.* found cytogeneticity in both adults and children who were exposed to O<sub>3</sub>, but associations between traffic proximity and micronucleus frequency were detected only in children, suggesting that children may be more susceptible to genotoxicity caused by traffic pollution [38]. Similarly, Valverde *et al.* demonstrated that exposure to a polluted urban atmosphere with high ozone concentrations in Mexico promoted DNA damage in young adults [39]. The genotoxic effect of O<sub>3</sub> is due to the production of a cascade of free radicals that then react with DNA and affect genomic integrity. At relatively high concentrations, the reaction products can cause extensive DNA damage, and this genotoxic lesion might lead to a mutagenic impact, as observed by the formation of DNA micronuclei [39].

Our data from the pollen abortion test were highly correlated with the NO<sub>2</sub> concentration along the urban-rural gradient in Porto Alegre, demonstrating that this assay is an effective marker of the degree of urbanization and could be used in monitoring networks as a marker of urban air pollution effects. Despite no difference was found in buccal micronucleus of high and intermediate population area, human and vegetal exposure were interrelated when comparing high populated and low populated areas in Porto Alegre. Studies that use both plant bioindicators and human biomarkers to evaluate the genotoxic effects of air pollution are still rare. In a study performed in the Amazon region,

micronuclei frequency in *Tradescantia pallida* and human alveolar cells both indicated genotoxic effects of organic PM collected during intense biomass burning period [18].

There were some limitations to this study. We did not measure additional pollutants released in vehicular emissions that are demonstrated genotoxicants, such as PM, PAHs and SO<sub>2</sub>. In addition, we did not measure individual exposures to O<sub>3</sub> and NO<sub>2</sub>, which precludes studying individual exposures to genotoxic pollutants in the context of an urban-rural gradient. Finally, we were not able to assess vegetal biomonitoring in summer because the flower buds of *Bauhinia variegata* grow only in winter (July and August).

We associated human and plant biomonitoring in a city using the micronucleus frequency assay in buccal epithelial cells in humans and the pollen abortion assay in *Bauhinia variegata*. These results are strongly associated with the urbanization gradient near heavily trafficked roads, the population distribution and the concentration of air pollution among areas with high, intermediate or low population density in Porto Alegre.

## **5. Conclusions**

In conclusion, high concentrations of air pollutants in Porto Alegre are related with both human and plant genotoxicity. In this study, we have shown that the degree of urbanization is associated with the degree of air pollution, which in turn is related to the level of genotoxic stress in children and plants.

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## **Author Contributions**

Alan da Silveira Fleck participated in study design, all analysis and manuscript preparation. Mariana Vieira carried out the pollen abortion assay, passive sampling and data revision. Claudia Rhoden and Sergio Amantea participated in the study design,

revision of the results and manuscript preparation. All authors have read and approved the final manuscript.

### Conflicts of Interest

The authors declare no conflict of interest.

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## 5.2. Artigo a ser submetido à revista *Environmental Research*

### **Biocummulation of traffic-related elements in human nails and tree barks in an urban-rural gradient.**

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## **Abstract**

Heavy metals are among the most toxic compounds that affect our environment. In this context, several studies have employed biomonitoring from both human and vegetal matrices as an alternative tool to assess the effects of heavy metals present in the atmosphere. The aim of this study was to evaluate the accumulation of elements in tree bark and children's nails in areas with varying population density and to compare the pattern of distribution of those elements between the two matrices. Element accumulation in tree bark was assessed in trees located near highways in areas with high (Area A), intermediate (Area B) and low (Area C) population density. Elements accumulated in nails were measured by ICP-MS in students living near these areas. NO<sub>2</sub> and O<sub>3</sub> concentrations were determined by passive sampling. Moreover, Principal component analysis (PCA) was used to obtain an overview of the data as well as to identify possible sources that explain its distribution. We report here that Ba, Cd, Co, Cu, Mg, Mn, Ni, Pb, Sb, Sr, V and Zn were at higher concentrations in tree bark in Area A, while Cd, Co, Cu, Mg, Mn, Ni and Sr were at higher levels in students' nails of Area A as well. PCA of the samples demonstrated that vehicular traffic explained 66.4 % of the variance in tree bark and 50.8 % of the variance in nails. In conclusion, high levels of traffic-related trace elements were found accumulated in human and vegetal matrices, being associated with the degree of urbanization. In addition, a relationship was demonstrated between human nails and tree bark as bioindicators of exposure to metals.

Keywords: metals, air pollution, bioindicator, nail, tree bark, principal component analysis.

## **1. Introduction**

Heavy metals are among the most toxic compounds that affect our environment. These elements are mainly emitted in the atmosphere from anthropogenic sources like pesticides, industrial waste, mining operations and vehicular traffic. In urbanized cities, not only occupationally exposed workers may suffer due to environmental exposure, but also communities living in highly polluted areas (Mehra and Juneja, 2005). In this context, several studies have employed biomonitoring of both human and vegetal matrices as a tool to assess the effects of heavy metals present in the atmosphere (Baltreinaite et al., 2014; Garty, 2001; Huang et al., 2014).

Human nails are an attractive matrix for the assessment of environmental exposure to toxic elements since collection is noninvasive, the samples can be stored at room temperature and, unlike blood that reflects short-term concentration changes, nails can provide a continuous record of trace elements in the body (Wilhelm et al., 1991). The incorporation of elements occurs during keratin synthesis or by binding to sulfhydryl groups of proteins. Therefore, analysis of nails can provide information about dietary intake of essential elements as well as exposure to toxic compounds over the preceding months (Hornos Carneiro et al., 2011). Several studies using this biological matrix have estimated the exposure to metals from different sources including fish intake (Oyoo-Okoth et al., 2010), groundwater consumption (Normandin et al., 2014) and mining activity (Ndilila et al., 2014).

In addition to nails providing information about environmental exposure to heavy metals in humans, tree barks are vegetal bioindicators that also accumulate these elements, reflecting their concentration in the atmosphere. The outer layer of the bark is considered an effective long-term bioaccumulator of heavy metals due to its porous structure as well as its capacity for accumulation and retention of aerosol particles (Schulz et al., 1999). The elements deposited in the outer layer are physically separated from trace elements taken up from the soil (Schelle et al., 2008).

The aim of this study was to evaluate element accumulation in tree bark and children's nails in areas with varying population density and to compare the pattern of distribution of these compounds between the two matrices. This study is an extension of preliminary research that evaluated the genotoxic effects of air pollution in the same areas.

In that study, the authors observed a relation between vegetal (pollen abortion assay) and human biomarkers of genotoxicity (micronucleus in buccal cells) in the same subjects assessed in this research (Fleck et al., 2014).

## **2. Material and Methods**

### **2.1. Study Population and Area**

Porto Alegre is located in southern Brazil and is the capital of the state of Rio Grande do Sul. The city has 1,467,823 inhabitants distributed over 496,827 km<sup>2</sup>. The climate is humid-subtropical, with consistent and above-average precipitation throughout the year.

Monitoring groups were selected near major streets in three areas with different population profiles: Area A: Protásio Alves Avenue (30°02'22.44"S/51°10'35.13"W) — high population density (11,458 pop/km<sup>2</sup>); Area B: Cavallhada Avenue (30°05'49.69"S/51°13'44.39"W) — intermediate population density (5,205 pop/km<sup>2</sup>); Area C: Juca Batista Avenue (30°09'38.07"S/51°11'13.14"W) — low population density (2,660 pop/km<sup>2</sup>). The distance between each area is approximately 7 km.

Students at public schools in the three areas were invited to participate in the study. To minimize the chance of cross-contamination of pollutants, only children living near their respective monitoring areas were selected. Thus, students living more than 3 km from their respective sampling site were excluded from the study. Students with declared active smoking were also excluded from the study. Confounding factors, including children's distribution, socioeconomic status, age, gender and passive smoking were controlled for by applying the socioeconomic section of the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire (Solé et al., 2006).

The study was approved by the Ethics Committee of the Federal University of Health Sciences of Porto Alegre (No. 315.260).

### **2.2. Passive Sampling of Nitrogen Dioxide (NO<sub>2</sub>) and Ozone (O<sub>3</sub>)**

NO<sub>2</sub> was measured because its concentrations are related to vehicular traffic and the degree of urbanization (Esplugues et al., 2010; Gauderman et al., 2005) while O<sub>3</sub> is a

marker of secondary pollution. Measurements were performed in winter 2013 (June and July) and summer 2014 (February and March).

For NO<sub>2</sub> passive sampling, cellulose filters (n = 18 per group) (37 mm, Energética, Rio de Janeiro, Brazil) were impregnated with 2 mL of triethanolamine absorbent solution and dried at 37 °C for 24 hours. Filters were then inserted into open diffusion tubes and placed at monitoring sites for 14 days. Blanks were obtained by exposing a filter in the same conditions but sealed from atmospheric contact. Nitrite ions produced were extracted with methanol and reacted with sulfanilamide and 8-anilino-1-naphthalenesulfonic acid (ANSA) (Lodge, 1988). The solutions were analyzed by spectrophotometry at 550 nm (Perkin Elmer, Waltham, MA, United States).

For O<sub>3</sub> passive sampling, filters (n = 18 per group) were impregnated with 400 µL indigotine disulfonate (IDS) solution, inserted into open diffusion tubes and placed at monitoring sites for 8 h. Blanks were obtained from filters exposed in the same conditions but sealed from air contact. After exposure, the filters were placed into glass tubes with 5mL of distilled water and sonicated in an ultrasonic bath for 5 min. The tubes were then centrifuged for 10 min at 3800 rpm to clear filter debris and the supernatants analyzed by spectrophotometry at 610 nm (Perkin-Elmer Lambda 35, Perkin-Elmer do Brazil, SP, Brazil) (Scheeren and Adema, 1996).

## **2.3. Accumulated Elements in Tree Bark and Human Nails.**

### **2.3.1 Materials**

All reagents used were of analytical reagent grade except nitric acid (HNO<sub>3</sub>), which was previously purified in quartz sub-boiling stills (Kürner Analysentechnik, Rosenheim, Germany). A clean lab and laminar flow hood were used to prepare the solutions. High-purity deionized water (resistivity 18.2 MΩ-cm) obtained from a Milli-Q water purification system (Millipore, Milli-Q RiOs, Bedford, MA) was used throughout. Plastic bottles and glassware were cleaned by soaking in 10 % (v/v) HNO<sub>3</sub> for 24 h, rinsed five times with Milli-Q water, and dried in a class 100 laminar flow hood before use. All operations were performed on a clean bench.

### 2.3.2. Instrumentation

Determination of chemical elements in the samples was performed using a PE ELAN DRC II inductively coupled plasma–mass spectroscopy (ICP-MS) instrument (Perkin Elmer, Waltham, United States). The ICP-MS was operated with Pt sampler and skimmer cones, both purchased from Perkin Elmer. Argon at 99.999 % (White Martins, São Paulo, Brazil) was used. Multi-element stock solution containing 10 mg/L was obtained from Perkin-Elmer (Perkin Elmer, Waltham, MA, United States).

### 2.3.3. Sample collection and preparation

Tree bark from *Bauhinia variegata* (n = 27) was collected by hand at 0, 100 and 200 meters from the main road of the respective area in winter 2013, summer 2014 and winter 2014. The samples were washed in distilled water and dried an oven at 50 °C until they were completely desiccated. Samples were stored in plastic bags at 4 °C until analysis. Briefly, samples (50–80 mg) in duplicate were digested with 2 ml of sub-boiling nitric acid at 80 °C for 6 h. After 24 h at room temperature, samples were diluted and analyzed by ICP-MS. Two reference materials (Mess-3 and Pine needles 1575a) provided by the National Research Council of Canada (NRCC) and the National Institute of Standards and Technology (NIST) were used to assure quality control of data. The obtained values were in good agreement with the provided reference or certified values.

Nails (n = 102) were clipped with a stainless steel instrument and placed inside metal-free plastic tubes. After the washing procedure using acetone and Triton X-100, samples were dried in a class 100 laminar flow hood. The nails were cleaned, weighed (10–20 mg) and 1 mL of 50 % (w/v) TMAH solution was added. Afterwards, the tubes were incubated at room temperature for 12 h. The volume was made up to 10 mL with 1 % (v/v) HNO<sub>3</sub> and directly analyzed by ICP-MS following the method reported by Batista et al. (Batista et al., 2008). In all analyses, rhodium was added as an internal standard to a final concentration of 10 µgL<sup>-1</sup>. Hair samples provided by the *Institut National de Santé Publique du Québec*, Canada were used as reference material.

## 2.4. Statistical Analysis

The means of NO<sub>2</sub>, O<sub>3</sub> and element concentrations were compared by one-way ANOVA followed by Bonferroni's *post hoc*. Principal Component Analysis (PCA) was used to obtain an overview of the data as well as to identify possible sources that explain its distribution. The influence of confounding factors was assessed using a Chi-Square test and one-way ANOVA. In addition, the association between the elements was assessed using Pearson Correlation.

Analysis of the data was performed using the Sigma Plot 12.0 software and IBM SPSS Statistics 20.0. The level of significance for these analyses was set at 5%.

## 3. Results

### 3.1. Study Population and Area

The hourly mean traffic flow was collected from public traffic data (Public Transport and Circulation Company — EPTC) in a single period in each monitoring area. Area A averaged 5060 vehicles per hour, 4054 vehicles per hour were counted in Area B and 1607 vehicles per hour passed through Area C.

Table 1 shows the results of possible confounding factors assessed in students from the different areas. The results show no statistically significant difference in age ( $P = 0.067$ ), gender ( $P = 0.155$ ), socioeconomic status ( $P = 0.249$ ) or exposure to passive smoke ( $P = 0.897$ ) among areas A, B and C.

### 3.2. Passive Sampling of NO<sub>2</sub> and O<sub>3</sub>

NO<sub>2</sub> was clearly different in concentration between the areas. The levels are consistent with the results provided by vehicular flow and population density, ratifying the different profile of vehicular traffic in each region. The accumulated concentration of NO<sub>2</sub> after the 3 exposure periods was  $42.19 \pm 4.42 \mu\text{g}/\text{m}^3$  in Area A,  $26.93 \pm 5.48 \mu\text{g}/\text{m}^3$  in Area B and  $15.86 \pm 5.91 \mu\text{g}/\text{m}^3$  in Area C. All groups were significantly different from each other ( $P < 0.001$ ) (Figure 1a).

O<sub>3</sub> concentrations showed a different pattern from NO<sub>2</sub>. O<sub>3</sub> concentration in the high population area and the intermediate population area were not significantly different ( $P = 1.000$ ). However both areas had higher levels of O<sub>3</sub> compared with Area C ( $P = 0.003$ ). The

concentration in Area A was  $39.78 \pm 11.11 \mu\text{g}/\text{m}^3$ , whereas the mean concentration in Area B was  $39.87 \pm 11.09 \mu\text{g}/\text{m}^3$  and  $29.96 \pm 11.20$  in Area C (Figure 1b).

### 3.3. Accumulated Elements in Tree Bark and Human Nails.

Table 2 shows the concentrations of accumulated elements in tree bark of *Bauhinia variegata*. Cd ( $P = 0.004$ ), Zn ( $P < 0.001$ ), Mg ( $P = 0.034$ ), Sb ( $P < 0.001$ ), Ba ( $P < 0.001$ ), Co ( $P < 0.001$ ), Cu ( $P = 0.016$ ), Mn ( $P = 0.010$ ), Pb ( $P = 0.018$ ), V ( $P = 0.010$ ), Ni ( $P = 0.007$ ) and Sr ( $P < 0.001$ ) were at higher concentrations in Area A compared to Area B, Area C or both. Area B had elevated concentrations of Cd ( $P = 0.004$ ) compared to Area C, as well as levels of P ( $P = 0.02$ ) statistically higher than in Area A. Furthermore, As ( $P = 0.015$ ) in Area C was measured at higher concentrations than Area B.

The analysis of elements in students' nails showed increased concentrations of Mg ( $P = 0.018$ ), Cd ( $P = 0.044$ ), Co ( $P < 0.001$ ), Cu ( $P < 0.001$ ), Mn ( $P = 0.004$ ), Ni ( $P = 0.006$ ) and Sr ( $P = 0.032$ ) in Area A compared to the other areas. At the same time, the element P ( $P = 0.045$ ) in Area B was elevated in comparison with Area C (Table 3). Fe, Se, Sb and As were below the detection limit. Correlations between elements in both matrices are presented in Tables 4 and 5.

PCA of tree bark identified four factors with eigenvalues  $> 1$ . These factors explained 83.1 % of the total variance in the data set. In addition, PCA of nails identified three main factors which explained 68.4 % of the variance among the samples (Table 6).

## 4. Discussion

The main objective of this study was to measure elements accumulated in vegetal and human matrices in regions with different population profiles, and to compare the pattern of these compounds between the two matrices. Thus, three areas with different population density, vehicular flow and air pollution levels were selected. In addition, areas in the inner city mostly exposed to air pollution by vehicular traffic were selected, minimizing the influence of industrial sources.

PCA was used to obtain an overview of the data and to identify possible sources of contamination. The PCA of tree bark revealed four main factors that explained 83.1 % of the variance. Factor 1 explained the largest proportion of the total variance (54.3 %) and

included high scores of Al, V, Fe, Pb, As, Ni, Cr, Cu, Se and Cd, as well as intermediate scores of Zn, Co, Sb, Mn and Hg. This factor is explained mainly by vehicular emissions and crustal emissions. Fe, Cr and Al, components of crustal soil, are predominantly attributed to resuspended road dust. Pb is present in crustal materials, but the high levels found in Area A may be explained by previous deposition of Pb from industry or by tailpipe emissions from combustion of leaded gasoline before it was phased out. Industrial sources and tailpipe emissions prior to the phaseout of leaded gasoline have previously been identified as the causes of Pb enrichment in roadway dirt. The strong correlation among Al, Fe and Pb supports the hypothesis of a common source for these elements. In addition, V, Cd, Pb, Cr, Cu, Ni, Zn and Se are attributed to diesel tailpipe emissions and gasoline tailpipe emissions (Claiborn et al., 2002; Park and Kim, 2005; Schauer et al., 2006).

Factor 2 contributed 12.1 % of the variance and included high loadings of Ba, Sr, Sb, Co, Zn and Mn. This factor is also explained by the influence of vehicular traffic. Sb is considered a marker of brake wear (Dietl et al., 1997). In addition, this type of emission is also associated with elevated concentrations of Ba, Mn, Zn, Sr (Schauer et al., 2006). The positive correlation between Sb, Ba, Zn and Sr supports the possibility of a common source suggesting that the concentration of these trace elements in the environment may be affected by brake wear emissions. Additionally, Zn can also be released in the atmosphere by tire wear emissions. This element is also used as antioxidant in engine oils (Andrade et al., 2012). Factor 3 (11.3 %) comprised the elements Mg, Hg and P. These elements are associated with soil composition. Factor 4, which explained only 5.4% of the total variance, included only the element Rb. The concentration of Rb in the tree bark can be explained by root uptake from the soil (Berg et al., 1995).

PCA of students' nails showed three main factors that explained 68.4 % of the total variance. Factor 1 was related with the largest proportion of the variance (50.8 %) and included the elements V, Hg, Cu, Sr, Ba, Ni, Mn, Cd and Pb. All these elements were present in Factors 1 and 2 of the PCA of tree bark and they can also be explained by exposure to air pollution by vehicular traffic. Some of these trace elements could also be associated with tobacco smoke (Caruso et al., 2014; Duran et al., 2012). However, all students that reported active smoking were excluded from the study. In addition, a Chi-

square analysis of exposure to passive smoking showed no difference among the groups and there was also no difference in trace element levels in the nails of students exposed or not exposed to passive smoking (Table 7), which minimizes the likelihood of tobacco smoke being a source of trace elements in Factor 1. Factor 2, which contributed with 10.2% of the variance, has high scores of Mg, Rb, Mn and Zn. These are essential elements and they are associated with dietary uptake. Factor 3 (7.4%) is composed only of Co. Since cobalt is presented alone in this factor, the authors could not attribute a specific source. However, this element may be associated with both dietary uptake and atmospheric contamination (Samecka-Cymerman and Kempers, 1999).

In this study, the region of the city with high population density showed elevated levels of trace elements accumulated in tree bark and nails of the residents. Most of these elements are toxic metals as well as known markers of air pollution. Furthermore, all elements found in higher concentrations in nails from Area A (Mg, Ba, Cd, Co, Cu, Mn, Ni, Sr) were also accumulated at higher levels in tree bark from the same area. This pattern — allied with the PCA of the samples, NO<sub>2</sub> concentration and vehicular flow — reinforces the hypothesis that vehicular traffic was the main source of the elements. A few studies have previously also assessed vehicular traffic as a source of metals in human nails. Were et. al. observed higher concentration of Cd and Pb in nails of students from an urban area of Nairobi compared with children living and studying in a rural area. Although Cd levels were attributed to industrial sources, significantly higher Pb levels in nails were observed in children whose school was near a highway (Were et al., 2008). Pb was also found in elevated concentration in nails of traffic policemen, showing significant and positive correlations with the duration of exposure and markers of kidney damage (Mortada et al., 2001).

In a preliminary study, the results of human and plant biomonitoring using the micronucleus frequency assay in buccal epithelial cells and the pollen abortion assay were correlated in the same students and trees evaluated in the present study. The results were strongly associated with the urbanization gradient near roads with heavy traffic, the population distribution and the concentration of air pollution among the areas with high, intermediate or low population density in Porto Alegre (Fleck et al., 2014). These results are in agreement with the findings of trace element distribution in humans and trees,

showing that — under the present study conditions — the results found in vegetal biomonitoring are comparable with the results found in human biomonitoring.

## 5. Conclusion

In conclusion, high levels of trace elements related to traffic-related air pollution were found accumulated in human and vegetal matrices, being associated with the degree of urbanization. In addition, the authors were able to demonstrate a relationship between human nails and tree bark as bioindicators of exposure to metals.

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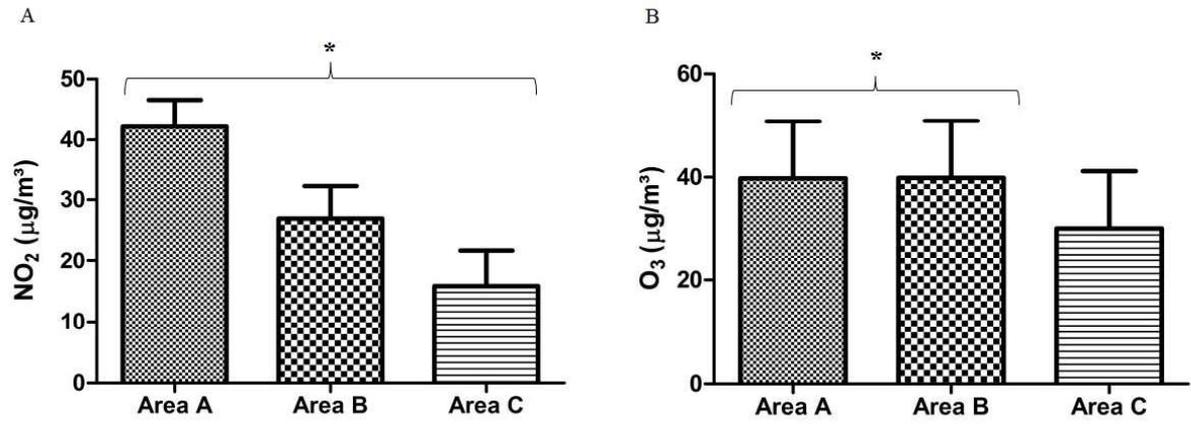
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## Tables and Figures

Figure 1



**Table 1:** Students profile from areas of high (A), intermediate (B) and low (C) population density.

	Area A (n=40)	Area B (n=30)	Area C (n=32)	<i>P</i>
Age (Years)	13.7 ± 1.4	13.7 ± 1.3	13.1 ± 1.2	0.067
Gender				
Male	11	15	12	0.155
Female	29	15	21	
Socioeconomic Status				
≤ 2 Minimum Wage	25	19	15	0.249
> 2 Minimum Wage	15	11	18	
Passive Smoking				
Yes	24	19	19	0.897
No	16	11	14	

Tests performed: One-way ANOVA and Chi-Square.

Table 2: Elements concentration in tree barks of *Bauhinia variegata* from areas of high (A), intermediate (B) and low (C) population density.

Elements ( $\mu\text{g/g}$ )	Area A (n = 9) Mean $\pm$ S.D	Area B (n = 9) Mean $\pm$ S.D	Area C (n = 9) Mean $\pm$ S.D	P
Fe	329.61 $\pm$ 181.56	204.71 $\pm$ 186.01	200.42 $\pm$ 117.83	0.083
Cd	0.12 $\pm$ 0.05 <sup>#</sup>	0.12 $\pm$ 0.07 <sup>#</sup>	0.05 $\pm$ 0.02	0.004
Zn	68.88 $\pm$ 28.51* <sup>#</sup>	36.10 $\pm$ 17.26	31.27 $\pm$ 12.40	<0.001
Mg	846.38 $\pm$ 180.19 <sup>#</sup>	819.80 $\pm$ 323.07	626.47 $\pm$ 214.44	0.034
P	633.90 $\pm$ 181.67	1,309.89 $\pm$ 872.97 <sup>###</sup>	819.80 $\pm$ 315.58	0.002
Hg	0.044 $\pm$ 0.030	0.049 $\pm$ 0.042	0.040 $\pm$ 0.042	0.834
Sb	0.30 $\pm$ 0.19* <sup>#</sup>	0.06 $\pm$ 0.06	0.03 $\pm$ 0.03	<0.001
Al	187.86 $\pm$ 105.90	127.91 $\pm$ 129.79	153.81 $\pm$ 138.86	0.364
Ba	967.77 $\pm$ 435.96* <sup>#</sup>	382.23 $\pm$ 189.70	261.14 $\pm$ 106.92	<0.001
Co	0.59 $\pm$ 0.39* <sup>#</sup>	0.17 $\pm$ 0.14	0.18 $\pm$ 0.14	<0.001
Cu	18.39 $\pm$ 7.38*	11.16 $\pm$ 6.81	13.90 $\pm$ 8.03	0.016
Mn	64.52 $\pm$ 30.79*	36.01 $\pm$ 22.83	54.02 $\pm$ 28.60	0.010
Pb	1.10 $\pm$ 0.66 <sup>#</sup>	0.67 $\pm$ 0.56	0.54 $\pm$ 0.49	0.018
Se	0.21 $\pm$ 0.07	0.24 $\pm$ 0.11	0.25 $\pm$ 0.08	0.474
Rb	3.23 $\pm$ 2.27	5.46 $\pm$ 3.79	5.33 $\pm$ 4.00	0.111
Ni	1.00 $\pm$ 0.33 <sup>#</sup>	0.77 $\pm$ 0.29	0.68 $\pm$ 0.24	0.007
V	0.80 $\pm$ 0.48*	0.33 $\pm$ 0.36	0.48 $\pm$ 0.36	0.010
As	0.16 $\pm$ 0.05	0.12 $\pm$ 0.06	0.18 $\pm$ 0.07*	0.015
Cr	2.55 $\pm$ 0.66	2.08 $\pm$ 0.97	2.32 $\pm$ 0.7	0.223
Sr	155.75 $\pm$ 49.37* <sup>#</sup>	94.37 $\pm$ 22.67	90.17 $\pm$ 28.48	<0.001

\*Statistically higher than Area B. <sup>#</sup>Statistically higher than Area C. \*<sup>#</sup>Statistically higher than Area B and Area C. <sup>###</sup>Statistically higher than Area A.

S.D: Standard Deviation. Test performed: One-way ANOVA followed by Bonferroni's *post hoc*.

Table 3: Elements concentration in students nails from from areas of high (A), intermediate (B) and low (C) population density.

Elements ( $\mu\text{g/g}$ )	Area A (n = 40) Mean $\pm$ S.D	Area B (n = 30) Mean $\pm$ S.D	Area C (n = 32) Mean $\pm$ S.D	P
Mg	34.11 $\pm$ 19.17 <sup>#</sup>	29.82 $\pm$ 9.88	24.13 $\pm$ 11.18	0.018
P	279.89 $\pm$ 189.01	280.64 $\pm$ 134.56 <sup>#</sup>	185.40 $\pm$ 124.27	0.045
Hg	0.011 $\pm$ 0.009	0.008 $\pm$ 0.007	0.010 $\pm$ 0.008	0.344
Ba	3.07 $\pm$ 2.50	2.70 $\pm$ 1.65	2.76 $\pm$ 1.95	0.090
Cd	0.18 $\pm$ 0.10* <sup>#</sup>	0.12 $\pm$ 0.07	0.11 $\pm$ 0.07	0.044
Co	0.086 $\pm$ 0.054* <sup>#</sup>	0.039 $\pm$ 0.029	0.025 $\pm$ 0.018	<0.001
Cu	10.84 $\pm$ 5.7* <sup>#</sup>	7.59 $\pm$ 4.00	5.55 $\pm$ 2.38	<0.001
Mn	1.30 $\pm$ 1.10 <sup>#</sup>	1.03 $\pm$ 0.69	0.66 $\pm$ 0.47	0.004
Pb	0.55 $\pm$ 0.37	0.57 $\pm$ 0.31	0.46 $\pm$ 0.39	0.425
Rb	1.33 $\pm$ 0.75	1.26 $\pm$ 0.57	1.32 $\pm$ 0.78	0.919
Zn	104.01 $\pm$ 33.89	102.41 $\pm$ 68.32	97.71 $\pm$ 49.31	0.869
Ni	1.86 $\pm$ 1.55*	0.94 $\pm$ 0.74	1.26 $\pm$ 1.03	0.006
V	0.084 $\pm$ 0.052	0.095 $\pm$ 0.065	0.086 $\pm$ 0.052	0.719
Sr	1.54 $\pm$ 0.89 <sup>#</sup>	1.42 $\pm$ 0.72	1.09 $\pm$ 0.41	0.032

\*Statistically higher than Area B. <sup>#</sup>Statistically higher than Area C. \*<sup>#</sup>Statistically higher than Area B and Area C. S.D: Standard Deviation.

Test performed: One-way ANOVA followed by Bonferroni's *post hoc*.

Table 4: Correlation coefficients for elements-to-elements in tree barks of Bauhinia variegata in all studied areas.

	Mg	P	Hg	Sb	Al	BA	Cd	Co	Cu	Mn	Pb	Se	Rb	Zn	Ni	V	As	Cr	Sr
Fe	0.151	-0.15	<b>0.44</b>	<b>0.73</b>	<b>0.96</b>	<b>0.39</b>	<b>0.67</b>	<b>0.65</b>	<b>0.74</b>	<b>0.65</b>	<b>0.94</b>	<b>0.52</b>	-0.3	<b>0.72</b>	<b>0.85</b>	<b>0.97</b>	<b>0.68</b>	<b>0.72</b>	<b>0.41</b>
Mg		<b>0.64</b>	<b>0.40</b>	0.13	0.12	<b>0.33</b>	0.26	<b>0.28</b>	<b>0.40</b>	<b>0.37</b>	0.12	0.28	0.14	<b>0.29</b>	<b>0.36</b>	0.11	-0.66	<b>0.53</b>	<b>0.43</b>
P			0.18	-0.24	-0.11	-0.09	0.08	-0.17	0.08	0.02	-0.16	0.26	<b>0.36</b>	-0.11	-0.01	-0.15	-0.13	0.191	-0.07
Hg				-0.03	<b>0.49</b>	-0.02	0.15	0.04	<b>0.39</b>	<b>0.36</b>	<b>0.40</b>	<b>0.43</b>	-0.05	0.14	<b>0.30</b>	<b>0.43</b>	0.220	<b>0.53</b>	0.11
Sb					<b>0.63</b>	<b>0.69</b>	<b>0.63</b>	<b>0.81</b>	<b>0.65</b>	<b>0.56</b>	<b>0.74</b>	0.15	-0.33	<b>0.90</b>	<b>0.69</b>	<b>0.71</b>	<b>0.44</b>	<b>0.49</b>	<b>0.57</b>
Al						<b>0.26</b>	<b>0.61</b>	<b>0.54</b>	<b>0.76</b>	<b>0.60</b>	<b>0.94</b>	<b>0.52</b>	-0.31	<b>0.63</b>	<b>0.79</b>	<b>0.97</b>	<b>0.72</b>	<b>0.67</b>	<b>0.27</b>
Ba							<b>0.43</b>	<b>0.70</b>	<b>0.48</b>	<b>0.62</b>	<b>0.34</b>	0.21	-0.19	<b>0.77</b>	<b>0.45</b>	<b>0.34</b>	0.07	<b>0.44</b>	<b>0.86</b>
Cd								<b>0.57</b>	<b>0.52</b>	<b>0.45</b>	<b>0.64</b>	<b>0.45</b>	-0.11	<b>0.69</b>	<b>0.72</b>	<b>0.62</b>	<b>0.29</b>	<b>0.55</b>	0.34
Co									<b>0.60</b>	<b>0.81</b>	<b>0.62</b>	<b>0.33</b>	-0.3	<b>0.76</b>	<b>0.71</b>	<b>0.65</b>	<b>0.44</b>	<b>0.54</b>	<b>0.72</b>
Cu								<b>0.72</b>			<b>0.71</b>	<b>0.47</b>	-0.27	<b>0.73</b>	<b>0.75</b>	<b>0.75</b>	<b>0.53</b>	<b>0.73</b>	<b>0.40</b>
Mn											<b>0.56</b>	<b>0.59</b>	-0.29	<b>0.65</b>	<b>0.66</b>	<b>0.67</b>	<b>0.50</b>	<b>0.70</b>	<b>0.65</b>
Pb												<b>0.44</b>	-0.26	<b>0.68</b>	<b>0.81</b>	<b>0.94</b>	<b>0.63</b>	<b>0.64</b>	<b>0.40</b>
Se													0.19	<b>0.29</b>	<b>0.61</b>	<b>0.51</b>	<b>0.47</b>	<b>0.67</b>	<b>0.33</b>
Rb														-0.20	-0.07	-0.34	-0.19	-0.03	-0.10
Zn															<b>0.75</b>	<b>0.68</b>	<b>0.41</b>	<b>0.67</b>	<b>0.66</b>
Ni																<b>0.80</b>	<b>0.62</b>	<b>0.77</b>	<b>0.52</b>
V																	<b>0.70</b>	<b>0.66</b>	<b>0.36</b>
As																		<b>0.52</b>	0.10
Cr																			<b>0.54</b>

Bolds values are significant at  $P < 0.005$ . Test performed: Pearson correlation.

Table 5: Correlation coefficients for elements-to-elements in nails in all studied areas.

	P	Hg	Ba	Cd	Co	Cu	Mn	Pb	Rb	Zn	Ni	V	Sr
Mg	<b>0.569</b>	-0.035	<b>0.344</b>	<b>0.351</b>	0.0701	<b>0.253</b>	<b>0.628</b>	<b>0.350</b>	<b>0.453</b>	0.119	<b>0.304</b>	0.174	<b>0.453</b>
P		<b>0.263</b>	<b>0.326</b>	<b>0.385</b>	0.006	<b>0.454</b>	<b>0.493</b>	<b>0.381</b>	<b>0.401</b>	0.102	<b>0.472</b>	<b>0.386</b>	<b>0.475</b>
Hg			<b>0.642</b>	<b>0.409</b>	0.001	<b>0.759</b>	<b>0.380</b>	<b>0.324</b>	<b>0.296</b>	<b>0.399</b>	<b>0.554</b>	<b>0.864</b>	<b>0.701</b>
Ba				<b>0.585</b>	0.020	<b>0.678</b>	<b>0.692</b>	<b>0.405</b>	<b>0.362</b>	<b>0.441</b>	<b>0.515</b>	<b>0.682</b>	<b>0.811</b>
Cd					0.111	<b>0.673</b>	<b>0.702</b>	<b>0.592</b>	<b>0.316</b>	<b>0.262</b>	<b>0.719</b>	<b>0.498</b>	<b>0.560</b>
Co						0.0355	0.115	0.036	-0.033	0.024	0.060	0.099	0.049
Cu							<b>0.622</b>	<b>0.560</b>	<b>0.439</b>	<b>0.475</b>	<b>0.713</b>	<b>0.826</b>	<b>0.727</b>
Mn								<b>0.653</b>	<b>0.337</b>	<b>0.269</b>	<b>0.564</b>	<b>0.630</b>	<b>0.777</b>
Pb									<b>0.337</b>	<b>0.215</b>	<b>0.400</b>	<b>0.506</b>	<b>0.508</b>
Rb										<b>0.387</b>	<b>0.378</b>	<b>0.376</b>	<b>0.438</b>
Zn											<b>0.275</b>	<b>0.325</b>	<b>0.408</b>
Ni												<b>0.621</b>	<b>0.519</b>
V													<b>0.820</b>

Bolds values are significant at  $P < 0.005$ . Test performed: Pearson correlation.

Table 6: Explained variance and loadings for the extracted factors for tree barks and nails of students from all areas.

Tree Bark					Nails			
Factor	F1	F2	F3	F4	Factor	F1	F2	F3
Variance (%)	54.3	12.1	11.3	5.4	Variance (%)	50.8	10.2	7.4
Al	.946				V	.921		
V	.914				Hg	.912		
Fe	.909	.335			Cu	.847	.341	
Pb	.873	.323			Sr	.757	.459	
As	.839				Ba	.722	.372	
Ni	.821	.486			Ni	.689	.325	
Cr	.709	.385	.438		Mn	.643	.602	
Cu	.694	.408			Cd	.631	.406	
Se	.688		.326	.347	Pb	.556	.427	
Cd	.617	.459			Mg		.878	
Ba		.942			Rb		.698	
Sr		.870			P	.340	.631	
Zn	.499	.794			Zn		.510	
Co	.441	.791			Co			.942
Sb	.496	.776						
Mn	.552	.563	.302					
Mg		.366	.829					
Hg	.463		.707					
P			.688	.474				
Rb				.834				

Table 7: Elements concentration in nails of students exposed and not exposed to passive smoking in all studied areas.

Elements ( $\mu\text{g/g}$ )	Not Exposed (n = 41) Mean $\pm$ S.D	Exposed (n = 61) Mean $\pm$ S.D	<i>P</i>
Mg	27.67 $\pm$ 11.24	31.20 $\pm$ 17.13	0.249
P	221.76 $\pm$ 115.73	272.79 $\pm$ 177.45	0.108
Hg	0.009 $\pm$ 0.008	0.010 $\pm$ 0.007	0.675
Ba	3.42 $\pm$ 2.33	3.11 $\pm$ 2.39	0.533
Cd	0.15 $\pm$ 0.09	0.14 $\pm$ 0.09	0.371
Co	0.04 $\pm$ 0.03	0.05 $\pm$ 0.04	0.363
Cu	6.6 $\pm$ 4.6	6.8 $\pm$ 4.8	0.865
Mn	0.93 $\pm$ 0.71	1.10 $\pm$ 0.96	0.347
Pb	0.49 $\pm$ 0.37	0.52 $\pm$ 0.38	0.646
Rb	1.23 $\pm$ 0.70	1.38 $\pm$ 0.71	0.311
Zn	100.07 $\pm$ 38.57	110.51 $\pm$ 74.68	0.412
Ni	1.19 $\pm$ 1.05	1.35 $\pm$ 1.18	0.546
V	0.08 $\pm$ 0.04	0.09 $\pm$ 0.05	0.351
Sr	1.32 $\pm$ 0.63	1.45 $\pm$ 0.90	0.418

S.D: Standard Deviation. Test performed: Student's t-test.

### Figure Captions

**Figure 1:** NO<sub>2</sub> (A) and O<sub>3</sub> (B) concentrations measured by passive sampling in all monitoring areas. A) \* All groups were significantly different from each other ( $P < 0.001$ ). B) \* Area A and Area B statistically higher than Area C ( $P=0.003$ ). Data are expressed as the mean  $\pm$  standard deviation. Test performed: One-way ANOVA followed by Bonferroni's *post hoc*.

## **6. Conclusão**

Diante dos resultados encontrados, conclui-se que as concentrações de NO<sub>2</sub> e O<sub>3</sub> foram mais elevadas na área de maior densidade populacional na cidade de Porto Alegre; Tal área também demonstrou maior dano genotóxico em células vegetais - medido pelo ensaio do aborto polínico – e maior acúmulo, em cascas de árvore, de elementos relacionados a emissões veiculares; avaliações de biomonitoramento humano demonstraram a maior taxa de dano genotóxico - medido pelo ensaio do micronúcleo em mucosa bucal – e acúmulo de metais em unhas de estudantes de escolas públicas da área de maior densidade populacional.

Em conclusão, áreas com diferentes concentrações de poluentes demonstraram possuir um padrão dependente do gradiente de urbanização ao qual refletiu no dano genotóxico humano e vegetal nestas áreas, levando também a um perfil similar de acúmulo de elementos em matriz vegetal e humana; demonstrando, assim, associação entre biomonitoramento humano e vegetal dos efeitos poluição atmosférica.

## **7. Anexos**

### **7.1. Anexo 1 – Normas da revista Environmental Research**

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Submission to this journal proceeds totally online and you will be guided stepwise through the creation and uploading of your files. The system automatically converts your files to a single PDF file, which is used in the peer-review process. As part of the Your Paper Your Way service, you may choose to submit your manuscript as a single file to be used in the refereeing process. This can be a PDF file or a Word document, in any format or lay-out that can be used by referees to evaluate your manuscript. It should contain high enough quality figures for refereeing. If you prefer to do so, you may still provide all or some of the source files at the initial submission. Please note that individual figure files larger than 10 MB must be uploaded separately.

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If your article includes any Videos and/or other Supplementary material, this should be included in your initial submission for peer review purposes.

Divide the article into clearly defined sections.

Please ensure your paper has consecutive line numbering - this is an essential peer review requirement.

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Please ensure the figures and the tables included in the single file are placed next to the relevant text in the manuscript, rather than at the bottom or the top of the file.

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Regardless of the file format of the original submission, at revision you must provide us with an editable file of the entire article. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier: <http://www.elsevier.com/guidepublication>). See also the section on Electronic artwork.

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For original full-length and short communications:

*Introduction* should be as concise as possible, without subheadings.

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*Results and Discussion* may be combined and may be organized into subheadings.

For commentaries and articles related to environmental policy, alternate formats will be accepted but should include an Introduction describing the problem in terms that a general reader will understand. All statements of fact need to be referenced and papers that make use of newly acquired data must include a Materials and methods section as well as a Results and Discussion section.

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Divide your article into clearly defined and numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2, ...), 1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer to 'the text'. Any subsection may be given a brief heading. Each heading should appear on its own separate line.

### ***Introduction***

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

### ***Material and methods***

Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference: only relevant modifications should be described.

### ***Results***

Results should be clear and concise.

### ***Discussion***

This should explore the significance of the results of the work, not repeat them. A combined Results and Discussion section is often appropriate. Avoid extensive citations and discussion of published literature.

## *Conclusions*

The main conclusions of the study may be presented in a short Conclusions section, which may stand alone or form a subsection of a Discussion or Results and Discussion section.

## *Appendices*

If there is more than one appendix, they should be identified as A, B, etc. Formulae and equations in appendices should be given separate numbering: Eq. (A.1), Eq. (A.2), etc.; in a subsequent appendix, Eq. (B.1) and so on. Similarly for tables and figures: Table A.1; Fig. A.1, etc.

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- **Corresponding author.** Clearly indicate who will handle correspondence at all stages of refereeing and publication, also post-publication. **Ensure that the e-mail address is given and that contact details are kept up to date by the corresponding author.**
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*Materials and methods* should be sufficiently detailed to enable the experiments to be reproduced or the study design to be understood fully.

*Results and Discussion* may be combined and may be organized into subheadings.

For commentaries and articles related to environmental policy, alternate formats will be accepted but should include an Introduction describing the problem in terms that a general reader will understand. All statements of fact need to be referenced and papers that make use of newly acquired data must include a Materials and methods section as well as a Results and Discussion section.

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A concise and factual abstract is required. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

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## **Highlights**

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Immediately after the abstract, provide a maximum of 5 keywords, using American spelling and avoiding general and plural terms and multiple concepts (avoid, for example, "and", "of"). Be sparing with abbreviations: only abbreviations firmly established in the field may be eligible. These keywords will be used for indexing purposes.

## **Abbreviations**

Define abbreviations that are not standard in this field in a footnote to be placed on the first page of the article. Such abbreviations that are unavoidable in the abstract must be defined at their first mention there, as well as in the footnote. Ensure consistency of abbreviations throughout the article.

*Abbreviations* should follow the usage established by *Chemical Abstracts*. Please restrict the use of acronyms, especially non-standard ones, as much as possible.

### **Acknowledgements**

Collate acknowledgements in a separate section at the end of the article before the references and do not, therefore, include them on the title page, as a footnote to the title or otherwise. List here those individuals who provided help during the research (e.g., providing language help, writing assistance or proof reading the article, etc.).

*Acknowledgments* should be brief and should precede the references. In agreement with the Commission on Publication Ethics, authors must submit full information on sources of funding and other support for their work that is presented in their paper.

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### **References**

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References should be cited in the text by the author's name and year of publication. References should be listed alphabetically in an unnumbered list at the end of the paper in the following style:

Baeklund, M., Pedersen, N.L., Bjorkman, L., Vahter, M., 1999. Variation in blood concentrations of cadmium and lead in the elderly. *Environ. Res.* 80, 222-230.

Letourneau, D.K., 1997. Plant-arthropod interactions in agroecosystems. In: Jackson, L.E.(Ed.), *Ecology in Agriculture*. Academic Press, San Diego, pp. 239-290.

Morgan, W.K.C., Seaton, A. (Eds.), 1995. *Occupational Lung Diseases*, 3rd ed. Saunders, Philadelphia, pp. 308-373.

References drawn from the worldwide web must include the date in which the material was accessed.

The names of journals should be abbreviated according to the latest available edition of *Index Medicus* or *Chemical Abstracts Service Source Index*. Only articles that have been published or are in press should be included in the references. " Manuscript in preparation," " personal communication," and " unpublished observation" should be cited as such in the text.

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Journal names should be abbreviated according to the List of Title Word Abbreviations: <http://www.issn.org/services/online-services/access-to-the-ltwa/>.

Only articles that have been published or are in press should be included in the references. "Manuscript in preparation," "personal communication," and "unpublished observation" should be cited as such in the text.

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One author has been designated as the corresponding author with contact details:

- E-mail address.
- Full postal address . All necessary files have been uploaded, and contain:
- Keywords
- All figure captions
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Further considerations

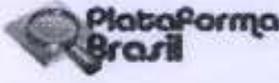
- Manuscript has been 'spell-checked' and 'grammar-checked'
- All references mentioned in the Reference list are cited in the text, and vice versa
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## 7.2. Anexo 2 – Carta de aceite do Comitê de Ética em Pesquisa (CEP)

<b>UNIVERSIDADE FEDERAL DE CIÊNCIAS DA SAÚDE DE PORTO ALEGRE</b>		
<b>PARECER CONSUBSTANCIADO DO CEP</b>		
<b>DADOS DO PROJETO DE PESQUISA</b>		
<b>Título da Pesquisa:</b> Avaliação dos Efeitos da Exposição Humana a Contaminantes Atmosféricos Associada ao Biomonitoramento da Qualidade do Ar		
<b>Pesquisador:</b> Cláudia Ramos Rhoden		
<b>Área Temática:</b>		
<b>Versão:</b> 2		
<b>CAAE:</b> 15035313.7.0000.5345		
<b>Instituição Proponente:</b> Universidade Federal de Ciências da Saúde de Porto Alegre		
<b>Patrocinador Principal:</b> Financiamento Próprio		
<b>DADOS DO PARECER</b>		
<b>Número do Parecer:</b> 315.260		
<b>Data da Relatoria:</b> 20/06/2013		
<b>Apresentação do Projeto:</b>		
<p>Estudos realizados anteriormente no Laboratório de Estresse Oxidativo e Poluição Atmosférica - UFCSPA demonstram a eficácia do uso da Pata de Vaca (<i>Bauhinia variegata</i>) como um bioindicador da poluição atmosférica. Entretanto, são escassos na literatura os trabalhos que abordam a análise de diversos parâmetros de biomonitoramento, concomitantemente, em uma única espécie vegetal, como a influência da poluição atmosférica na taxa de aborto polínico, acúmulo de metais em cascas de árvore e estresse oxidativo em folhas. A união de ensaios de acumulação e sensibilidade é importante para determinar o perfil dos poluentes que agem sobre o bioindicador.</p> <p>Além disso, a comparação entre ensaios de biomonitoramento e exposição humana é importante para a determinação do grau de exposição humana aos poluentes atmosféricos locais e seus efeitos.</p>		
<b>Endereço:</b> Rua Sarmiento Leite, 245		
<b>Bairro:</b>		<b>CEP:</b> 90.050-170
<b>UF:</b> RS	<b>Município:</b> PORTO ALEGRE	
<b>Telefone:</b> (51)303-8804	<b>E-mail:</b> cep@ufcspa.edu.br	

Continuação do Parecer: 315.260

**Objetivo da Pesquisa:**

Avaliar a qualidade do ar em áreas com diferentes gradientes populacionais próximas a uma via de intenso tráfego automotor - em distâncias crescentes em relação à fonte poluidora - por meio de diversos parâmetros de biomonitoramento em uma única espécie vegetal, bem como correlacionar dados de biomonitoramento vegetal com marcadores de exposição humana e monitoramento passivo.

**Avaliação dos Riscos e Benefícios:**

Desconfortos e riscos esperados: O aluno poderá sentir um pequeno desconforto no momento da coleta de amostra da mucosa bucal e coleta da unha, porém estes procedimentos não apresentam nenhum risco. Caso haja qualquer desconforto em responder às perguntas do questionário ou tiver dúvidas sobre suas respostas, você pode me chamar para discutirmos juntos a sua dúvida. Se em algum momento você quiser parar de responder ao questionário por questões pessoais, você poderá parar. A direção da escola assim como o professor estão de acordo com a realização deste estudo na escola.

Os benefícios que se pode obter: o participante e/ou representante legal será informado sobre os resultados da pesquisa, após análise de todos os dados em conjunto. Não há despesas pessoais para os participantes em qualquer fase do estudo. Também não haverá compensação financeira relacionada a esta participação. Se existir qualquer despesa adicional, ela será absorvida pelo orçamento da pesquisa. O pesquisador se compromete em utilizar os dados coletados no estudo somente para fins da pesquisa.

**Comentários e Considerações sobre a Pesquisa:**

A comparação entre ensaios de biomonitoramento e exposição humana é importante para a determinação do grau de exposição humana aos poluentes atmosféricos locais e seus efeitos.

**Considerações sobre os Termos de apresentação obrigatória:**

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Bairro: CEP: 90.050-170  
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Telefone: (51)303-8804 E-mail: cep@uicspa.edu.br

Continuação do Parecer: 315-290

Todos os termos estão contemplados.

**Recomendações:**

**Conclusões ou Pendências e Lista de Inadequações:**

As solicitações foram plenamente atendidas pelos pesquisadores.

**Situação do Parecer:**

Aprovado

**Necessita Apreciação da CONEP:**

Não

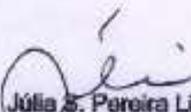
**Considerações Finais a critério do CEP:**

De acordo com o Parecer do Relator.

PORTO ALEGRE, 25 de Junho de 2013

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Assinador por:  
José Geraldo Vernet Taborda  
(Coordenador)

  
Júlia S. Pereira Lima  
Vice-Coordenadora CEP/UFCSPA

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