MARCELLE DAFRÉ MARTINELLI

Aporte e deposição de elementos químicos marcadores de poluição atmosférica em fragmentos florestais na região metropolitana de Campinas, São Paulo

Tese apresentada ao Instituto de Botânica da Secretaria do Meio Ambiente, como parte dos requisitos exigidos para a obtenção do título de DOUTOR em BIODIVERSIDADE VEGETAL E MEIO AMBIENTE, na Área de Concentração de Plantas Vasculares em Análises Ambientais.

SÃO PAULO
2015

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Ficha Catalográfica elaborada pelo **NÚCLEO DE BIBLIOTECA E MEMÓRIA**

Martinelli, Marcelle Dafré
M375a Aporte e deposição de elementos químicos marcadores de poluição atmosférica em fragmentos florestais na região metropolitana de Campinas, São Paulo / Marcelle Dafré Martinelli -- São Paulo, 2015.
134 p. il.

Tese (Doutorado) -- Instituto de Botânica da Secretaria de Estado do Meio Ambiente, 2015.
Bibliografia.

1. Poluição atmosférica. 2. Florestas. 3. Solos tropicais. I. Título.

CDU: 502.55

Dedico este trabalho
aos meus amores
Fábio, Arthur e Maya.

“O conhecimento real não é construção de alguns dias. É obra do tempo”.

(André Luiz)

Agradecimentos

Esta tese de doutorado é fruto de muita parceria e colaboração de grandes pesquisadores, instituições e de amigos queridos que Deus colocou em meu caminho para tornar tudo mais fácil e por isso aí vão meus agradecimentos mais que especiais:

À Dra Marisa Domingos pela orientação, total apoio, sinceridade e disponibilidade para tirar minhas dúvidas em nossas micro-reuniões, por telefone e até mesmo e muito por whatsapp. Obrigada por me oferecer tantas oportunidades de crescimento pessoal e científico durante todos estes anos desde o mestrado.

À Dra Ana Maria G. Figueiredo pela co-orientação e ajuda que nos ofereceu durante todo esse tempo, inclusive nos guiando até outros grandes colaboradores.

À Dra Mitiko Saiki pelo uso do laboratório e moinho de bola de ágata do IPEN/CNEN.

À Dra Mirian C.S. Rinaldi pela atenção e esclarecimentos técnicos e científicos durante a execução de análises laboratoriais e pela colaboração no capítulo relacionado à deposição seca e úmida.

A toda equipe do Instituto de Astronomia, Geofísica e Ciências Atmosféricas (IAG) da USP, em especial à funcionária Rosana Astolfo, pela análise dos filtros de MP_{10}.

Ao Dr Américo A. F. S. Kerr, do Instituto de Física da USP, por me apresentar seu aluno Luis Mendes, que carinhosamente disponibilizou seu tempo e paciência para me ensinar a ajustar os dados obtidos no IAG.

À Dra Jacinta Enzweiler, que viabilizou a análise dos teores totais dos elementos no solo no laboratório do Instituto de Geofísica da UNICAMP com a ajuda das funcionárias Lúcia e Aparecida e também pela contribuição na elaboração do capítulo referente ao solo.

Ao Dr Raphael Bragança Alves Fernandes do Departamento de Solos e Centro de Ciências Agrárias da UFV, pela atenção dispendida desde o primeiro contato, permitindo que seu querido aluno Júlio me auxiliasse com a extração por DTPA e também pela contribuição na elaboração do capítulo solo.

Ao Dr Rubens C. L. Figueira do Instituto Oceanográfico da USP e Alexandre Salaroli por me ensinarem o método de extração de metais 3050B (US EPA).

Aos pesquisadores da Ecologia: Márcia Inês Lopes, Regina M. Moraes e Eduardo P.C Gomes que dividiram comigo durante as disciplinas todo seu amor e conhecimento pela Botânica e pelo solo.

A equipe de coleta do grupo Paulínia: Carla e Patrícia pela liderança e ensinamentos, Valdis pelas risadas e por abrir nosso caminho nas matas, Leonardo, Andressa, Cristiane, Patrícia Giampaoli, Ana Paula, Marcela, Solange, Amariles e demais “coletores não-oficiais” não só pelas amostras coletadas, mas pelos momentos de cansaço divididos.

A todos os amigos que fiz na Ecologia durante todo esse tempo: as Patês, Carlota, Andressa Maria, Leo, Ane, Jessbers, Day, Pedrones, Mazinha, Mari, Jessiquinha, Yu e em especial ao meu amigo querido e Doutor em estatística Ricks que sempre teve paciência e disposição para me ensinar, além de me ajudar em qualquer problema que fosse e também a minha amiga-irmãzinha Ana Paula por tudo o que compartilhamos durante todos estes anos, o que inclui amostras, armários, orientadora e principalmente momentos, felizes e tristes mas que ficarão pra sempre em nossas memórias.

Ao Programa Nacional de Desenvolvimento da Botânica (PNADB), CAPES, pela bolsa de doutorado concedida e ao programa SISBIOTA-BRASIL (proc. CNPQ 563335/2010; proc. FAPESP 2010/52319-2) pelo suporte financeiro.

Às Áreas de Relevante Interesse Ecológico “Mata da Santa Genebra” e “Matão de Cosmópolis”, Cooperativa Agropecuária Holambra e Fazenda Meia Lua, por permitirem a coleta de folhas, solo e deposição atmosférica em suas propriedades.

A Coordenação Especial para Restauração de Áreas Degradadas-CERAD e Paulo R. T. Ortiz pela elaboração da figura 1 incluída nos diferentes capítulos.

A toda a minha família, em especial meus pais e marido, que sempre me motivaram a ir atrás dos meus sonhos e que se orgulham de mim, mesmo sem entender ao certo o que tanto estudo.

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Resumo

O material particulado atmosférico, proveniente de fontes naturais ou antropogênicas, pode promover o aporte de elementos potencialmente tóxicos, como Cr, Cu, Ni, Pb, Zn, em ecossistemas florestais através da deposição seca e/ou úmida, promovendo seu acúmulo em diferentes compartimentos dos ecossistemas, como solo e comunidade vegetal. Programas de biomonitoramento de qualidade do ar, utilizando espécies vegetais, permitem avaliar a distribuição temporal e espacial destes poluentes e seu impacto aos ecossistemas. Em tese, esses programas poderiam ser úteis na Região Metropolitana de Campinas (RMC), que abriga um vasto parque industrial, incluindo a maior refinaria de petróleo do país, que tem uma intensa atividade agrícola (principalmente de cana-de–açúcar) e um alto fluxo veicular. Essa diversidade de fontes de poluição margeia os fragmentos florestais que ainda restam na região. O presente estudo foi proposto visando a testar as seguintes hipóteses: os fragmentos florestais remanescentes nesta região têm recebido um excesso de elementos traço, sendo que o nível de aporte destes elementos varia espacialmente e sazonalmente; o nível de disponibilidade de elementos traço no solo depende de sua origem e propriedades físicas dos solos; a capacidade de acúmulo foliar de elementos químicos e seu potencial para o biomonitoramento diferem entre as espécies arbóreas nativas. Assim, a deposição seca e úmida de elementos traço foi caracterizada nas proximidades de dois fragmentos florestais (um próximo ao pólo industrial em Paulínia e outro próximo a fontes urbanas em Campinas). Os níveis totais e disponíveis de elementos traço nos solos, assim como o acúmulo de elementos traço na folhagem das três espécies arbóreas mais abundantes (*Astronium graveolens* Jacq., *Croton floribundus* (L.) Spreng. e *Piptadenia gonoacantha* (Mart.) J.F. Macbr), foram determinados nesses mesmos fragmentos e em outros dois próximos a atividades agrícolas, em Cosmópolis e Holambra. A variação sazonal dos teores dos elementos químicos estudados na deposição seca e úmida não foi claramente definida, no entanto, muitos elementos ocorreram em maiores concentrações em amostras de MP_{10} durante a estação seca, em ambos os locais estudados. Al e Fe, elementos ligados à ressuspensão de solo, foram os mais abundantes na deposição seca e úmida. A água de chuva, em Campinas e Paulínia, mostrou-se moderadamente enriquecida por Cu, Pb e Zn. O MP_{10} teve enriquecimento moderado de Cu e alto enriquecimento de Mo em ambas as áreas. Não houve variação sazonal na concentração dos elementos traço avaliados no solo e a variação espacial foi influenciada não somente pelas fontes de poluição como pelas rochas matrizes do solo. A disponibilidade dos elementos no solo da região foi influenciada pelo pH, teor de matéria orgânica e de argila, capacidade de troca catiônica (CTC) e presença de óxidos de Al, Fe e Mn. Concluiu-se que o fragmento de Campinas foi mais exposto à poluição de elementos traço no solo do que os outros fragmentos, considerando as concentrações totais e disponíveis destes elementos, atributos físicos e químicos do solo e a estimativa dos níveis de contaminação. Folhas de *Croton floribundus* e, em menor grau, de *P. gonoacantha*, demonstraram maior habilidade em acumular a maioria dos elementos analisados do que as folhas de *A. graveolens*. Durante a estação seca, foram obtidos os maiores teores foliares de grande parte dos elementos avaliados em todas as espécies, possibilitando o uso das três para monitoramento da sazonalidade no nível de poluição pelos mesmos. *A. graveolens* foi a espécie mais indicada para discriminar a variação espacial dos elementos potencialmente tóxicos, na Região Metropolitana de Campinas. Análises de agrupamento indicaram que a deposição seca e MP_{10} foram fontes mais importantes dos elementos avaliados para as 3 espécies arbóreas do que o solo e a água de chuva, tanto em Campinas quanto em Paulínia.

Palavras-chave: Floresta Estacional Semidecidual; fragmentos florestais; espécies arbóreas nativas; Região Metropolitana de Campinas; elementos potencialmente tóxicos; biomonitoramento passivo.

Abstract

The atmospheric particulate matter, from natural or anthropogenic sources, may promote an input of potentially toxic elements, such as Cr, Cu, Ni, Pb and Zn, in forest ecosystems through dry and/or wet deposition. They may be accumulated in soil and vegetation, among other ecosystem compartments. Biomonitoring programs of air quality, using plant species, allow to evaluate temporal and spatial distribution of air pollutants and their impact on ecosystems. In theory, these programs might be useful in the Campinas Metropolitan Region (CMR), which houses a great industrial park, including the largest petroleum refinery in the Brazilian territory, significant agricultural activities (especially sugarcane) and intense vehicular traffic. A variety of pollution sources surrounds the remaining forest remnants of the region. The present study was proposed to test the following hypothesis: the forest remnants in this region have received a high input of trace elements, which a spatial and temporal variations in the element input occur; the availability of trace elements in the soil depends on parental material and physical properties of soil; leaf accumulation capacity of trace elements and consequently their potential use in biomonitoring programs differ among native tree species. Therefore, the dry and wet deposition of trace elements was evaluated in the vicinity of two forest remnants (one close to industrial complex in Paulínia and the other close to urban sources in Campinas). Total and available contents of trace elements in soils, as well as the leaf accumulation in the three most abundant tree species (*Astronium graveolens* Jacq., *Croton floribundus* (L.) Spreng. and *Piptadenia gonoacantha* (Mart.) JF Macbr) were also determined in those remnants and in two other forest remnants surrounded by agricultural activities, which are located in Cosmópolis and Holambra cities. The seasonal variation in the studied chemical element concentrations in samples of dry and wet deposition was not clearly defined; however, many elements were measured in higher concentrations in PM_{10} samples during dry season, in both sampling sites. Al and Fe, usually related to soil resuspension, were the most abundant elements in dry and wet deposition. The rainwater in Campinas and Paulínia was moderately enriched by Cu, Pb and Zn. PM_{10} showed moderate enrichment of Cu and high enrichment of Mo, in both areas. There was no seasonal variation in the concentration of trace elements evaluated in the soil.The spatial variation was influenced not only by pollution sources but also by the parental material of soils. The element availabilitywas influenced in this region by pH, organic matter and clay content, cation exchange capacity (CEC) and the presence of Al, Fe and Mn oxides in soils. The forest remnant from Campinas was more exposed to pollution of trace elements in the soil than the other remnants, considering total and available concentrations of studied elements, physical and chemical soil attributes and the soil contamination level estimates. Leaves of *Croton floribundus*, followed by those of *P.gonoacantha* revealed a greater ability to accumulate most of the elements analyzed than leaves of *A. graveolens*. The highest foliar concentrations of the most trace elements occurred in all species during the dry season, allowing their use to detect seasonal variations in trace element pollution by analyzing leaf concentrations. *A. graveolens* proved to be the most suitable species to discriminate spatial variation of potentially toxic elements in Campinas Metropolitan Region. Cluster analysis indicated that dry deposition and PM_{10} were more important sources of trace elements to the three tree species than the rainwater and soil in the forest remnant of Campinas and Paulínia.

Key-words: Semideciduous Forest; forest remnants; tree native species; Campinas Metropolitan Region; potentially toxic elements; biomonitoring.

Prefácio

Esta tese foi dividida em 5 capítulos. O primeiro traz conceitos gerais sobre a poluição atmosférica e suas fontes, com enfoque no material particulado, que pode ser enriquecido por "metais pesados", termo este discutido segundo a literatura atual ainda neste capítulo. Os conceitos ecológicos relacionados ao aporte e deposição desses elementos químicos nos ecossistemas naturais são apresentados, assim como os meios de absorção e possíveis danos à vegetação causados por estes elementos. O capítulo introdutório, é finalizado com aspectos importantes sobre o biomonitoramento e com a apresentação de justificativas, hipóteses e objetivos gerais deste trabalho.

Os capítulos 2 a 4 são prévias de artigos científicos, parcialmente prontos para a submissão às revistas internacionais visando à publicação (Atmospheric Research, Environmental Science and Pollution Research e Ecological Indicators, respectivamente). Por isso são independentes entre si, contendo em cada um deles: resumo, palavras-chave, introdução, material e métodos, resultados, discussão, conclusões e referências bibliográficas. Desta forma, foram redigidos em inglês, embora ainda sem a revisão de um especialista na língua. Cada um destes capítulos se refere a avaliação de elementos químicos marcadores de poluição atmosférica em diferentes matrizes, todas coletadas em fragmentos florestais da Região Metropolitana de Campinas. O capítulo 2 é referente à deposição seca e úmida e teve como colaboradora a Dr^{a} Mirian Cilene Spasiani Rinaldi, pesquisadora do Instituto de Botânica. O capítulo 3 é relacionado ao solo e contou com a colaboração da Dr^{a} Jacinta Enzweiler da Universidade de Campinas (UNICAMP) e do Dr Raphael Bragança Alves Fernandes da Universidade de Viçosa (UFV). O capítulo 4 refere-se às folhas das espécies arbóreas nativas da região (*Astronium graveolens* Jacq., *Croton floribundus* Spreng. e *Piptadenia gonoacantha* (Mart.) J. F. Macbr).

O capítulo 5 traz uma discussão geral, com uma abordagem integrada de todas as matrizes avaliadas, a fim de compreender a origem dos elementos químicos observados nas folhas das espécies arbóreas estudadas. Ao final deste capítulo são apresentadas as conclusões gerais do estudo.

Capítulo 1

Introdução geral

Conceitos gerais

Poluição atmosférica pode ser definida como a presença na atmosfera de substâncias ou energia em tal quantidade e duração que podem causar danos à vida, estruturas e materiais construídos pelo homem ou provocar mudanças metereológicas e/ou climáticas (Yang & Omaye 2009). É um grave problema ambiental enfrentado principalmente por áreas extremamente populosas e industrializadas no mundo (Carreras & Pignata 2001; Kumar et al., 2015).

Os poluentes atmosféricos podem ser emitidos por fontes naturais, como vulcões, queimadas espontâneas, por organismos anaeróbios ou por fontes antrópicas, como fertilizantes, pesticidas, água de irrigação contaminada, queima de biomassa, combustão de carvão e óleo, queima de combustíveis por emissões veiculares, incineração de resíduos urbanos e industriais e principalmente mineração, fundição e refinamento (Freedman 1995; Leyval et al., 1997; Monks et al. 2009; Morselli et al., 2003). No entanto, desde a Revolução Industrial, o ambiente tem sido submetido constantemente à emissão e deposição de elementos químicos de origem antrópica. Conseqüentemente, alguns desses elementos têm-se acumulado nos ecossistemas, tanto terrestres como aquáticos, em maiores proporções do que esperado, colocando em risco toda a biota, dos produtores aos consumidores, devido ao seu potencial tóxico (Adriano et al., 2004; Bini & Bech 2014).

O material particulado atmosférico é um dos constituintes mais importantes da poluição atmosférica (Gioda et al., 2011), uma vez que pode conter elementos tóxicos, como metais pesados, cujo lançamento no ambiente tem aumentado muito nas últimas décadas (Bini & Bech 2014).

Têm sido chamados de metais pesados os metais ou metalóides com densidade relativamente alta (> 5 g cm^{-3}), especialmente metais de transição como Pb, Cd e Hg, devido ao grau de toxicidade. Alguns estudiosos consideram este termo impreciso e inapropriado, uma vez que tende a ser usado de forma pejorativa, relacionando-os sempre à idéia de contaminação e prejuízo da qualidade ambiental (Alloway 2013; Kabata-Pendias 2010).

No entanto, cabe lembrar que alguns destes chamados metais pesados são micronutrientes necessários para o crescimento das plantas, como zinco (Zn), cobre (Cu), manganês (Mn), níquel (Ni) e cobalto (Co), e são requeridos em pequenas quantidades (ao contrário dos macronutrientes como enxofre, nitrogênio, fósforo e potássio, que são requeridos em maior quantidade). Porém qualquer excesso desses elementos essenciais pode promover respostas biológicas desfavoráveis (Adriano et al., 2004). Por outro lado, elementos como arsênio (As), mercúrio (Hg), prata (Ag), antimônio (Sb), cádmio (Cd), chumbo (Pb) e urânio (U) não têm nenhuma função conhecida como nutriente, podendo ser mais ou menos tóxicos para os organismos (Schützendübel & Polle 2002).

Assim, deu-se preferência neste estudo ao uso do termo elemento traço, que está mais relacionado à abundância na crosta terrestre e nos seres vivos (< 1000 mg kg^{-1}) e é amplamente utilizado, por incluir os metais/metalóides pesados e também os metais e não-metais de baixa densidade como boro (B), iodo (I) e flúor (F), que não necessariamente causam os mesmos problemas ambientais e toxicológicos que os metais pesados (Alloway 2013; Kabata-Pendias 2010). Todos os elementos da tabela periódica são chamados de elementos-traço, excetuando-se oxigênio (O), silício (Si), alumínio (Al), ferro (Fe), cálcio (Ca), sódio (Na), potássio (K), magnésio (Mg), fósforo (P) e titânio (Ti), que, por constituirem mais de 99% do teor de elementos totais da crosta terreste, são chamados de elementos maiores (Alloway 2013).

Os elementos Ba, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Sb, Se, Sr e Zn têm sido chamados de elementos potencialmente tóxicos, uma vez que em altas concentrações podem afetar a qualidade dos ecossistemas e prejudicar os seres vivos (Birani et al., 2015; Davis et al., 1978). Dentre estes elementos, alguns podem ser marcadores de fontes crustais como Fe e Mn, enquanto que Zn, Mo, Sb de fontes veiculares, Cr, Co, Ni e Se de fontes industriais e Cd de atividades agrícolas (Calvo et al., 2013; Celo & Dabek-Zlotorzynska 2010; Nagajyoti et al., 2010).

O aporte, ou seja, a entrada destes elementos nos ecossistemas ocorre por meio da deposição atmosférica (seca e úmida) do material particulado. A contribuição destas diferentes formas de deposição para o fluxo total dos elementos tóxicos nos ecossistemas pode ser bastante significativa em áreas urbanizadas e industrializadas (Oliva & Espinosa, 2007; Olowoyo et al., 2010). A permanência desses elementos nos diferentes compartimentos do ecossistema, como a comunidade vegetal e solo, pode promover efeitos em longo prazo, mesmo depois de interrompidas as emissões de poluentes que os contêm, como a bioacumulação, que é o aumento da concentração de um elemento com a idade ou tamanho do organismo e também como a biomagnificação, que é o aumento do teor de um determinado elemento nos organismos a medida que vai sendo transferido para níveis tróficos superiores (Nagajyoti et al., 2010; Prasad et al., 2001).

A composição e concentração dos elementos traço no solo estão relacionadas tanto ao material de origem (rocha) sobre o qual o solo se formou, quanto à deposição atmosférica seca e úmida provenientes de fontes antropogênicas de poluição, resultantes dos processos de urbanização e industrialização. Uma vez incorporados ao solo, os elementos podem permanecer por vários milhares de anos (Dai et al., 2004; Moraes & Jordão 2002; Nagajyoti et al., 2010).

Os elementos traço no solo podem estar presentes nas formas de íons livres, de complexos solúveis e de íons trocáveis, ou podem também estar ligados a compostos orgânicos, ou formar compostos insolúveis. Podem ainda, fazer parte da estrutura dos minerais silicáticos. A toxicidade destes elementos, depende de sua disponibilidade no solo, ou seja, das frações, cujas formas químicas possam ser absorvidas (forma iônica, quelada ou complexada) por diferentes organismos, como as plantas (Clemente et al., 2003; Dao et al., 2013; Leyval et al., 1997; Zeng et al., 2011).

A disponibilidade destes elementos no solo para as plantas depende de diversos fatores, como pH, teor de matéria orgânica, de argila, de óxidos de ferro e alumínio, capacidade de troca catiônica e especiação química, entre outros (Dai et al., 2004; Zeng et al., 2011).

A absorção radicular é uma das vias de entrada destes elementos nas plantas e pode ocorrer passivamente através do fluxo de massa, que está associado ao gradiente de potencial hídrico provocado pela absorção de água, e por difusão, causada pelo movimento térmico ao acaso dos íons em direção à raiz, em virtude do gradiente de concentração gerado na superfície radicular (Silva et al., 1998). Pode, ainda, ocorrer por transporte ativo, atravessando a membrana plasmática das células epidérmicas radiculares (Lopes et al. 2012).

A absorção foliar é outra via de entrada e depende muito das condições climáticas, da espécie vegetal e respectivo estado fisiológico (Uzu et al., 2010). A morfologia foliar, composição química e estrutural das ceras foliares e a quantidade de tricomas facilitam a captura de material particulado atmosférico, via deposição seca ou úmida nas superfícies externas das cascas e de folhagens e, por consequência, a entrada foliar de elementos traço ali presentes (Cape 2009; Kabata-Pendias 2010; Saebo et al., 2012; Sawidis et al., 2001).

A entrada excessiva de elementos potencialmente tóxicos nas plantas pode resultar em diversas alterações em processos fisiológicos, causadas em níveis celulares/moleculares, como inativações enzimáticas, bloqueio de grupos funcionais de moléculas metabolicamente importantes, deslocando ou substituindo elementos essenciais e rompendo membranas celulares (Hall 2002; Rascio & Navari-Izzo 2011). Uma conseqüência comum da maioria dos estresses bióticos e abióticos, incluindo o excesso destes elementos nas plantas, é o aumento da produção de espécies reativas de oxigênio (EROs), que podem causar a oxidação inespecífica de proteínas e membranas lipídicas ou podem causar danos ao DNA, resultando em desordens no metabolismo, como inibição da fotossíntese e respiração, cloroses, diminuição do crescimento e eventual morte do indivíduo (Gratão et al., 2005; Guala et al., 2010; Gupta et al., 2007; Meers et al. 2005; Schützendübel & Polle 2002).

Algumas espécies vegetais, no entanto, conseguem sobreviver em ambientes com alto teor de elementos tóxicos, pois restringem a entrada excessiva destes elementos (excludentes), ou toleram o estresse causado por eles (tolerantes), imobilizando e/ou compartimentabilizando estes elementos em suas células (Agrawal et al., 2011; Leyval et al., 1997; Martínez-Sánchez et al., 2012) e até mesmo melhorando seu sistema de defesa antioxidante a fim de neutralizar os efeitos do estresse oxidativo (Rascio & Navarro-Izzo 2011).

As plantas tolerantes acumulam estes elementos tóxicos em seus tecidos foliares, sem apresentar nenhuma injúria visível, como necroses e descolorações, sendo chamadas então de acumuladoras ou bioacumuladoras (Figueiredo et al., 2007; Massa et al., 2010). Estas plantas são ferramentas comumente utilizadas no biomonitoramento, que consiste no uso de respostas de plantas ou de associações de plantas a fim de detectar ou predizer mudanças no ambiente e observar essas mudanças em função do tempo (De Temmerman et al., 2004).

O biomonitoramento permite avaliar não somente a distribuição temporal como espacial dos poluentes, possibilitando a avaliação de vários locais simultaneamente, indicando o impacto da poluição do ar em comunidades e ecossistemas, e detectando os efeitos na biodiversidade, além de oferecer suporte e conhecimento científico para estabelecer padrões de qualidade do ar, com base nos valores-limite que causam efeitos sobre as plantas (De Temmerman et al. 2004; Wannaz et al. 2006).

O biomonitoramento pode ser passivo quando plantas são observadas *in situ*, constituindo importante método para o estudo das interações entre plantas e os poluentes em seu ambiente natural, ou pode ser ativo, quando cultivares específicas são levadas de forma padronizada para pontos específicos de monitoramento (De Temmerman et al. 2004; Domingos et al. 2002; Falla et al. 2000).

Programas de biomonitoramento passivo para avaliar a qualidade do ar, têm sido padronizados por órgãos ligados à comunidade européia, tais como ICP – Forests (*International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests*), ICP – Vegetation (*International Co-operative Programme on Effects of Air Pollution on Natural Vegetation and Crops*), que avaliam e monitoram os efeitos dos poluentes sobre florestas e culturas agrícolas da Europa. O nível de contaminação atmosférica por metais pesados tem sido determinado nesses programas por meio de espécies representativas das florestas européias (UNECE 2010).

Justificativas e Hipóteses

Embora pouco estudados, o aporte e deposição de elementos traço nos ecossistemas tropicais/subtropicais, muitas vezes fragmentados, tem ocorrido supostamente em proporções capazes de causar danos à biota, devido ao crescente uso e ocupação do solo pelo homem nestas regiões, que são prováveis fontes de emissão de material particulado no ambiente.

O biomonitoramento passivo, utilizando espécies representativas das florestas como biomonitoras de elementos potencialmente tóxicos, pode vir a ser de grande aplicabilidade no território brasileiro, considerando a alta biodiversidade encontrada nas florestas brasileiras, principalmente quando feito em concomitância com o monitoramento destes elementos em outras matrizes de deposição (solo) e de aporte (água de chuva e material particulado seco). Essa abordagem ecossistêmica pode ser aplicada na Região Metropolitana de Campinas (RMC).

A RMC, localizada no centro-leste do Estado mais populoso do Brasil (São Paulo), possui diversos fragmentos de Floresta Estacional Semidecidual, subtipo de domínio da Mata Atlântica, caracterizada pela alta diversidade de plantas e por apresentar um número de espécies de árvores que perdem parcialmente as folhas durante a estação seca (Freitas et al., 2010; Lira et al., 2012; Oliveira-Filho e Fontes, 2000), que estão cercados por inúmeras fontes de material particulado existentes. A região abrange 20 cidades com uma população total de aproximadamente 3 milhões de habitantes, que ocupam uma área de 3,8 mil km^2. Desde a expansão urbana que ocorreu durante a década de 70, a RMC têm se tornado altamente industrializada. Atualmente a RMC abriga, 13.290 indústrias químicas, agroquímicas e petroquímicas, dentre outro segmentos (http://www.campinas.sp.gov.br), incluindo a maior refinaria de petróleo em território brasileiro. A RMC também apresenta numerosas rodovias com intenso tráfego veicular, com uma frota de aproximadamente 1,3 milhões de veículos (Ueda & Tomaz 2011). Além disso, atividades agrícolas e agroindustriais são bastante expressivas na região. A contaminação do solo e de água subterrânea com substâncias químicas, como pesticidas organoclorados e elementos químicos (principalmente Al, Cr, Fe, Mn e Pb), já foram verificadas na área industrial de Paulínia, sendo também constatados casos de contaminação de moradores da região por As e Pb, todos atribuídos às atividades de uma fábrica de pesticidas que funcionou durante 20 anos (de 1975 a 1995) na região (Suassuna, 2001).

Sendo assim, as hipóteses levantadas neste estudo foram: 1) os fragmentos florestais remanescentes nesta região têm recebido um excesso de elementos traço, contidos no material particulado emitido pelas referidas fontes de poluição; 2) o nível de incorporação dos elementos traço varia espacialmente na RMC, em função da maior ou menor proximidade de fontes de emissões antrópicas específicas; também varia em função da sazonalidade, devido as condições climáticas, especialmente o regime pluviométrico, que condiciona a sazonalidade nas concentrações atmosféricas de material particulado; 3) o nível de disponibilidade de elementos traço no solo pode variar entre os diferentes fragmentos de floresta, devido às diferentes origens e propriedades físicas dos solos descritas anteriormente para a região de estudo por Lopes et al. (2015); 4) a capacidade de acúmulo foliar de elementos químicos e consequentemente seu potencial para o biomonitoramento diferem entre as espécies arbóreas nativas dos fragmentos florestais, devido as variações em suas características morfológicas e fisiológicas.

Objetivos gerais

Visando a testar as hipóteses propostas, este trabalho foi realizado em fragmentos florestais da RMC com os seguintes objetivos gerais:

- Verificar se há variação espacial e sazonal no aporte de elementos químicos marcadores de poluição atmosférica, de origem natural ou antrópica , via deposição seca e úmida;
- Avaliar a poluição potencial do solo por elementos traço nos solos, considerando suas concentrações disponíveis e totais, bem como os atributos do solo (pH, teor de matéria orgânica, de argila e de óxidos de alumínio, ferro e manganês, capacidade de troca de cátions);
- Identificar, entre as três espécies arbóreas representadas por maior número de indivíduos, qual a com maior potencial para biomonitoramento passivo desses elementos na região de estudo.

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Capítulo 2

Atmospheric deposition of major and trace elements in Semideciduous Atlantic Forest remnants, due to land use expansion in São Paulo State, SE Brazil

Capítulo a ser submetido ao periódico Atmospheric Research

Atmospheric deposition of major and trace elements in Semideciduous Atlantic Forest remnants, due to land use expansion in São Paulo State, SE Brazil

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Abstract

Forest ecosystems near urban and industrial areas are constantly exposed to atmospheric deposition of trace and major elements, but few studies have been conducted in the last remnants of Atlantic Forest, surrounded presently by different particulate pollution sources. The main goal of this study was to assess the magnitude of seasonal and spatial dry and wet deposition of major and trace elements from natural and anthropogenic sources next to two Semideciduous Atlantic forest remnants located in an urban area (CA) and in an industrial area (PA) of São Paulo State, enlarging the knowledge on the level of environmental contamination in the Brazilian territory. Samplings of rainwater, dry deposition and PM_{10} were carried out in both sites, during 2012/2013 wet and 2012 dry seasons. Concentrations of major and trace elements (Al, Cr, Cu, Fe, Mn, Mo, Ni, Zn, among others) in rainwater and dry deposition samples were determined using Inductively Coupled Plasma - Optical Emission Spectrometry (ICP OES) and of PM_{10} were determined by energy dispersive X-ray fluorescence spectrometer (EDXRF). The pH of rainwater samples and PM_{10} mass were also determined. Rainwater samples from industrial site (PA) was more acidic than those from the urban site (CA). Al and Fe were the most abundant elements daily deposited through rain and particulate matter. The daily dry deposition flux exceeded daily wet flux of most the elements during all studied period in both sampling sites. Although the seasonal variability in dry and wet deposition was not clearly defined, PM_{10} samples were more concentrated in several major and trace elements during dry season in both sites. Higher element concentrations were evidenced in PM_{10} samples from PA site. The moderate enrichment of rainwater and/or PM_{10} samples by Cu, Pb and Zn and high enrichment in PM_{10} by Mo suggested that these elements originated from anthropogenic sources in both sites, endangering the few remaining Semideciduous Atlantic forest remnants in São Paulo State.

Key-words: major and trace elements; Atlantic forest remnants; wet and dry deposition; PM_{10}; enrichment factor.

Introduction

Major and trace elements composing particulate matter can be emitted to the atmosphere from natural sources, such as soil dust, sea sprays and volcanic activity (Song and Gao 2011; Vuai and Tokuyama 2011). Al, Fe and Mn, abundantly found in Oxisols, for example, prevail in the atmospheric particles associated to natural sources in tropical regions (Farahmandkia et al., 2010; Song and Gao 2011). However, the atmospheric concentrations of many other elements have increased significantly due to anthropogenic sources (Al-Momani 2003; Cong et al., 2010), such as industries and power plants, vehicular traffic, mining operations, waste incinerators (Basak and Alagha 2010; Mijic et al., 2010; Morselli et al., 2003; Song and Gao 2011) and agricultural practices (Nanos and Martín 2012; Sun et al., 2013). Trace elements like Cr, Cd, Cu, Zn, Ba, Pb, V and Ni are usually associated to anthropogenic sources (Mijic et al., 2010).

The atmospheric deposition of these enriched particles on forest ecosystems occur in increasing distances from the emission sources, depending on the particle aerodynamic diameter, meteorological conditions and surface roughness (Kabata-Pendias 2010; Oliva and Mingorance 2004; Saebo et al., 2012). Therefore, long-range transport of these pollutants through the atmosphere and consequent impacts in remote ecosystems has been observed (Cong et al., 2010; Ejelonu 2011; Gandois et al., 2010, Song and Gao 2011; Vuai and Tokuyama 2011).

Particles containing major and trace elements may be incorporated in different compartments of the forest ecosystems by wet and dry deposition and induce toxic effects on the plant community, among other components of biota (Mijic et al., 2010; Morselli et al., 2003). Chlorosis, reduced plant growth, productivity decrease, reduced nutrient uptake and disorders in plant metabolism are among the effects commonly described on the plants (Gratão et al., 2005; Guala et al., 2010; Gupta et al., 2007).

Some studies, including Fornaro and Gutz (2006), Gioia et al. (2010), Mateus et al. (2013) and Quiterio et al. (2005), have focused on the atmospheric deposition of chemical elements in Brazil. However, few studies have been conducted in metropolitan regions, where diversified land uses have polluted the last remnants of forest ecosystems. Although still not assessed, the enhanced dry/wet deposition of major and trace elements due to the expansion of land occupation has supposedly been among the environmental risks posed to Atlantic Forest remnants located in the most developed Brazilian State, where large metropolitan conglomerations are found, such as the Campinas Metropolitan Region (CMR). We may also assume that deposition of major and trace elements varies seasonally as a consequence of the seasonal climate that affect particulate matter concentrations and spatially due to the distribution of specific natural and anthropogenic sources in that region.

So, the present study was proposed aiming to assess the magnitude of seasonal and spatial dry and wet deposition of major and trace elements from natural and anthropogenic sources next to Semideciduous Atlantic forest remnants in São Paulo.

Material and methods

Study Area

The sampling campaigns of dry and wet deposition were performed next to two Atlantic Forest remnants located in Campinas (22˚49'S - 47˚06'W; altitude: 621 m; 0.05 km far from forest) and Paulínia (22°41'S - 47°07'W; altitude: 581 m; 2 km far from forest) cities, indicated as CA and PA respectively in Fig. 1. The natural vegetation of the remnants is classified as Semideciduous forest, a subtype of the Brazilian Atlantic Forest domain, which is characterized by high plant diversity and a number of tree species that partially lose their leaves during the dry season (Oliveira-Filho and Fontes, 2000). The soils of the selected forest remnants are classified as Oxisols, according USDA Soil Taxonomy (Lopes et al., 2015).

The sites belong to the Campinas Metropolitan Region (CMR). This region is located in the central-eastern of São Paulo State, in Southeast Brazil, in an area of 3.8 thousand km² and is inhabited by 2.8 million people (www.campinas.sp.gov.br). Both are inserted in the most urbanized and industrialized portions of CMR, as represented in Fig. 1. The CA site is mainly surrounded by urban areas and exposed to emissions from intense road traffic. The PA site is about 3 km far from the industrial complex that comprehends numerous chemical, agro-chemical and petrochemical industries, including the largest petroleum refinery of the country. In addition, soil and groundwater contamination of toxic chemicals, such as organochlorine pesticides and chemical elements (mainly Al, Cr, Fe, Mn and Pb), as well as blood contamination of residents with As and Pb, were verified in the industrial area of Paulínia during twenty years (from 1975 to 1995) of activities of a pesticide factory

(Suassuna, 2001). CA site is farther from the industries (about 10 km).Both sites are surrounded by extensive lands dedicated to sugarcane production, among other crops, as represented in Fig. 1.

The predominant climate in the CMR is Cwa (humid subtropical zone with dry winter and hot summer), according to Koeppen’s classification (Alvares et al., 2013). It is characterized by a hot and rainy season between October and March and a dry season between April and September, as shown in the climate diagram included in Moura et al. (2014). The wind speed ranges from 0 to 9.1 m s^{-1} and the wind dominant direction is SE–NW (Fig.1).

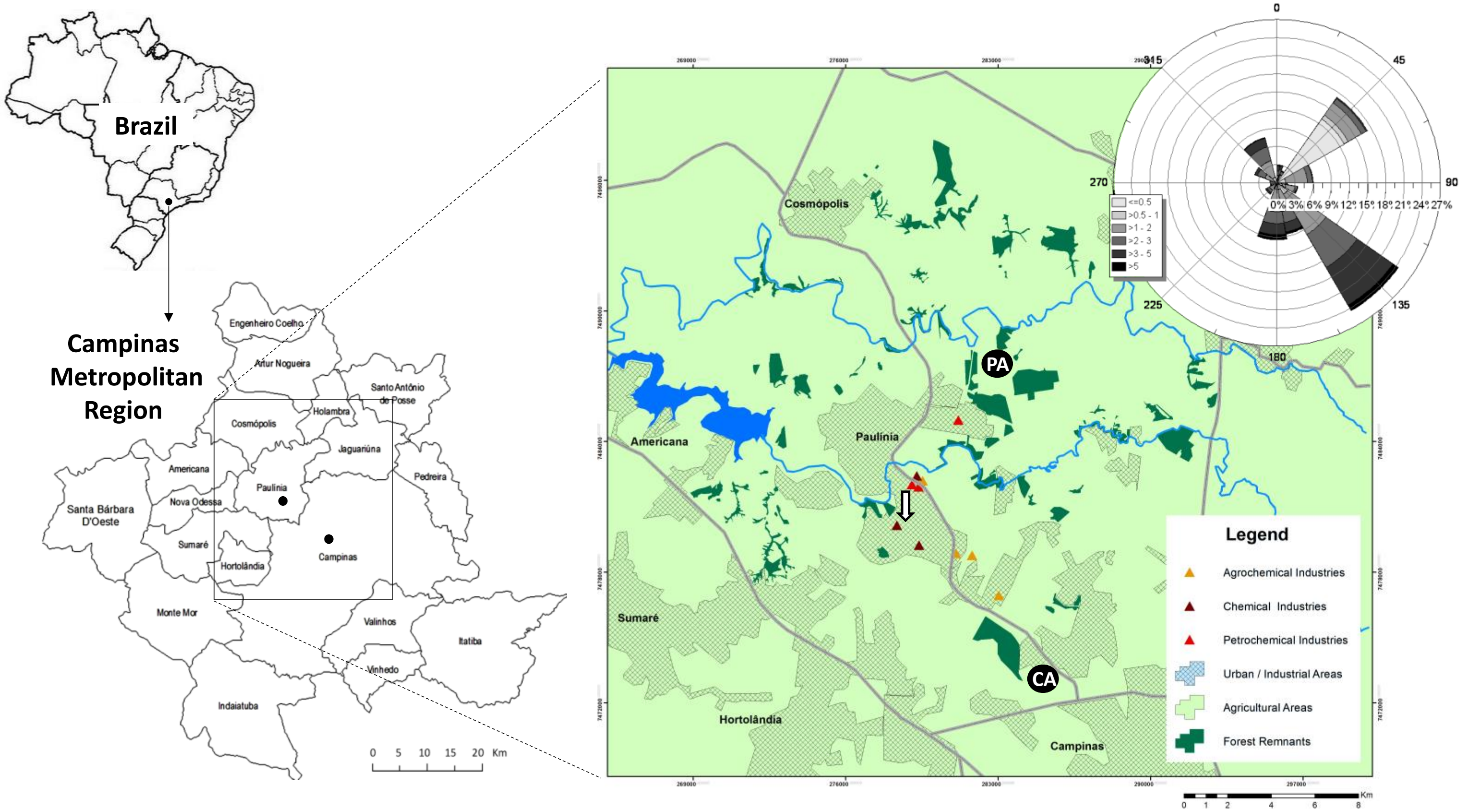

Figure 1. Map showing the sampling sites, located in Paulínia (PA) and Campinas (CA) cities respectively, belonging to Campinas Metropolitan Region, São Paulo State of Brazil (modified from Domingos et al., 2015). (data of winds obtained from the air quality monitoring station of Environmental Company of São Paulo State (CETESB) installed in Paulínia city and downloaded from www.cetesb.sp.org.br - arrow symbol).

Sampling and chemical analysis

Wet and dry deposition samples

The rainfall events and dry deposition were concurrently sampled in each site using an atmospheric precipitation sampler (APS) designed by Loda Etretonics Company, which was 1.3 m high from the ground (Fig. 2). These samplers contained a precipitation sensor that allowed the collection of wet and dry deposition separately. The container for dry deposition collections was lined with a Teflon bag to avoid the loss of particles. The sampling campaigns were performed during two wet seasons (from January to March 2012 and 2013) and one dry season (from June to October 2012).

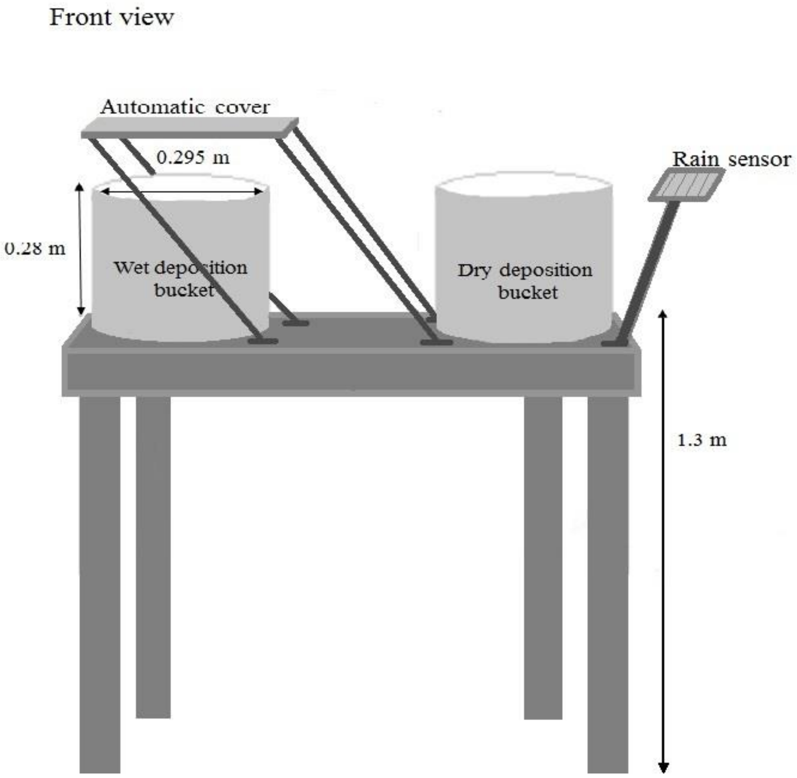

Figure 2. Illustration of the atmospheric precipitation sampler

Fourteen and 34 rainwater samples were obtained during wet seasons and 7 and 9 during dry season in CA and PA, respectively.

The unfiltered rain samples were immediately acidified with extra pure nitric acid, reaching pH ≤ 2, in plastic bottles and then kept at 4˚C, following the protocol proposed by EPA (1982). Aliquots of the non-acidified rainwater were used to determine the pH values.

The Teflon bag used for dry deposition samplings were removed at the end of each season, resulting in one sample from each season in CA and three samples in PA (two of them collected during the wet seasons and one during the dry season). The bags were maintained at room temperature, washed with 800 mL of HNO_3 solution (10%) just before analyses and the resulting solution was evaporated up to reach a volume of 10 mL. This final solution and rain samples were acid digested according US EPA method 3050B (1996) and 3005A (1992), respectively. Certified reference materials were used to ensure quality control.

The concentrations of Al, As, B, Ba, Cd, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sr, Tl, V and Zn in both materials were determined by using Inductively Coupled Plasma - Optical Emission Spectrometry (ICP OES) (US EPA method 6010C - 2007). Their detection limits, in µg L^{-1}, were 18.2, 1.89, 10.8, 0.43, 0.6, 0.28, 0.85, 25, 0.7, 1.05, 1.92, 0.87, 2, 1.85, 0.69 and 0.76 respectively.

All plastic recipients were cleaned with 10% HNO_3 solution to avoid any metal contamination.

PM_{10} samples

Sampling of PM_{10} occurred during 4-8 days in 2012 wet season (January to March) only in PA sampling site. In 2013 wet season (January to March) and 2012 dry season (June to August), sampling of PM_{10} occurred in both study areas not concurrently. The sampling periods of PM_{10} coincided with those of wet and dry deposition.

Each PM_{10} sample from both sites was obtained by using a high-volume sampler (AGVPM10, Energética), which measured the amount of ambient air that passed during 24 hours through a quartz microfiber filter (515 cm^2, Whatman). Five and 13 PM_{10} samples were collected during wet seasons and 5 and 4 samples during the dry season in CA and PA, respectively. All filters were weighed before and after samplings to gravimetric analyses.

Pieces of 3 cm^2 were cut from these sampled filters for analyses of Al, Br, Ca, Cl, Cr, Cu, Fe, K, Mg, Mn, Mo, Ni, S, Sr, Ti, Zn and Zr by energy dispersive X-ray fluorescence spectrometer (EDX 700 HS, Shimadzu). The detection limits were calculated according to the volume of each sample and the minimum limits were, in $\mu g\ m^{-3}$, 0.02 for Al and Mg; 0.0002 for Br; 0.008 for Ca; 0.001 for Cl, Cu, Fe, Sr, Ti, Zn and Zr; 0.0005 for Cr, Mn; 0.003 for K; 0.01 for Mo; 0.0003 for Ni and 0.002 for S.

Data presentation and statistical methods

The elements that were detected in less than 2 samples (wet/dry deposition and PM_{10}) were disregarded.

The volume weighted mean (VWM) concentration for all detected elements and pH was calculated, according to Liptzin et al. (2013) as:

$$\mathbf{VWM} = \sum^{n} \mathbf{C_i V_i} / \sum^{n} \mathbf{V_i}$$

Where C_i is the concentration from the event i, V_i is the volume of precipitation from the event i, and n is the number of samples during the study period.

The detection frequency of each element in the rainwater and PM_{10} samples (N of samples in which the element was detected/total N of samples) were also estimated.

Daily wet and dry deposition fluxes during both seasons were calculated, by dividing the concentration of each element in the rain or particulate sample by the number of days in the sampling period and by the collector area, following method described by Birgül et al. (2011) and Cong et al. (2010). The daily wet and dry deposition fluxes were expressed as µg

m^{-2} d^{-1} or mg m^{-2} d^{-1}. The ratios between daily dry and wet deposition fluxes were also calculated.

A two-way variance analysis, followed by multiple comparison analysis (Holm-Sidak test), when necessary, were conducted to evaluate the significance of differences in rainwater pH and elements encountered in rainwater and PM_{10} among sampling sites (factor 1) and seasons (factor 2). Interactions of both factors were also tested. Data were $\log_{10}$, rank or square root transformed, when necessary to satisfy assumptions of normality and equal variances. These statistical analysis were performed using Sigma Plot software 11.0.

Factor analysis (FA) was used to examine the correlation between the elements analyzed in PM_{10}, employing Principal Components Method and raw Varimax rotation. Only factors extracted with eigenvalues > 1.0 and factor loadings greater than 0.7 were considered. This analyses was carried out using STATISTICA software. The data was previously standardized.

The enrichment factor (EF), a measurement of the accumulation level of metals in the environment according to many authors (Kara et al., 2014; Kim et al. 2012; Lynam et al. 2015; Odabasi et al. 2002; Özsoy and Örnektekin 2009; Song and Gao 2011), was calculated using the equation below. This ratio helped to determine which elements in PM_{10} and rainwater were from anthropogenic sources and which elements were from natural sources.

$$\mathbf{EF_i = [(C_i/C_{ref})]_{rain\ or\ PM10} / [(C_i/C_{ref})_{soil}]}$$

Where: EF_i is the enrichment factor of trace element i, C_i is the element concentration in PM_{10} or rainwater. Crustal elements like Al, Fe, Li, Mn, Sc, Ti, or Zr have been used as the reference element. In the present study C_{ref} is the reference concentration of Fe in soils of Campinas Metropolitan Region (CMR). The soil concentrations of Fe were obtained from

Fadigas et al. (2006), considering that soils of CMR were classified as Oxisol according Lopes et al. (2015).

The reference concentrations of Ba, Cr, Cu, Mo, Ni, Pb and Zn in soils from São Paulo State were obtained in a guideline published by the Environmental Company of São Paulo State (CETESB, 2014). EFs < 10 indicate no enrichment by anthropogenic sources; $10 <$ EFs < 100 indicate moderate anthropogenic enrichment, and elements with EFs > 100 indicate highly anthropogenic enrichment (Farahmandkia et al., 2010; Özsoy and Örnektekin 2009).

The nonparametric Mann–Whitney rank sum test ($p < 0.05$), using SIGMA PLOT (Version 11.0), was used to compare EF of rainwater and EF of PM_{10} between the sampling sites (CA and PA).

Results

General characterization of environmental conditions during the sampling period

The accumulated rainfall was 1113 and 1049 mm during the wet seasons (2012/2013 Jan-Mar) and 301 and 332 mm during the dry season (2012 Jun-Oct) in CA and PA, respectively. The average PM_{10} levels were higher in PA than in CA during the dry season, ranging from 26 and 23 µg m^{-3} in the wet seasons (2012/2013 Jan-Mar) to 36 and 39 µg m^{-3} in the dry season (2012 Jun-Oct) in CA and PA, respectively. The highest monthly means of PM_{10} were observed in September/2013 (CA: 46 µg m^{-3}; PA: 53 µg m^{-3}) (Fig. 3; data from the air quality monitoring station managed by Environmental Company of São Paulo State, available in www.cetesb.sp.org.br).

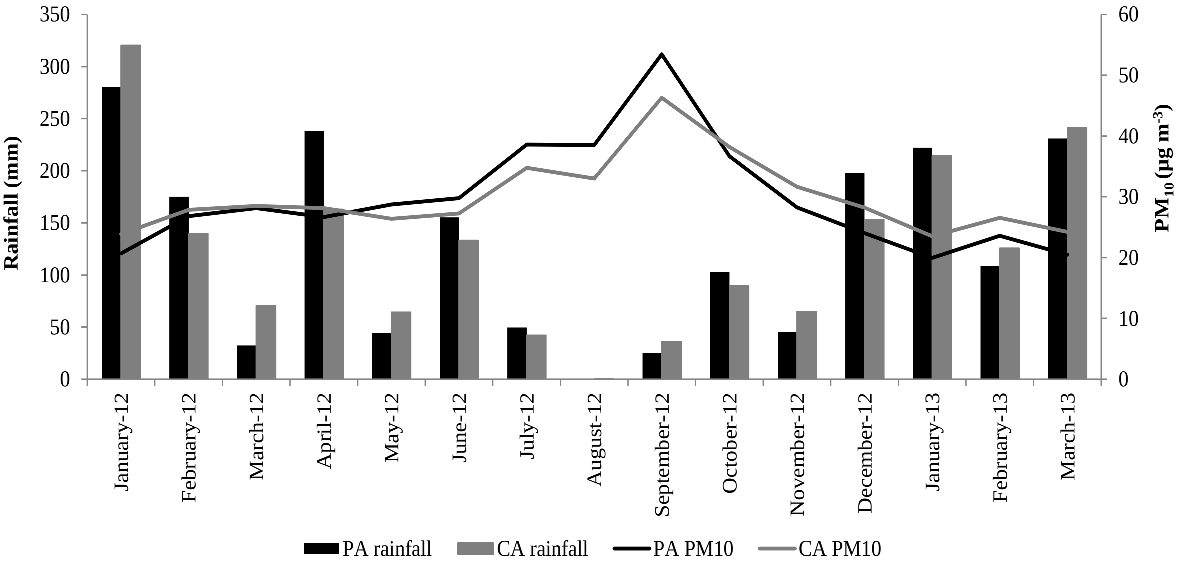

Figure 3. Monthly rainfall sum (mm) and average PM_{10} (µg m^{-3}) in PA and CA sampling sites during the study period (data of rainfall of PA was obtained from meteorological station of the region and data of CA from UNICAMP station, downloaded from www.cepagri.unicamp.br). PM_{10} data were obtained from the air quality monitoring station of Environmental Company of São Paulo State (CETESB) installed at Paulínia and Campinas cities and downloaded from www.cetesb.sp.org.br.

Dry and wet deposition in the sampling sites

The rainwater pH ranged from 3.2 to 7.5 in PA and 5.5 to 7.5 in CA, but no significant differences between studied areas and seasons were proved. Volume weighted mean pH (VWM pH) was 6.3 and 4.2 during the wet seasons and 6.7 and 5.2 in the dry season for CA and PA, respectively (Table 1).

Al, Ba, Cr, Cu, Fe, Mn and Zn concentrations were above the analytical detection limits in 10 to 100% of rainwater samples collected in all seasonal campaigns. Detectable levels of Sr were not only found in CA during the wet season. Mo and Pb appeared in 10 to 30% of samples from the dry period in both sites. Ni and V were rarely detected in the rain samples. In both sampling sites, Fe was the most concentrated element in rainwater, followed by Al and Zn. Cr was the least abundant element in this study (Table 1).

No interactions were found between both factors (study areas and seasons). The levels of major and trace elements in rainwater did not differ significantly between both sampling sites. The seasonal effect (factor 2) was observed for few elements. Rainwater samples collected during wet seasons was more concentrated in Cr than those from the dry season. The opposite condition was observed for Mn and Zn concentrations that were higher during dry season (Table 1).

Table 1. VWM - volume-weighted mean, Med - median, Min - minimum , Max - maximum concentrations (µg L^{-1}) and Freq. - detection frequency (%) of trace elements in rainwater sampled in Campinas (CA) and Paulina (PA) sites, during wet (2012-2013) and dry (2012) seasons. *Detected in no more than 2 samples during the season. Distinct lower case letters indicate significant differences ($p< 0.05$) among seasons, in each sampling site. The absence of letters means that average values did not differ significantly among seasons, sampling sites and also indicate that there was no interaction between these factors.

Sites	**Seasons**		**Al**	**Ba**	**Cr**	**Cu**	**Fe**	**Mn**	**Mo**	**Ni**	**Pb**	**Sr**	**V**	**Zn**	**pH**
CA	**wet**	VWM	56	6.0	3.5	2.5	82	4.2	3.2	5.5	7.3	–	–	9.5	6.3
		Mean	54	8.0	2.8 **a**	3.5	82	4.7 **b**	3.2	5.2	15	–	–	13.2 **b**	6.7
		Med	53	4.5	1.0	2.8	68	3.3	2.4	3.9	1.8	–	–	10.5	6.8
		Min	19	0.7	0.7	1.2	34	1.2	1.1	2.0	1.3	–	–	3.5	5.5
		Max	112	21	12	9.5	173	13	6.8	11	55	–	–	32	7.4
		Freq.	36	100	43	86	43	100	29	29	29	*	*	100	–
	dry	VWM	53	3.6	0.6	2.0	60	12.6	–	–	–	9.7	–	26	6.7
		Mean	120	7.3	0.7 **b**	4.5	117	34 **a**	–	–	–	19	–	30 **a**	6.8
		Med	28	3.8	0.5	1.3	47	9.8	–	–	–	2.4	–	17	6.9
		Min	18	1.6	0.4	0.9	28	1.7	–	–	–	2.1	–	6.7	5.8
		Max	618	34	1.6	21	482	193	–	–	–	53	–	111	7.5
		Freq.	100	100	100	86	86	100	*	*	*	43	*	100	–
PA	**wet**	VWM	22	2.0	0.9	1.8	49	2.5	2.9	–	1.6	2.8	–	6.9	4.2
		Mean	23	2.5	1.2 **a**	2.1	43	3.0 **b**	3.3	–	1.7	2.6	–	10 **b**	5.8
		Med	23	1.3	0.8	2.0	39	2.2	2.0	–	0.9	2.5	–	7.3	6.2
		Min	21	0.5	0.6	1.0	26	0.9	1.4	–	0.9	2.3	–	2.3	3.2
		Max	24	17	5.1	4.6	74	11	6.6	–	3.2	3.0	–	85	7.5
		Freq.	9	94	32	76	44	94	21	*	9	9	*	100	–
	dry	VWM	129	4.0	0.9	2.2	114	9.9	–	–	–	5.2	1.7	39	5.2
		Mean	297	5.0	0.9 **b**	2.6	298	18 **a**	–	–	–	11	2.9	35 **a**	6.6
		Med	96	2.4	0.6	1.8	61	7.5	–	–	–	11	1.7	37	7.0
		Min	19	0.6	0.3	1.1	36	2.6	–	–	–	3.4	1.3	3.1	4.5
		Max	1273	17	3.0	5.3	1532	99	–	–	–	19	5.6	72	7.4
		Freq.	70	90	100	60	80	100	*	*	*	20	30	100	–
p1 (factor 1)			0.690	0.144	0.791	0.530	0.954	0.274	0.950	—	0.389	0.755	—	0.863	0.327
p2 (factor 2)			0.138	0.063	**0.004**	0.466	0.147	< **0.001**	—	—	—	0.437	—	< **0.001**	0.072
p3 (factor 1 x 2)			0.065	0.345	0.241	0.161	0.073	0.533	—	—	—	0.817	—	0.233	0.311

p1< 0.05: indicates significant effects of factor 1 (forest remnants); **p2< 0.05:** indicates significant effects of factor 2 (seasons); **p3< 0.05:** indicate significant interactions between factor 1 and 2.

Al and Fe were daily deposited in higher proportions than the other elements, in both areas and seasons, via dry deposition/DDF (Campinas/CA: > 2.5 mg Al m^{-2} d^{-1}; > 1.5 mg Fe m^{-2} d^{-1}; Paulínia/PA: > 7 mg Al m^{-2} d^{-1}; > 3 mg Fe m^{-2} d^{-1}). Considering wet deposition fluxes (WDF), Fe was the most abundant element in Paulínia/PA during both rainy (0.07 mg m^{-2} d^{-1}) and dry seasons (0.11 mg m^{-2} d^{-1}). Enhanced WDF of Fe was also evidenced in Campinas/CA during wet season (0.05 mg m^{-2} d^{-1}) and of Fe and Al during the dry season (0.03 mg m^{-2} d^{-1}). The DDF of Mn (>100 μg m^{-2} d^{-1}) in both seasons and WDF of Pb (>20 μg m^{-2} d^{-1}) during the rainy season were also evidenced in CA. DDF (>80 μg m^{-2} d^{-1}) and WDF (> 30 μg m^{-2} d^{-1}) of Zn were also detached in both seasons in PA. The DDF of Al and Fe, in both seasons, seemed to be higher in PA than in CA, while DDF of Mn appeared to be higher in CA (Table 2).

All elements were preponderantly deposited by means of DDF (D/W > 1; Table 2), during both seasons and sampling sites, except Mo, during rainy season in both sites, when WDF surpassed DDF (D/W < 1).

Table 2. Daily fluxes of dry (DDF) and wet (WDF) deposition and ratio between dry and wet deposition flux (D/W) of trace elements, during the dry and wet seasons in Campinas (CA) and Paulínia (PA) sites.

	CA						**PA**					
	wet season			**dry season**			**wet season**			**dry season**		
	DDF	**WDF**	**D/W**	**DDF**	**WDF**	**D/W**	**DDF**	**WDF**	**D/W**	**DDF**	**WDF**	**D/W**
Al	2.6	0.02	115	2.8	0.03	103	8.1	0.01	1112	7.5	0.08	91
As	0.4	—	—	0.2	—	—	0.8	—	—	0.5	—	—
Ba	31.5	12.1	2.6	54.2	1.8	29	13.8	8.1	1.7	18.5	4.1	4.5
Cd	0.7	—	—	0.11	—	—	1.0	—	—	0.3	—	—
Cr	12.5	5.2	2.4	7.3	0.3	24	10.4	0.6	16	8.7	0.9	10
Cu	13.7	4.5	3.1	17.2	0.9	19	11.7	4.9	2.4	11.7	1.3	9.3
Fe	1.8	0.05	37	1.6	0.03	58	4.1	0.07	56	3.3	0.11	31
Mn	139	10.5	13	134	6.4	21	37.5	9.8	3.8	59.0	10.2	5.8
Mo	0.6	3.3	0.2	0.2	—	—	0.3	3.0	0.1	0.2	—	—
Ni	6.1	2.2	2.8	3.7	—	—	4.8	—	—	4.2	—	—
Pb	20.9	11.2	1.9	6.3	—	—	13.3	2.0	6.5	3.8	—	—
Sr	—	—	—	—	1.2	—	—	0.6	—	—	0.8	—
V	4.8	—	—	5.3	—	—	21.4	—	—	23.9	0.8	32
Zn	203	27.4	7.4	45.2	13.0	3.5	302	30.6	9.8	82.4	39.8	2.1

DDF, WDF and TDF of Al and Fe were represented in mg m^{-2} d^{-1}, while others elements were in µg m^{-2} d^{-1}.

PM_{10} deposition in the sampling sites

All elements were measured over the detection limits in the majority of PM_{10} samples (frequency detection between 60% and 100%). Interaction between both factors (sites and seasons) was only observed for PM_{10}, K and Mn levels. No significant differences were proved among seasons and sites for Br, Cl, Cu, Mg, Mo, S, Sr, Zn and Zr (Table 3).

Significantly higher concentrations of PM_{10}, Al, Ca, Fe, Ti and Mn were verified during dry season than during wet season next to the Paulínia industrial complex (PA site). Similar seasonal differences were proved in Campinas (CA site) for Al, Ca, Fe and Ti.

Al, Fe, Ti and Cr concentrations in PM_{10} were significantly higher in PA than in CA, during both seasons. Similar spatial differences were proved for PM_{10}, K and Mn during the dry season.

Only two samples, one in CA during 2013 wet season (151 µg m^{-3}) and other in PA during 2012 dry season (145 µg m^{-3}), exceeded the daily limit (120 µg m^{-3}) proposed by CETESB (2013), which should not be exceeded more than once per year.

The total mass of analyzed elements accounted for an average of 12.6 % and 18.7 % of PM_{10} mass, in CA and PA respectively (Fig. 4). More than a half of these proportions were due to the sum of the most abundant elements (Al, Fe and Mg) observed in PM_{10} in both sites. Some elements contributed less than 0.1% to these results, such as Br, Cr, Mn, Ni, Sr, Zn and Zr in Campinas and Br, Cl, Cr, Mn, Sr and Zr in Paulínia.

Table 3. Med - median, Min - minimum, Max - maximum concentrations and Freq - detection frequency (%) of elements in PM_{10} sampled in Campinas (CA) and Paulínia (PA) sites, during wet (2012-2013) and dry (2012) seasons. * Detected in no more than 2 samples during the season. Distinct lower case letters indicate significant differences ($p < 0.05$) among seasons, in each sampling site. Distinct capital letters indicate significant differences ($p < 0.05$) among sampling sites in the same season. The absence of letters means that average values did not differ significantly among seasons, sampling sites and also indicate that there was no interaction between these factors.

Sites	**Seasons**		**PM_{10}**	**Al**	**Ca**	**K**	**Fe**	**Mg**	**S**	**Ti**	**Br**	**Cl**	**Cr**	**Cu**	**Mn**	**Mo**	**Ni**	**Sr**	**Zn**	**Zr**
			µg m^{-3}								**ng m^{-3}**									
CA	**wet**	Mean	53 **Aa**	1.80 **Bb**	0.25 **Ab**	0.41 **Aa**	0.36 **Bb**	1.31	0.35	0.03 **Bb**	4.0	223.3	1.9 **Ba**	79.9	—	126.8	—	8.1	43.0	—
		Med	29	1.01	0.16	0.24	0.14	0.83	0.15	0.01	4.1	30.3	1.4	55.0	—	81.5	—	4.6	11.1	—
		Min	16	0.62	0.01	0.10	0.06	0.30	0.07	0.004	0.8	6.3	0.9	33.9	—	26.2	—	2.3	0.9	—
		Max	151	5.34	0.70	1.25	1.26	4.15	1.09	0.11	7.0	836.8	3.4	202.7	—	318.0	—	20.9	133.8	—
		Freq.	—	100	100	100	100	100	100	100	100	100	60	100	*	80	*	80	100	*
	dry	Mean	27 **Ba**	1.99 **Ba**	0.46 **Aa**	0.26 **Ba**	1.10 **Ba**	0.56	0.17	0.15 **Ba**	1.9	56.5	2.0 **Ba**	100.8	9.4 **B**	35.1	0.8	5.8	14.2	4.0
		Med	25	1.56	0.35	0.23	0.82	0.52	0.18	0.10	1.9	49.8	1.8	101.1	8.0	34.3	0.6	5.1	12.8	2.6
		Min	18	1.30	0.23	0.18	0.57	0.33	0.13	0.08	0.0	6.8	0.9	74.1	4.2	18.9	0.5	1.7	2.4	0.9
		Max	43	4.08	0.88	0.42	2.51	0.93	0.25	0.37	3.4	119.5	3.9	128.5	19.7	52.8	1.2	11.3	30.8	10.0
		Freq.	—	100	100	100	100	100	100	100	100	80	100	100	100	80	60	80	100	80
PA	**wet**	Mean	31 **Ab**	2.41 **Ab**	0.23 **Ab**	0.20 **Ab**	0.82 **Ab**	0.98	0.20	0.16 **Ab**	3.5	24.1	19.5 **Aa**	45.5	6.7 **b**	607.2	64.4	3.9	347.2	5.9
		Med	23	1.88	0.19	0.19	0.63	1.12	0.16	0.08	2.9	18.6	16.2	27.5	4.9	73.2	80.6	4.7	42.0	3.8
		Min	13	0.67	0.05	0.09	0.11	0.33	0.06	0.01	0.6	4.0	1.0	7.2	1.4	16.0	27.8	1.0	1.1	0.9
		Max	77	6.91	0.71	0.40	3.08	1.51	0.57	0.58	7.8	57.4	42.8	103.9	17.6	1841.9	92.7	5.4	1043.4	19.3
		Freq.	—	100	100	100	100	100	100	100	77	46	62	77	69	85	38	38	85	46
	dry	Mean	98 **Aa**	7.22 **Aa**	1.20 **Aa**	0.71 **Aa**	5.28 **Aa**	1.24	0.41	0.77 **Aa**	7.0	39.6	7.1 **Aa**	75.6	40.5 **Aa**	34.5	—	14.4	49.7	18.7
		Med	91	7.28	1.08	0.75	4.93	1.23	0.41	0.72	7.2	39.8	6.2	76.0	39.0	40.0	—	13.9	59.9	18.5
		Min	63	4.55	0.79	0.32	3.31	0.76	0.05	0.48	3.8	4.4	4.9	13.9	19.4	15.5	—	7.3	22.6	9.7
		Max	145	9.78	1.86	1.02	7.94	1.73	0.75	1.16	9.8	74.5	11.3	136.5	64.5	48.0	—	22.4	66.6	28.2
		Freq.	—	100	100	100	100	100	100	100	100	100	100	100	100	75	*	100	75	100
p1 (factor 1)			0.053	**0.002**	0.106	0.294	**0.003**	0.107	0.745	< **0.001**	0.103	0.500	**0.007**	0.078	0.442	0.619	–	0.494	0.177	0.059
p2 (factor 2)			0.088	**0.005**	< **0.001**	**0.024**	< **0.001**	0.826	0.641	< **0.001**	0.707	0.797	0.756	0.174	0.085	0.072	–	0.136	0.321	0.106
p3 (factor 1 x 2)			**0.005**	0.096	0.329	**0.013**	0.419	0.176	0.385	0.056	0.107	0.743	0.685	0.930	**0.019**	0.578	–	0.077	0.541	0.458

p1< 0.05: indicates significant effects of factor 1 (forest remnants); **p2< 0.05:** indicates significant effects of factor 2 (seasons); **p3< 0.05:** indicate significant interactions between factor 1 and 2.

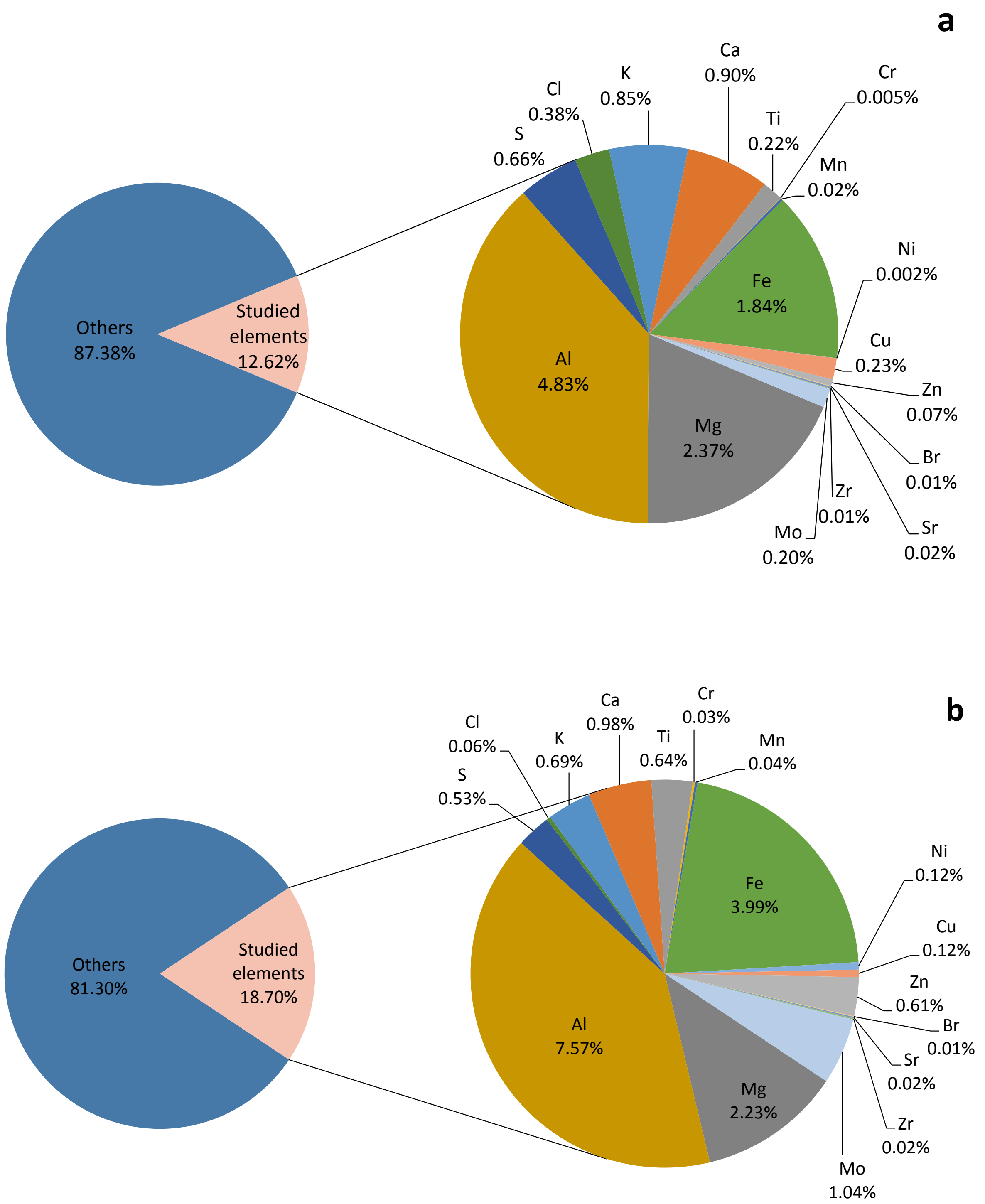

Figure 4. Proportional contribution of major and trace elements and other non-analyzed components to the average total mass of PM_{10} in CA (a) and PA (b) during all studied period.

Factor analysis indicated that approximately 93% of the variance was explained by the first three factors (Table 4). Al, Ca, Ti, Mn and Fe were uploaded in factor 1 (56% of total variance), Cr and Zn in factor 2 (21% of total variance) and Mg, S, K and Cu in factor 3 (16% of total variance).

Table 4. Factor loadings (varimax raw rotation) for major and trace elements in PM_{10} samples from Paulínia (PA) and Campinas (CA) sampling sites during the study period. Those components with a factor loading > 0.7 are shown in bold.

	Factor 1	**Factor 2**	**Factor 3**
Mg	0.017	0.134	**0.908**
Al	**0.887**	0.118	0.366
S	0.404	0.042	**0.877**
K	0.536	-0.142	**0.782**
Ca	**0.919**	-0.218	0.285
Ti	**0.987**	0.122	0.044
Cr	0.031	**0.995**	0.011
Mn	**0.956**	-0.125	0.161
Fe	**0.987**	-0.099	0.105
Cu	0.059	-0.028	**0.850**
Zn	-0.128	**0.987**	0.050
Br	0.634	0.364	0.560
% of variance	56	21	16

Enrichment Factor of rainwater and PM_{10} samples

The enrichment factor of rainwater ($EF_{rainwater}$) was lower than 10 for Mn and Cr (in both sampling sites) and for Ba (in PA), indicating that the rainwater was non-enriched with these elements by anthropogenic sources. The $EF_{rainwater}$ of Ba (in CA), Cu, Pb and Zn (in both sites) were above 10 and below 100, indicating a moderate anthropogenic enrichment. $EF_{rainwater}$ for all elements was similar in CA and PA sites (Fig. 5a).

The enrichment of PM_{10} (EF_{PM10}) with Mn, Cr and Zn, in both study sites, was below 10, indicating that PM_{10} was not enriched with these elements by anthropogenic emissions.

The EF_{PM10} of Cu was between 10 and 100, in CA and PA, representing a moderate enrichment. EF_{PM10} of Mo was above 100, revealing a high enrichment by anthropogenic sources. Only the EF_{PM10} of Cu was significantly higher in samples from CA than from PA (Fig.5b).

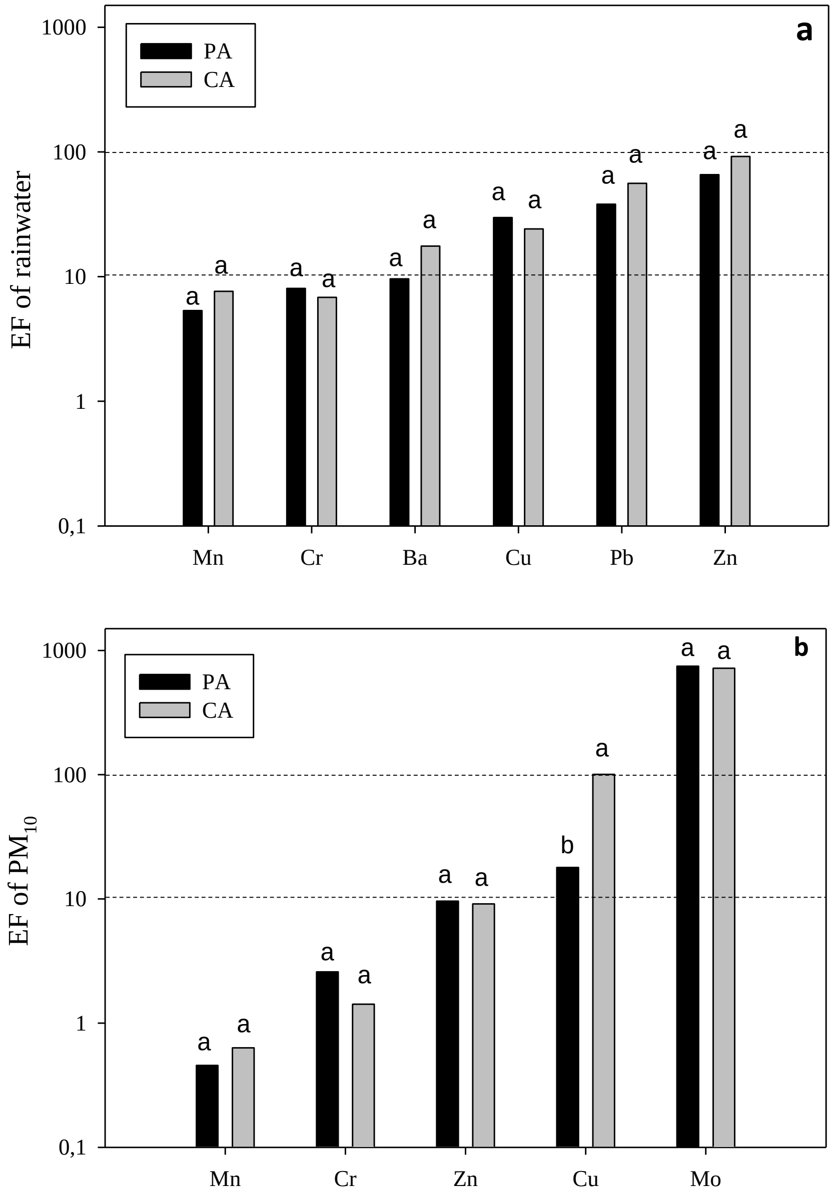

Figure 5. Medians of enrichment of elements in rainwater (a) and PM_{10} (b) samples from CA and PA during all the studied period. Scale of y axis was adjusted by $\log_{10}$ to facilitate the median visualization. Distinct letters indicate significant differences ($p < 0.05$) among sampling sites.

Discussion

The VWM pH of rainwater from the industrial site (PA) in the CMR was similar to that described by Banerjee (2008) in an industrial area of India (VWM pH = 4.54) and by Park et al. (2015) next to industries in Korea (VWM pH= 4.84). The VWM pH observed in our urban area (CA) was higher than that measured in the rainwater collected in urban areas of São Paulo (VWM pH = 5.19) (Leal et al., 2004), but similar to the rainwater pH measured by Mouli et al. (2005) in an urban area of India (VWM pH= 6.78). Values of pH below 5.6, which characterize the acid precipitation, were only measured in 38% and 5% of samples from the industrial (PA) and urban sites (CA), respectively. The rain alkalinization in the urban area may be attributed to the intense agricultural activities that surround both sampling sites, as also mentioned by Cunha et al. (2009) and Uygur et al. (2010). The acidic rain events observed specifically in Paulínia may be possibly associated to the proximity of the sampling location to the largest Brazilian petrochemical industry and to the contribution of secondary aerosols (SO_4 and NO_3).

Al and Fe, the most abundant elements found in the wet and dry deposition and in PM_{10} samples from our study region, were also detected in high proportions by other authors (Contini et al., 2012; Lim et al., 2010; Oliveira et al., 2012; Uygur et al., 2010). These elements possibly originated from re-suspension of soil-dust (Calvo et al., 2013). Zn, which was the only element detected in all rainwater samples, could be associated to anthropogenic sources, taking into consideration the moderate enrichment estimated ($10<EF<100$) and the result of factor analysis. Oliveira et al. (2012) also observed that Zn was one of most abundant elements in soluble fractions of rainwater samples collected in a rural site of São Paulo.

Considering total element concentrations (including soluble and insoluble fractions) measured in the rain events, Hu and Balasubramanian (2003) found lower values of VWM Al and Fe (18.4 and 23.9 µg L^{-1} respectively) in an industrial area of Singapore than the levels observed in our studied sites in both seasons. Conko et al. (2004) also measured lower VWM

Fe (25 µg L^{-1}) in an urban area (USA) than we measured in PA and CA sites and lower values of VWM Al (57 µg L^{-1}) than we observed during dry season in PA. In contrast, almost all trace elements (Al, Cr, Cu, Fe, Mn, Pb, Sr and Zn) in rainwater sampled in PA and CA were measured in lower concentrations than in an urban area of Turkey (Özsoy and Örnektekin 2009). Their measurements of VWM Al and Fe (485 and 743 µg L^{-1} respectively) were about 4-6 times larger than our highest results. The authors discussed that the high concentrations in the urban area in Turkey were consequences of pollution emissions from various industries, such as petroleum refinery, thermal power plant and soda, chromium, fertilizer, cement and glass producers.

Higher contribution of dry deposition to total input of elements was also observed by Sakata and Asakura (2011), at least for Al, Cr, Cu, Mn and Ni. This result might be explained by the fact that the gravitational dry deposition is an efficient process in removing large particles from the atmosphere, due to their shorter atmospheric lifetimes (Grantz et al., 2003; Sakata and Asakura 2011).

In general, the lower ratio between dry and wet deposition fluxes during wet season (mainly in the CA site) may be explained by rainfall increases in rainy periods (> 60 mm) that reduce dry deposition, according to Connan et al. (2013). In addition, finer particles, which are more enriched by trace elements (Lü et al., 2012), are removed more efficiently by wet deposition than by dry deposition because they remain suspended in the atmosphere for longer periods than the coarse particles (Zheng et al., 2005). As a consequence, higher concentrations of major and trace elements in particles with aerodynamic diameter of 10µm or lower are generally registered during dry periods, which seemed to have occurred is the sampling sites of the present study.

The average PM_{10} concentrations next to major industries located in Paulínia city (PA site) during dry seasons (98 µg m^{-3}) was higher than the mean concentrations measured in industrial areas of Korea (Lim et al., 2010), Greece (Manalis et al., 2005) and RJ/Brazil

(Gioda et al., 2011) (87, 55 and 71 µg m^{-3} respectively). This increased levels of PM_{10} may be associated not only to the diverse industrial sources in that location, but also to typical meteorological conditions during the dry season in the CMR, including low temperature and rain precipitation (Fig. 2) that restrain the pollutant dispersion (Kulshrestha et al., 2009).

Factor analysis (FA) applied to elemental composition of PM_{10} permitted to infer about the main sources of major and trace elements analyzed. Factor 1 grouped elements of typical crustal origin (Al, Ca, Ti, Mn and Fe) according Moreno et al. (2006). Factor 2 joined elements from industrial-traffic sources (Cr and Zn) according Mijic et al. (2010) and elements included in Factor 3 (Mg, S, K and Cu) may be related to agricultural practices, among other sources (Christofoletti et al. 2013; Nanos and Martín 2012).

Sugarcane has been extensively cultivated in the studied region. Its fertirrigation has been from vinasse, a liquid by-product of the sugar-ethanol industry, which is acidic and contains high levels of organic matter. Vinasse is characterized by high concentrations of K, SO_4, Mg and Cu (2056, 710, 237 and 0.35 respectively in mg L^{-1}) (Christofoletti et al., 2013).

Enrichment estimates indicated that Mn and Cr in rainwater and PM_{10} samples were not from anthropogenic sources in both sampling sites. Although the $EF_{rainwater}$ in both areas showed a moderate enrichment of Zn, this effect was not evidenced by the EF_{PM10}. As PM_{10} in CA is more enriched by Cu than PA, this trace element, which is a tracer of brake lining and tire wear (Celo and Dabek-Zlotorzynska 2010), seemed to have originated from intense vehicular emissions during the experimental period in Campinas. The $EF_{rainwater}$ showed that only CA is moderately enriched by Ba, which may also be associated to vehicular emissions according to Pant and Harrison (2013). The high enrichment of PM_{10} by Mo in both sampling locations can be from industrial-traffic sources, since this element is used in metallurgy for hardening alloys and emitted through vehicle tailpipes, as it is a component of catalysts (Calvo et al. 2013, Kabata- Pendias 2010).

Conclusions

Rainwater samples from industrial site in the Campinas metropolitan region was more acidic than those from the urban site. Al and Fe were the most abundant elements found in all wet and dry deposition, indicating the important contribution of crustal sources, mainly soil dust to the atmospheric deposition in the study region.

All studied elements, except Mo, were deposited in higher proportions by dry than wet deposition, during both seasons and sampling sites (Campinas/CA and Paulínia/PA).

Although the seasonal variability in the concentration levels of major and trace elements in dry and wet deposition was not clearly defined, PM_{10} samples were more concentrated in both sites during dry season. Spatial variation, mainly concerning PM_{10} deposition, was detached. Higher element concentrations were evidenced in PM_{10} samples from the industrial site.

The close variation over time and in both locations in the Al, Ca, Ti, Mn and Fe levels in PM_{10}, revealed by factor analysis, was an indicative of the contribution of crustal sources in the study region. Similarly, the close associations of Cr and Zn and of Mg, S, K and Cu in PM_{10} indicated the presence of industrial-traffic and agricultural sources of elements respectively.

The moderate enrichment of rainwater and/or PM_{10} samples by Cu, Pb and Zn and high enrichment in PM_{10} by Mo suggested that these elements originated from anthropogenic sources in both sites, endangering the few remaining Semideciduous Atlantic forest remnants in São Paulo State.

Acknowledgments

The authors thank *Programa SISBIOTA-BRASIL* (proc. CNPQ 563335/2010; proc. FAPESP 2010/52319-2) for financial support; CAPES for offering a Ph.D. scholarship to the first author; ARIE *“Mata de Santa Genebra”* for permitting the collections in its property;

Technician Rosana Astolfo from *Laboratório de Análise-dos-Processos Atmosféricos/Instituto de Astronomia, Geofísica e Ciências Atmosféricas/USP* and Professor Américo A. F. S. Kerr and his student Luís Henrique M. Santos from *Instituto de Física/*USP for helping in all steps of PM_{10} analyses. Dr. Patrícia Giampaoli for illustrating the atmospheric deposition sampler.

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Capítulo 3

Trace elements in soils of remnants of Semideciduous Atlantic Forest due to land use expansion in Southeast Brazil

Capítulo a ser submetido ao periódico Environmental Science and Pollution Research

Trace elements in soils of remnants of Semideciduous Atlantic Forest due to land use expansion in Southeast Brazil

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Abstract

Anthropogenic input of trace elements in tropical soils has increased over the years due to land use expansion, but little research has been conducted on this field in Brazilian soils. Trace element pollution of soils from the remaining of Semideciduous Atlantic Forest in São Paulo State, has supposedly occurred, since the forest remnants are surrounded by numerous anthropogenic sources of trace elements, including cities, petrochemical, chemical and agrochemical industries, roads with intense traffic and extensive agricultural lands. The main goal was to evaluate the potential soil pollution by trace elements in four forest remnants located in different cities (Campinas, Cosmópolis, Holambra and Paulínia) of the second largest Metropolitan Region of São Paulo State. Soil samples (0- 20 cm) were collected during dry and wet seasons at the forest edge and in forest interior. Total concentrations of As, Ba, Cr, Cu, Mo, Ni, Pb, Sr, V and Zn were determined by X-ray fluorescence spectrometer and available contents of Cu, Mn, Ni, Pb and Zn were determined after DTPA extraction using ICP OES. Physical and chemical attributes of soils were also determined. Reference levels and calculated contamination factors of trace elements were used to qualify and quantify soil pollution in these remnants. No seasonal and edge effects affected the total and available content of trace elements in the forestry soils. The soil from Campinas forest showed to be more polluted than that from other studied forest remnants based on total and available concentrations, physical and chemical attributes and the inference of soil contamination levels. The total trace element concentrations and soil attributes (Al_2O_3, Fe_2O_3, MnO, pH, CEC, OM and clay) were effective to tentatively predict the available levels of Cu, Mn, Ni, Pb and Zn in soils from the forest remnants studied.

Key-words: Land use; Semideciduous Atlantic Forest; Tropical soils; Soil attributes; Trace elements; Total and available concentrations; Contamination factor

Introduction

Trace elements in soils can be originated either from anthropogenic activities or weathering of parent materials (Abreu et al., 2002; Alloway, 2013; Li et al., 2013). Presently, anthropogenic activities, such as mining or industrial processes, burning of fossil fuels, agricultural and livestock practices, agricultural irrigation and the disposal of domestic and industrial waste are major sources of trace element enrichment in soils (Leyval et al., 1997; Naidu et al., 2001; Shi et al., 2014; Zeng et al., 2011).

Both essential and nonessential trace elements in soils may become toxic to plants and other living organisms, depending on their chemical speciation and concentration levels (Naidu et al., 2001; Sarma et al., 2011). However, the toxicity level of trace elements to plants in particular depends on a number of factors (Alloway, 2013; Birani et al., 2015; Clemente et al., 2003; Dai et al., 2004; De Matos et al., 2001; Leyval et al., 1997; Teixeira et al., 2010; Zeng et al., 2011), including: 1) their availability in soil, either dissolved in soil solution or present in an exchangeable form in organic and inorganic soil components; the unavailable fraction, essentially bound to minerals, is of lower relevance under the ecotoxicological perspective; 2) the soil physical-chemical properties, such as mineralogical composition, pH, organic matter, clay content, cation exchange capacity, among others. Chlorosis, reduction in growth and nutrient uptake and disorders in whole metabolism are among the damaging effects caused by trace elements in plants (Guala et al., 2010).

Anthropogenic input of trace elements in tropical soils has supposedly been increasing over the last years due to land use expansion, probably causing adverse effects on the plant community in natural ecosystems (Bermudez et al., 2012; Hernandez et al., 2003). In spite of this concern, few studies have been conducted on this field in tropical soils from Brazil, as those included in Birani et al. (2015), De Matos et al. (2001) and Teixeira et al. (2010). The soil pollution by trace elements in the Campinas Metropolitan Region (CMR), located in the most populated State in Brazil (São Paulo), has theoretically occurred due to increasing land use. This region encompasses numerous anthropogenic sources of particulate matter, including: 20 cities with a total population of about three million inhabitants occupying an area of 3,645.16 km^2; a diversified industrial park located in Paulínia city, covering the largest petroleum refinery in Brazilian territory and many chemical and agrochemical plants; numerous roads with intense traffic of about one million of vehicles and extensive agricultural lands (CETESB, 2013). In addition, soil and groundwater contamination of toxic chemicals, such as organochlorine pesticides and trace elements (mainly Al, Cr, Fe, Mn and Pb), as well as blood contamination of residents with As and Pb, were verified in the industrial area of Paulínia resulted of twenty years (from 1975 to 1995) of activities of a pesticide factory (Suassuna, 2001). These diversified land uses in the CMR may have also induced trace element pollution of soils in the last remaining remnants of Semideciduous Atlantic Forest in the region. We may also raise the hypothesis that the total and available trace element concentrations in soil vary among forest remnants due to three reasons: a) differences in physical and chemical soil parameters previously described by Lopes et al. (2015); b) proximity to specific anthropogenic emission sources; c) edge effects resulting from forest fragmentation. The seasonal climate that determines marked dry and wet periods and consequently seasonal variations in the particulate matter levels (CETESB, 2006) may be another source of variation of soil pollution by trace elements in the CMR. Therefore, the main aim of this study was to evaluate the potential soil pollution by trace elements in forest remnants in the CMR on the basis of the hypotheses raised, by: 1) Determining the total and available concentrations of selected trace elements; 2) Investigating the impacts of edge effect, dry/wet seasons and proximity to the industrial complex in the concentration of these elements in the soil of different forest remnants; 3) Qualifying and quantifying potential trace element contamination of soils using reference levels and contamination factors; 4) Proposing a tentative multilinear model for predicting the levels of available forms of trace elements, based on soil attributes, such as pH, organic matter, clay content, cation exchange capacity and Al, Fe and Mn oxides and total levels of trace elements.

Material and methods

Study area and soil sampling

Soil was sampled from four forest remnants (FCA, FCO, FHO and FPA) located in different cities (Campinas, Cosmópolis, Holambra and Paulínia, respectively) of the CMR. FCA and FPA are closer to emission sources of particulate matter containing trace elements, among them urban areas and petrochemical/chemical/agrochemical industries, than FCO and FHO. All forests are surrounded by agriculture, mainly dedicated to sugarcane cultivation (Fig. 1; Table 1).

This natural vegetation is classified as Semideciduous forest, a subtype of the Atlantic Forest domain, and is characterized by high plant diversity and a number of tree species that partially lose their leaves during the dry season (Oliveira-Filho and Fontes, 2000). The soils of the selected forest remnants are classified as Oxisols (Prado, 1997), according USDA Soil Taxonomy, and were originated from different parental materials (diabase in FCA; diabase/sandstone/siltstone in FCO; sandstone in FHO; diabase/sandstone in FPA). The soils also differed concerning texture (clay in FCA and sandy clay loam in FCO, FHO and FPA).

The six most abundant tree species (circumference at breast height ≥ 30 cm were represented by 1295 ind.ha^{-1} on average (ranging from 1150 ind.ha^{-1} in FCA to 1575 ind.ha^{-1} in FCO). These species also totalized 53.0 $m^2.ha^{-1}$ of basal area on average (41.6 $m^2.ha^{-1}$ in FPA to 60.9 $m^2.ha^{-1}$ in FCA) (Table 1). Other phytosociological characteristics of the forest remnants studied are described in Domingos et al. (2015).

The predominant climate in the CMR is Cwa (humid subtropical zone with dry winter and hot summer), according to Köppen classification (Alvares et al., 2013). The hot and rainy season occurs between October and March, which is followed by a dry season between April and September (Moura et al., 2014). The winds predominantly originate from the Southeast (Fig. 1).

The concentration of atmospheric pollutants in the CMR is influenced by the predominant weather conditions and the diversity of pollution emission sources. The primary pollutants (particulate matter, nitrogen dioxide and sulfur dioxide) are in higher concentrations during autumn and winter months (May to August). The maximum hourly concentrations of PM_{10} registered from 2010 to 2012 in a monitoring station located in Paulínia city was 561 µg m^{-3}.

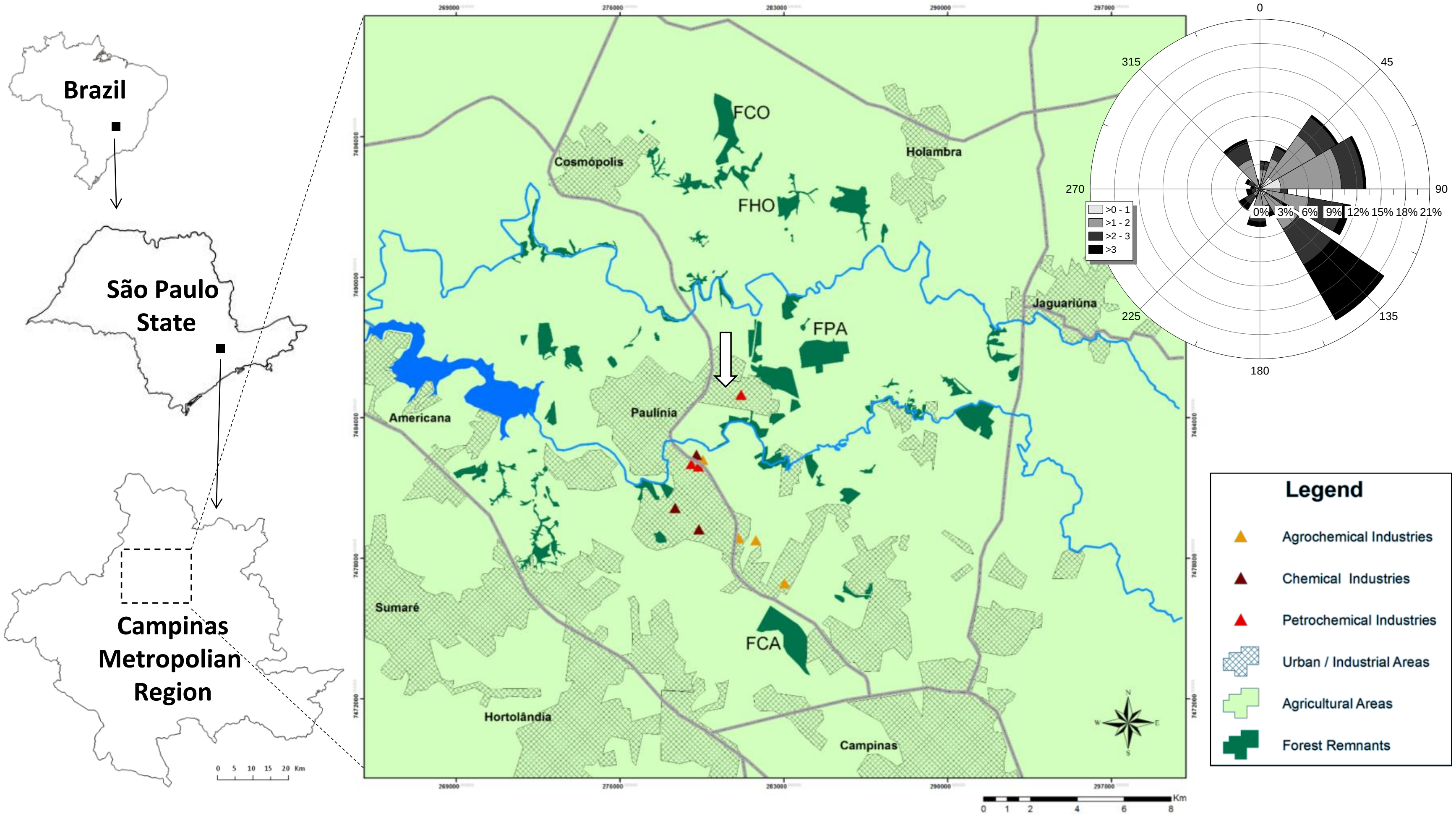

Figure 1. Location of the forest remnants studied (FCA; FCO; FHO; FPA) and the wind rose for the Campinas Metropolitan Region (modified from Domingos et al., 2015) (data obtained from a meteorological station located in inside the major petrochemical industry in Paulínia city, as indicated by white arrow).

Table 1. Descriptor parameters of studied forest remnants and respective soils in the Campinas Metropolitan Region, São Paulo, SP

Parameters	FCA	FCO	FHO	FPA
Cities	Campinas	Cosmópolis	Holambra	Paulínia
Latitude	22°49'17"S	22°37'22"S	22°39'21"S	22°42'47"S
Longitude	47°06'41"W	47°08'06"W	47°06'36"W	47°05'52"W
Size (ha)	235	120	35	190
Land use on surrounding	agricultural (sugarcane), urban	agricultural (sugarcane)	agricultural (citrus/ sugarcane)	agricultural (sugarcane), industrial
Distance to industrial area[1]	10	12	8	3.5
Soil texture [2]	clay	sandy clay loam	sandy clay loam	sandy clay loam
Parental material [2]	diabase	sandstone, diabase, siltstone	sandstone	diabase, sandstone
Tree density (N ind. ha-1)[3]	1150	1575	1200	1256
Basal area (m^2 ha^{-1}) [3]	60.9	51.9	57.6	41.6

[1] Distance (km) calculated from the center of the remnant to the major petrochemical industry of CMR (indicated by white arrow in Fig. 1).

[2] Data obtained from Lopes et al. (2015).

[3] Phytosociological data estimated considering the six most abundant tree species, according Domingos et al. (2015).

Soil samplings were carried out during the dry (Aug / Sep 2011) and the wet (Jan / Feb 2012) seasons. Four composite samples (each one obtained from 16-24 single sub-samples) at 0-20 cm depth were collected in four distinct locations of each forest remnant (Fig. 1). Two composite samples were collected at regular intervals along the forest edge, following the “transect sampling” recommended by IAEA (2004). The other two composite samples were collected inside the forests, starting about 100 m from their edges, randomly collecting individual soil samples in two grids that covered a total of 7,000 m^2 each, as recommended by the “systematic random sampling” (IAEA, 2004).

Chemical analysis

All composite samples of soil were air dried, passed through a 2 mm nylon sieve, homogenized and stored in plastic bags. One portion of each sample was finely ground in a mechanical agate grinder. A test-portion of 9.0 g of the milled powder was mixed with 1.5 g wax powder (Hoechst) and pressed as a pellet (40 mm diameter), which was used to determine the total content of trace elements by X-ray fluorescence spectrometry (Philips, PW 2404, Netherlands) (Zambello and Enzweiler 2002). Trace elements that are markers of pollution sources according to Calvo et al. (2013) and Celo and Dabek-Zlotorzynska (2010) (As, Ba, Cr, Cu, Mo, Ni, Pb, Sr, V, Zn) plus Al_2O_3, Fe_2O_3 and MnO were determined. The accuracy of the results was evaluated by the analysis of the soil reference materials GSS-2 and GSS-4. The obtained results were always within the 95% confidence interval of the reference value and its uncertainty, expressed by standard deviation. The detection limits, were 3 mg kg^{-1} for As, Cr, Ni and Pb; 10 mg kg^{-1} for Ba; 2 mg kg^{-1} for Cu, Sr and Zn; 1 mg kg^{-1} for Mo and 5 mg kg^{-1} for V.

The amount of available trace elements (Cu, Mn, Ni, Pb, Zn) was determined using the DTPA as extractor (Abreu et al., 2001). Three analytical replicates per sample were performed. The extracts were analyzed by Inductively Coupled Plasma Optical Emission Spectroscopy (Optima 7300 DV, Perkin-Elmer, USA). The detection limits were 0.36 mg kg^{-1} for Cu; 0.32 mg $kg^{-1}$for Mn; 0.23 mg kg^{-1} for Ni-and 1.3 mg kg^{-1} for Pb and Zn.

The soil pH was measured by dispersing a test portion in 0.01 mol L^{-1} $CaCl_2$ solution (Quaggio and Raij, 2001). Organic matter content was determined by colorimetry, after oxidation with $K_2Cr_2O_7.2H_2O$ and H_2SO_4 (Cantarella et al., 2001). The exchangeable cations (K^+, Ca^+, Mg^+) were simultaneously extracted from soils using ion-exchange resins, following the method proposed by Raij et al. (1986). Potential acidity (H+Al) was measured using buffer solution SMP according to Raij et al. (1987). Cation exchange capacity (CEC at pH 7) was calculated by summing the exchangeable cations (K^+, Ca^+, Mg^+) and potential acidity.

Soil texture that included the clay contents was determined by Bouyoucos method (EMBRAPA, 2011).

Quality Reference Levels and Contamination factor

The average total contents of As, Ba, Cr, Cu, Mo, Ni, Pb and Zn of each forest remnant soil sample was compared to the Quality Reference Levels (QRL; included in Table 3) proposed by the Environmental Company of São Paulo State (CETESB, 2014) for soils in São Paulo State. The QRL is the concentration of a substance in soil, which defines a soil as clean and is used to prevent soil pollution in São Paulo State (CETESB, 2005). Mean total contents lower than QRL indicated a clean soil.

The level of soil pollution was quantitatively defined by calculating a contamination factor (Cf^i) for average total contents of As, Ba, Cr, Cu, Mo, Ni, Pb, V and Zn, using the following formula suggested by Håkanson (1980), with adaptations to our regional scale:

$$\mathrm{Cf}^{\,i} = C^i/C_n^{\,i}$$

where C^i is the average total content of the trace element i in a forest remnant and C_n^i is the reference level (both included in Table 3) of the same element determined in non-contaminated soil samples from an area of maximum protection located in the CMR (Quináglia, 2001). The resulting ratios were categorized as follow to determine the trace element contamination level: $Cf^i \leq 1$ non–contamination; $1 < Cf^i \leq 3$, low contamination; $3 < Cf^i \leq 7$, moderate contamination, and $Cf^i > 7$, high contamination (modified from Håkanson, 1980; Qingjie et al., 2008).

Statistical analyses

Total and available trace element concentrations and soil attributes from the four forest remnants were described by means, medians, minimum and maximum values.

The nonparametric Mann–Whitney rank sum test ($p < 0.05$) was used to compare all soil parameters between seasons and sampling locations in the forest remnant (edge and interior). The nonparametric Kruskal-Wallis test (One Way Analysis of Variance on Ranks), followed by pairwise Tukey test applied to ranks ($p < 0.05$) when necessary, compared all soil parameters of the four forest remnants.

A cluster analysis, using STATISTICA for Windows (Version 7.1), was carried out using Ward's method as the joining rule and 1-Pearson r as the metric distance in order to verify the similarity level among the four soil forest remnants using total and available trace element concentrations, except elements, whose concentrations were below the detection limits at least in one forest remnant.

Factor analysis was performed with the same parameters used in cluster analysis by employing the Principal Components Method and normalized Varimax, also using STATISTICA for Windows (Version 7.1), to summarize associations among elements analyzed. Only factors extracted by eigenvalues > 2.0 and with factor loadings greater than 0.6 were considered.

Spearman's correlation coefficients were calculated, using SIGMA PLOT (Version 11.0), to determine the relationships between soil attributes (Al_2O_3, Fe_2O_3, MnO, pH, organic matter, cation exchange capacity and clay content) and available (Cu, Mn, Ni, Pb, Zn) and total concentrations (Cu, Ni, Pb, Zn).

Multilinear regression analyses were performed, using SIGMA PLOT (Version 11.0), in order to propose tentative models to predict the available trace element concentrations (Cu, Mn, Ni, Pb and Zn; dependent variables) in the soil of region based on linear combined associations among soil attributes (Al_2O_3, Fe_2O_3, MnO, pH, organic matter, cation exchange capacity and clay content) and total trace element concentrations, all of them adopted as independent variables. The data of all soil samples were included and analyzed by means of the backward stepwise method. After evaluation and eventual elimination of collinear independent variables, the adjustment procedure started from the saturated model (with all the independent variables present) and consisted of removing the variable with the smallest participation to explaining the variations in the availability of trace elements. New adjustments were successively made. At each step, the adjustment and the significance of the variables were evaluated, and only those that contributed significantly to predict the level of available trace elements in the soils were retained in the linear models with a significance level of $p<0.05$. The model proposed for av Pb started without organic matter and clay content because they did not correlate significantly with av Pb, as indicated by Spearman analysis.

All data were Log_{10}-transformed to ensure homogeneity of variances in the cluster and factor analyses, Spearman's correlations and multilinear regression models proposed for available Cu, Mn, Ni and Pb.

Results

None of the evaluated soil parameters differed significantly between edge and interior of forest remnants (data not shown). No significant differences were also detected between the dry and wet seasons (data not shown), even considering the distinct rainfall registered in both seasons (460.2 mm and 42.3 mm, respectively; www.agritempo.gov.br). Therefore, these data were treated as replicates for describing the results of total and available trace element concentrations, and attributes (pH, OM, clay, CEC, Al_2O_3, Fe_2O_3, MnO) of soils from FCA, FCO, FHO and FPA (Table 2).

Available contents of Ni were below the detection limit of the analytical method in soil samples from FCO and FPA and no available Pb was detected in soil samples from FCO, FHO and FPA. Total As was not only detected in the soil samples from FHO (Table 2).

Soil av Ni, tot As and Cr, pH and CEC did not vary significantly among all studied remnants. The soil of FCA contained significantly higher median contents of av Cu (4.8 mg kg^{-1}), tot Cu (81 mg kg^{-1}), tot Mo (3 mg kg^{-1}), tot Ni (22 mg kg^{-1}), tot Pb (13.7 mg kg^{-1}), tot V (219 mg kg^{-1}), tot Zn (51 mg kg^{-1}), organic matter (42 g dm^{-3}) and clay content (470 g kg^{-1}) than the other remnants. The soil of FHO was more concentrated in av Mn (77 mg kg^{-1}) than soil of FPA, in tot Ba (224 mg kg^{-1}) compared to the soils of FPA and FCO and in tot Sr (26 mg kg^{-1}) compared to soil of FCO (Table 2).

Table 2. Total (tot) and available (av) trace element concentrations, in mg kg^{-1}, and soil attributes (**OM**- organic matter, g dm^{-3}; **CEC**- cation exchange capacity, mmolc dm^{-3}; **Clay** – clay content, g kg^{-1} ;**Al_2O_3, Fe_2O_3** and **MnO**, oxides of aluminum, iron and manganese, respectively, content in percentage) from the four forest remnants evaluated (*n* = 32).

Soil Parameters		FCA			FCO			FHO			FPA		
		Mean	**Med**	**Range**	**Mean**	**Med**	**Range**	**Mean**	**Med**	**Range**	**Mean**	**Med**	**Range**
av	**Cu**	6.0	4.8 **a**	3.4 - 11.5	0.64	0.6 **b**	0.4 - 0.98	1.31	0.61 **b**	0.45 - 5	0.62	0.56 **b**	0.36 - 1.28
	Mn	75	61 **ab**	43 - 146	44	43 **ab**	27 - 65	83	77 **a**	24 - 152	32	27 **b**	11.7 - 58
	Ni	0.41	0.4 **a**	0.3 - 0.57	*n.d.*	*n.d.*	*n.d.*	0.37	0.32 **a**	0.23 - 0.81	*n.d.*	*n.d.*	*n.d.*
	Pb	2.0	1.9	1.3 - 3.07	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*	*n.d.*
	Zn	3.1	2.2 **a**	1.5 - 6.4	0.86	0.78 **b**	0.5 - 1.32	1.69	1.32 **ab**	0.74 - 3.6	0.85	0.81 **b**	0.37 - 1.7
tot	**As**	5.4	5.3 **a**	3.8 - 6.8	4.8	5.1 **a**	3 - 6.4	*n.d.*	*n.d.*	*n.d.*	4.3	3.9 **a**	3 - 6.2
	Ba	85	76 **ab**	41 - 153	54	52 **b**	38 - 78	234	224 **a**	110 - 561	47	42 **b**	33 - 95
	Cr	48	45 **a**	37 - 67	43	38 **a**	26 - 95	52	33 **a**	22 - 166	69	55 **a**	20 - 193
	Cu	109	81 **a**	46 - 225	6.9	6.3 **b**	3.9 - 11.5	6.4	5.3 **b**	3.5 - 14	7.5	6.1 **b**	3 - 14
	Mo	3.01	3.0 **a**	2.1 - 4.5	1.9	2.0 **b**	1.4 - 2.2	2.01	1.9 **b**	1.6 - 2.8	1.8	1.9 **b**	1 - 2.6
	Ni	23	22 **a**	17 - 32	6.09	6.1 **b**	3 - 9.7	4.3	3.7 **b**	3 - 8.3	4.7	3.5 **b**	3 - 7.7
	Pb	14.7	13.7 **a**	10.3 - 23	6.1	6.0 **b**	4.1 - 9.1	7.6	7.8 **b**	4.2 - 12	8.0	8.9 **b**	3.6 - 12
	Sr	20.8	19.1 **ab**	7.4 - 41	11.9	9.5 **b**	8.4 - 21	26.6	26 **a**	14 - 60	12.1	11.3 **ab**	8.5 - 19
	V	216	219 **a**	146 - 282	53	54 **b**	32 - 72	31.1	31 **b**	22 - 41	52	49 **b**	25 - 81
	Zn	50	51 **a**	29 - 69	15.6	15 **b**	10 - 25	15.1	14 **b**	10.9 - 23	15.3	15.2 **b**	9 - 23
Soil attributes	**pH**	4.7	4.8 **a**	3.8 - 5.3	4.2	4.1 **a**	3.8 - 5	4.5	4.5 **a**	3.8 - 4.9	4.2	4.1 **a**	3.5 - 5.2
	OM	43	42 **a**	32 - 57	24	24 **b**	22 - 26	24	23 **b**	18 - 36	25	25 **b**	15 - 35
	Clay	474	470 **a**	389 - 568	219	214 **b**	189 - 259	203	213 **b**	163 - 222	248	239 **b**	167 - 347
	CEC	116	118 **a**	99 - 142	79	81 **a**	66 - 89	71	71 **a**	50 - 105	90	81 **a**	56 - 138
	Al_2O_3	24.2	25 **a**	19.3 - 30	15.2	15.4 **a**	12 - 17.3	7.9	7.9 **b**	6.4 - 9.6	15.5	14 **ab**	9 - 23
	Fe_2O_3	12.3	9.7 **a**	5.9 - 23	2.63	2.6 **ab**	1.8 - 3.5	1.23	1.15 **b**	0.90 - 1.60	1.96	2.0 **b**	0.9 - 3
	MnO	0.07	0.06 **a**	0.04 - 0.13	0.02	0.02 **b**	0.02 - 0.03	0.03	0.03 **ab**	0.01 - 0.06	0.02	0.02 **b**	0.01 - 0.02

n.d.: non detected

Distinct letters indicate significant differences between median values of soil parameters in the forest remnants according Tukey Test ($p < 0.05$).

The cluster analysis (Fig. 2) separated the forest remnants according to the similarities or differences in their total and available trace element contents. The soil of FCA was completely separated at the linkage distance of 0.1 from the second group that included the other forest remnants. However, this second group was separated into two sub-groups at linkage distance of 0.05, the first composed by FHO and the other formed by FCO and FPA.

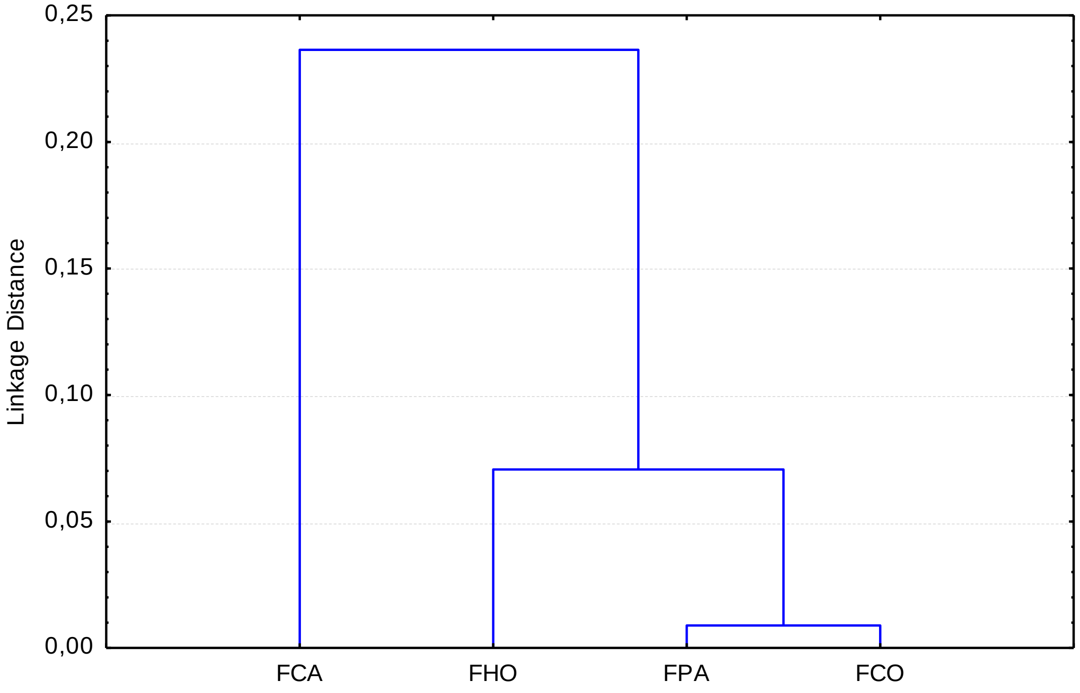

Figure 2. Tree dendrogram resulting from cluster analysis performed with total and available trace element concentrations (except av Ni, av Pb and tot As) measured in soil of the forest remnants studied.

The factorial analysis (Fig. 3) indicated that 77% of the data variability was explained by two factors. The first factor grouped most of the variables included in the analyses, including total and available forms of elements. The second factor was strongly explained by the total concentration of Ba and Sr and available Mn.

The trace elements uploaded in factor 1 (tot Cu, tot Mo, tot Ni, tot Pb, tot V, tot Zn, av Cu) were in higher levels in the soil sampled FCA than those in other forest soils (Table 2), explaining the isolation of FCA in cluster analysis. The few elements uploaded in factor 2 (tot Ba, tot Sr and av Mn) were found in high concentrations in the soil from FHO (Table 2).

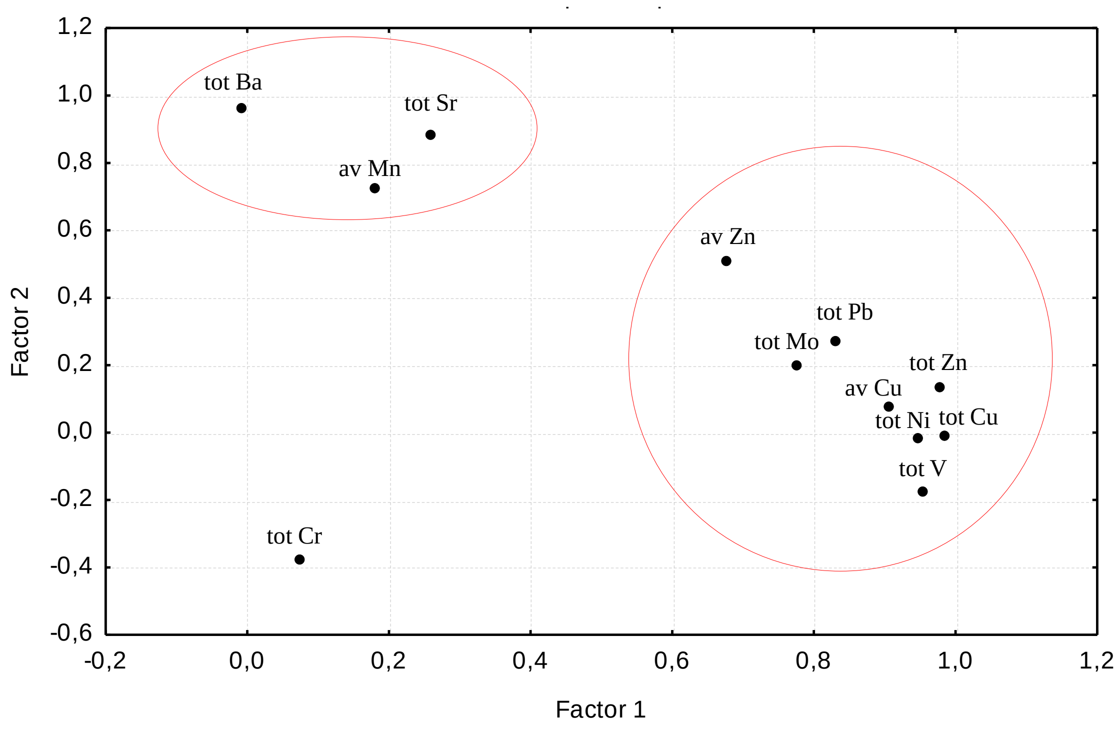

Figure 3. Plot resulting from factor analysis performed with total and available trace element concentrations (except av Ni, av Pb and tot As) measured in soils of the forest remnants studied. Variables inside the red circles were uploaded in factors 1 and 2 by factor loadings greater than 0.6.

Total content of Cr (factor loading below 0.6) was weakly associated with both factors as shown in Fig. 3, since this variable was similar among all studied forests (Table 2).

The mean values (C^i) of As, Ba, Cr, Cu and Ni exceeded the quality reference levels (QRL) in soils from FCA (Table 3). The concentration levels of Ba and Cr also exceeded QRL in soil samples of FHO, as well as As and Cr in soils from FCO and FPA. According to the contamination factors also presented in Table 3, a moderate contamination of As, Cu and V was estimated in soil samples from FCA. Soils of FCO and FHO were moderately contaminated by As and Ba, respectively, and the soils of FPA were moderately contaminated by As and Cr.

Table 3. Quality reference levels (QRL) proposed for São Paulo State, mean concentrations of trace elements (C^i) found in the four forest remnants, reference concentrations in a non-contaminated soil in the study region (C_n^i) and contamination factors **(**$Cf^{\,i}$) estimated in the soils from the forest remnants. QRL, C^i and C_n^i were represented in mg kg^{-1}.

	QRL†	**C^i**				**C_n^{i} ‡**	**$Cf^{\,i}$**			
		FCA	**FCO**	**FHO**	**FPA**		**FCA**	**FCO**	**FHO**	**FPA**
As	3.5	**5.4**	**4.8**	*n.d.*	**4.3**	1	6.2^{++}	5.5^{++}	n.d.	4.9^{++}
Ba	75	**85**	54	**234**	47	63	1.3^{+}	0.9	3.7^{++}	0.8
Cr	40	**48**	**43**	**52**	**69**	22	2.2^{+}	2.0^{+}	2.4^{+}	3.2^{++}
Cu	35	**109**	6.9	6.4	7.5	21	5.2^{++}	0.3	0.3	0.4
Mo	< 4	3.0	1.9	2.0	1.8	6	0.5	0.3	0.3	0.3
Ni	13	**23**	6.1	4.3	4.7	10	2.4^{+}	0.6	0.4	0.5
Pb	17	14.7	6.1	7.6	8.0	33	0.4	0.2	0.2	0.2
V	–	–	–	–	–	53	4.1^{++}	1.0	0.6	1.0
Zn	60	50	15.6	15.1	15.3	59	0.8	0.3	0.3	0.3

† Quality reference levels proposed by CETESB (2014)
‡ Reference concentrations measured by Quináglia (2001)
$^{+}$ Low contamination; $^{++}$ Moderate contamination
n.d.: non-detected.
Bold numbers indicate mean concentrations (C^i) that surpassed QRL.

The Spearman's correlation (Table 4) showed that all soil attributes, except pH, were significantly and positively related to available content of Cu and total content of Cu, Ni, Pb and Zn. The contents of Fe_2O_3, MnO, organic matter (OM), clay and cation exchange capacity (CEC) were positively correlated to av Ni. The content of MnO was also positively related to av Mn. Positive relationships among available contents of elements in soils were observed for Cu and Zn ($r = 0.76$), Mn and Ni ($r = 0.62$) and Mn and Zn (0.48). The correlation between available and total trace element contents was significant and positive for Cu ($r = 0.95$), Ni ($r = 0.58$) and Zn ($r = 0.70$).

The regression analyses (Table 5) indicated that the available concentrations of Cu, Mn, Ni, Pb and Zn may be predicted significantly and with high explicability ($R^2 > 0.9$, ranging from 0.92 for Ni and 0.99 for av Pb) by a linear combination of independent variables, which included total concentrations and soil attributes. Total trace element concentrations affected positively their respective available forms. Al_2O_3 was negatively correlated with av Pb and av Zn. Contents of Fe_2O_3 were negatively correlated with available Cu, Mn, Ni and Zn and positively with av Pb. The content of MnO was positively correlated with av Mn and av Ni and negatively related with av Pb and av Zn. Clay content was negatively related with av Cu. Soil pH affected negatively av Cu and av Pb. Organic matter affected positively the availability of Mn, Ni and Zn. CEC showed negative relationship with av Mn and av Zn.

Table 4. Spearman's correlation coefficients between (log) available and (log) total trace element concentrations and (log) soil attributes.

	Al_2O_3	Fe_2O_3	MnO	pH	OM	CEC	Clay	av Cu	av Mn	av Ni	av Pb	av Zn	tot Cu	tot Ni	tot Pb	tot Zn
Al_2O_3	1.00															
Fe_2O_3	0.93***	1.00														
MnO	0.34	0.49**	1.00													
pH	0.03	0.17	0.39*	1.00												
OM	0.73***	0.73***	0.59***	0.23	1.00											
CEC	0.80***	0.76***	0.35*	0.11	0.90***	1.00										
Clay	0.83***	0.83***	0.60***	0.07	0.81***	0.78***	1.00									
av Cu	0.64***	0.65***	0.52**	0.23	0.73***	0.64***	0.72***	1.00								
av Mn	-0.19	-0.08	0.74***	0.23	0.22	-0.06	0.16	0.09	1.00							
av Ni	0.43	0.56*	0.78***	0.15	0.76***	0.62*	0.51*	0.20	0.62**	1.00						
av Pb	0.35	-0.43	-0.59	0.42	-0.01	0.73*	-0.29	-0.31	-0.57	-0.36	1.00					
av Zn	0.24	0.28	0.64***	0.45**	0.62***	0.42*	0.44*	0.76***	0.48**	0.20	0.55	1.00				
tot Cu	0.78***	0.79***	0.47**	0.27	0.79***	0.76***	0.79***	0.95***	-0.02	0.39	-0.41	0.64***	1.00			
tot Ni	0.79***	0.87***	0.68***	0.19	0.83***	0.79***	0.86***	0.77***	0.16	0.58*	-0.36	0.50**	0.85***	1.00		
tot Pb	0.67***	0.67***	0.61***	0.31	0.85***	0.77***	0.86***	0.69***	0.22	0.68**	0.52	0.57***	0.77***	0.77***	1.00	
tot Zn	0.75***	0.79***	0.61***	0.32	0.87***	0.81***	0.84***	0.91***	0.12	0.53*	-0.31	0.70***	0.96***	0.88***	0.85***	1.00

*p < 0.05; ** p < 0.01; *** p < 0.001

OM- organic matter; **CEC-** cation exchange capacity

Table 5. Linear regression models to predict tentatively available trace element concentrations on the basis of total trace element concentrations and soil attributes.

Linear models	R^2	P	N
Log_{10}(av Cu) = 2 + 1.5 Log_{10}(tot Cu) - 0.55 $\text{Log}_{10}(Fe_2O_3)$ - 1.2 Log_{10}(clay) - 0.6 Log_{10} (pH)	0.984	< 0.001	32
Log_{10} (av Mn) = 3.7 -0.4 Log_{10} (Fe_2O_3) + 1.02 Log_{10}(MnO) + 0.7 Log_{10}(OM) -0.7 Log_{10}(CEC)	0.925	< 0.001	32
Log_{10} (av Ni) = -1.1 + 0.5 Log_{10} (tot Ni) - 0.6 $\text{Log}_{10}(Fe_2O_3)$ + 0.4 Log_{10}(MnO) + 0.7 Log_{10}(OM)	0.920	< 0.001	16
Log_{10} (av Pb)= -4.4 + 0.8 Log_{10}(tot Pb) - 0.9 $\text{Log}_{10}(Al_2O_3)$ + 2.3 $\text{Log}_{10}(Fe_2O_3)$ - 2.7 Log_{10}(MnO) - Log_{10} (pH)	0.999	0.001	8
av Zn = 0.74 + 0.2 tot Zn - 0.08 Al_2O_3 - 0.28 Fe_2O_3 - 37 MnO + 0.08 OM - 0.03 CEC	0.923	< 0.001	32

Only independent variables retained with a significance level of 95% (p < 0.05) by the backward stepwise analyses were included in the predictive linear models.

Discussion

The absence of seasonal variation in pH values and trace element contents in soil was also observed in other studies, such as that conducted by Chon et al. (1998). It is also a characteristic of deep and weathered soils, like Oxisols, due to the present low rate of weathering (Resende et al., 2002), which results in few short-term compositional changes. Moreover, the trace element accumulation in soil from anthropogenic sources is generally detected after a longer interval of time than that between the dry and wet sampling seasons.

The atmospheric deposition of trace elements is usually more pronounced on the forest edge than on the forest interior, as observed by Bergkvist et al. (1989), an effect that was not observed in the present study. This could indicate that atmospheric deposition has been similar over the entire forest remnants, regardless of the occurrence of trace element sources very close to the forest edge, such as extensive agricultural areas. The small area of the forest remnants and canopy disturbances are among the possible reasons for the mentioned similarity between edge and interior of forest in terms of trace element contamination. It is important to note that the soil attributes (pH, OM, clay, CEC, Al_2O_3, Fe_2O_3 and MnO) also did not differ between the edge and interior of the forests, contributing to explain these results, once they usually interfere in the available and total content of trace elements, as observed in this study (Table 4 and 5).

Inverse association between the distance from the industrial complex and total element concentration in soils, usually described by many authors (Jinmei and Xueping, 2014; Machender et al., 2011; Yaylalı-Abanuz, 2011), was not observed in the present study. Both the farthest and the closest remnants from the industrial complex (FCO and FPA, respectively) were very similar in terms of trace element contents. In addition, the highest total concentrations of most analyzed trace elements (Cu, Mo, Ni, Pb, V and Zn) (Table 2) were verified in the soils sampled in Campinas (FCA), one of the farthest forest remnants from the industrial complex. Three aspects can help to explain these results:

First, although the predominant wind direction is from Southeast during the year, the wind circulation during a single day (SSE and SE in early morning and evening, SSE, SE and NE in the morning and NNE, SSE, N, SSW and S in the afternoon) and the flat topography favor the mixture of air pollutants in the whole CMR (Bulbovas et al., 2015, Tresmondi and Tomaz, 2004). Second, despite of the proximity of remnants to the industrial complex, none of them (considering the prevailing winds; Fig.1) is straightly located downwind of industrial complex of the Campinas Metropolitan Region (CMR). Third, the total content of trace elements in a soil is the sum of the concentrations of elements derived from minerals in the geological parent material on which the soil has developed (lithogenic source) and inputs of anthropogenic sources (Alloway, 2013). Soils from FCO and FPA, for example, were originated from the same parental material, presenting similar physical and chemical attributes and trace element contents (Table 1). All the soils sampled in the study region have also received trace element inputs from petrochemical, chemical and agrochemical industries, vehicular traffic and agricultural activities.

Both probable natural and anthropogenic origins of trace elements in the soils evaluated in the CMR were also highlighted by using reference levels (QLR) and contamination factors, not only in the soil from FCA, but also from the other forest remnants.

Although the quality reference levels (QRL), established by legislation for preventing soil contamination in São Paulo State, were exceeded in FCA for most evaluated elements, a moderate contamination, based on contamination factor, was only found for As, Cu and V (Table 3). These trace elements require attention due to their potential toxicity to plants (Kabata-Pendias, 2010; Nagajyoti et al., 2010). Cu and V are present in high concentrations (90 mg kg^{-1} and 260 mg kg^{-1}, respectively, according to Alloway, 2013) in basalts (diabase), which originated the FCA soil. These elements are also usually related to traffic and industrial pollution sources, respectively (Calvo et al., 2013; Celo and Dabek-Zlotorzynska, 2010).

Moderate contamination of As, estimated in soils from three forest remnants (FCA, FCO and FPA), located in different distances of the industrial pool, indicated a common pollution source. This As input may attributed to a previous environmental contamination that occurred for twenty years in the industrial area of Paulínia, due to activities of a pesticide factory (Suassuna, 2001), as well as to agrochemical industries and agricultural practices that are widely distributed in the area, as this element is found in pesticides (Kabata-Pendias, 2010; Kumpiene et al., 2008; Wei and Yang, 2010).

Cr, which was found in higher levels than QRL in all remnants, may be considered a moderate contaminant only in the forest soil nearest to the industrial complex (FPA). In fact, this element is usually related to industrial emissions (Calvo et al., 2013; Mijic et al., 2010). Furthermore, Cr was another trace element that was related to the environmental contamination in the industrial area of Paulínia described by Suassuna (2001). Cr may have been also originated from the overspread agricultural practices in all the studied region, based on the results reported by Kumpiene et al. (2008), Udeigwe et al. (2011) and Wei and Yang (2010). It is highly toxic to plants and detrimental to their growth and development (Nagajyoti et al., 2010).

The moderate contamination estimated for Ba specifically in the superficial soil of FHO may indicate both lithogenic and agricultural sources. The sandstone, the parental material that originated that portion of soil in the CMR, is a natural source of Ba (300 mg kg^{-1}, on average, according to Alloway, 2013). Agricultural soils, which predominate in Holambra, may also present high contents of Ba (10 - 5000 mg kg^{-1}; Kabata-Pendias, 2010). This non-essential trace element is known to be toxic at elevated concentrations, inhibiting photosynthetic activity and plant growth (Alloway, 2013; Suwa et al., 2008).

The Spearman’s correlation was useful to obtain relationships among total, available trace element concentrations and soil attributes, revealing the strong and significant positive correlations between available concentration of Cu and total contents of Cu, Mn, Ni, Pb and

Zn with the soil attributes (Al, Fe and Mn oxides, CEC, and contents of organic matter and clay). However, as discussed below, multilinear models (Table 5) helped to better comprehend the role of total trace element concentrations as well as which soil attributes were in fact effective to predict tentatively the available levels of the studied trace elements in soils from the Campinas Metropolitan Region.

The positive relationship between total concentration of trace elements and their available forms (Table 5) was also observed for DTPA-extractable Cd, Cu, Pb and Zn by Dai et al. (2004) and by Ramos-Miras et al. (2011) for EDTA-extractable Cd, Cu, Pb and Zn.

Our models indicated a minor effect of soil pH on the trace element availability than other soil attributes. Birani et al. (2015), evaluating Oxisols from the Eastern Amazon (Brazil) and applying Pearson's correlation analyses, also found no relationship between soil pH and available content of all studied metals, extracted by a Mehlich-III solution. An inverse association between pH and the levels of available forms was only observed for Cu and Pb. Similar results were found by Ramos-Miras et al. (2011) for Cu and Ni, although they used EDTA as the extractable solution. It is well accepted that pH is one of the most important soil properties that determines the solubility of trace elements in soils (De Matos et al., 2001; Ramos-Miras et al., 2011). However, at pH values below 6.5, as observed in our study area, the magnitude of adsorption of trace elements may be controlled by the capacity of other isolated soil parameters to exchange cations, such as organic matter and clay content (Alleoni et al., 2005).

The positive correlations revealed by multilinear regression analyses among organic matter content (OM) and available forms of Mn, Ni and Zn showed that higher contents of this soil attribute may enhance the trace element availability to plants, providing metal chelates and increasing the solubility of either those with nutritional function or those potentially toxic to plants in soil solution (Zeng et al., 2011). Zeng et al. (2011) also found positive relation between EDTA-exctractable Mn and Zn and organic matter content.

Spearman correlation and multilinear regression analyses indicated positive associations between MnO and available Mn. Kämpf et al. (2011) also mentioned that the reduction and dissolution of Mn oxides increases the Mn mobility and availability to organisms. However, Al, Fe and Mn oxides usually absorb trace elements (Fijałkowski et al., 2012), decreasing their availability, our results contrasted with this general finding, once the multilinear regressions indicated a positive associations between MnO and available Ni and between Fe_2O_3 and available Pb. Birani et al. (2015) also found positive correlations between Al_2O_3 and available Cu, Mn, Ni, Pb and Zn and between Fe_2O_3 and av Cu and Ni. The availability dynamics of trace elements in tropical soils should be further investigated.

On the other hand, negative associations between the clay content and av Cu, and between CEC and av Mn and av Zn were found. Ramos-Miras et al. (2011) also found negative relation among clay content and available Cu, but using EDTA-extraction. These results might be expected because the reactive clay surfaces contribute to the retention of metals (Alleoni et al., 2005) and CEC is associated with adsorption of metals (Rieuwerts et al., 1998).

In brief, we could assume that the soil from FCA (located in the most urbanized portion of the studied region) is more polluted than that from the other forests studied, as shown by the variations in total and available concentrations and the inferences of soil contamination levels. However, this finding does not indicate necessarily a greater potential risk posed by trace elements to the plant community of that forest remnant, due to two main reasons. First, the term “availability” should not be confused with plant uptake, once a considerable number of authors found no correlation between extractable fraction in soil and plant contents (Maisto et al., 2004, Ramos-Miras et al., 2011). In addition, the total trace element contents are not indicative occurrences of adverse effects caused by trace elements on the forest ecosystem at present time (Birani et al., 2015; Luo et al., 2012), since they include not only the readily exchangeable ions, but also those trace elements more strongly bound within solid phases of the soil (Rieuwerts et al., 1998). These forms may only become gradually available for uptake by soil organisms, depending on many soil attributes such as pH, organic matter content, cation exchange capacity (CEC), oxidation-reduction status (Eh), the contents of clay contents, calcium carbonate, Fe and Mn oxides (Kumpiene et al., 2008; Udeigwe et al. 2011; Zeng et al., 2011).

Conclusions

The results obtained confirmed the hypothesis that total and available concentrations of trace elements in soils varied among forest remnants of CMR. It can be assumed that soils from FCA is more polluted than that from other forest remnants based on: variations in physical and chemical soil attributes, quality reference levels and contamination factors associated with the great variety of anthropogenic sources in the region.

No seasonal and edge effects on all soil parameters evaluated were observed. Inverse association between the distance from the industrial complex and total and available element concentrations in soils were also not proved. The quality reference levels proposed for soils in São Paulo State were surpassed for As, Ba, Cr, Cu and Ni in FCA, As and Cr in FCO and FPA and Ba and Cr in FHO. A moderate contamination of As, Cu and V were estimated in FCA soil, of As in FCO, of Ba in FHO and of As and Cr in FPA.

The total trace element concentrations and studied soil attributes (Al, Fe and Mn oxides, pH, CEC, organic matter and clay content) were effective to tentatively predict the available levels of the studied trace elements in soils from the forest remnants studied.

Acknowledgments

The authors thank *Programa SISBIOTA-BRASIL* (proc. CNPQ 563335/2010; proc. FAPESP 2010/52319-2) for the financial support; CAPES for offering a Ph.D. scholarship to the first author; ARIES *“Mata de Santa Genebra”* and *“Matão de Cosmópolis”* and *Cooperativa Agropecuária Holambra* for permitting the collections in their properties.

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Capítulo 4

Major and trace element leaf accumulation in native trees from the Semideciduous Atlantic Forest

Capítulo a ser submetido ao periódico Ecological Indicators

Major and trace element leaf accumulation in native trees from the Semideciduous Atlantic Forest

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Abstract

Forest ecosystems may have been affected by potentially toxic elements (PTEs), as they can be accumulated in leaves of their plant species. It is plausible to expect that these effects have mostly been evidenced in forest remnants next to anthropogenic sources, such as the remaining Semidiciduous Atlantic Forest located in Campinas Metropolitan Region (CMR), Southeast Brazil, which is surrounded by numerous industries, intense road traffic and agricultural land. We focused this study on forest remnants found in this little-known region with regard to pollution effects, aiming to: 1) Determine the leaf accumulation level of major and trace elements, including the PTEs, in the three most abundant native tree species (*Astronium graveolens, Croton floribundus* and *Piptadenia gonoacantha*) of four forest remnants, two of them located in the most urbanized/industrialized cities and the others in predominantly cities neighboring rural areas of CMR; 2) evaluate seasonal and spatial variations in the PTEs in leaves of all species; 3) determine which species better discriminate spatial variation of the PTEs. Samples were collected during the dry and wet seasons, at the edge and interior of the forest remnants. Major elements and trace elements were determined using inductively coupled plasma mass spectrometry (ICP MS) and rare-earth elements by instrumental neutron activation analysis (INAA). *Croton floribundus* showed higher capacity to accumulate most of the analyzed elements. The edge effect was not evidenced in all species. Higher concentrations of most analyzed PTEs were measured during dry season. *A. graveolens* was the most appropriate species to discriminate spatial variations and sources of PTEs to the forest remnants.

Key-words: Atlantic forest remnants; Campinas Metropolitan region; major and trace elements; potentially toxic elements; native tree species.

Introduction

Forest ecosystems cover about 30% of the Earth’s surface, and are ecologically indispensable in order to sustain nutrient and hydrological cycles, moderate climate, protect soils and act as carbon sinks, among many other roles in supporting human life and benefits (Gamfeldt et al., 2013; Matyssek et al., 2012). However, they have been affected over the last decades by significant reductions in their surface areas, air pollutants, global climate change, as well as diseases and pathogens (Amores and Santamaria, 2003; Pellissier et al., 2013), restraining the quality of the mentioned ecosystem services.

The effects of air pollutants on forest ecosystems, in particular, have been long recognized and may be caused by potentially toxic elements associated with the particulate matter captured by plant canopies through dry/wet deposition or deposited on the soil surface (Cape, 2009; De Nicola et al., 2008; Sawidis et al., 2011). The accumulation of elements in leaf tissues, both those non-essential (such as Pb, Cd and Hg) as well as those essential to plants (such as Ca, Mg, S, Co, Cu, Mn, Mo and Zn), can interfere and even disrupt plant metabolic processes, such as photosynthesis and respiration, protein synthesis and organelle development (Guala et al., 2010; Gupta et al., 2007; Nagajyoti et al., 2010;).

Although all chemicals are potentially toxic to organisms, As, Ba, Cd, Co, Cr, Cu, Fe, Hg, Mo, Ni, Pb, Sb, Se and Zn have been mostly referred as potentially toxic elements (PTEs), since their high concentrations are known to compromise ecosystem quality and affect the biosphere (Birani et al., 2015; Davis et al., 1978). Some plant species are more adequate than others to evaluate toxic element pollution, due to their low sensitivity and capacity to accumulate high levels of these elements in their leaf tissues (Hu et al., 2011). This ability depends on the structural, chemical and morphological leaf characteristics that govern foliar deposition, uptake and/or accumulation (Guzmán-Morales et al., 2011; Oliva and Mingorance, 2004). These species are powerful tools for determining geographic element distribution and their evolution over time (De Temmerman et al., 2004).

The studies using native plant species from the forest ecosystem, also referred to as passive biomonitoring by many authors, give a more realistic basis for evaluating potential effects caused by toxic element pollution and identifying their possible sources in a polluted environment (Falla et al., 2000). Such approach seems entirely applicable in tropical areas where the remaining forest ecosystems, which generally hold high plant diversity, are surrounded by numerous anthropogenic sources of PTEs, including cities, industrial pools, roads with intense traffic and extensive agricultural activities. This condition is depicted in the Campinas Metropolitan Region (CMR), central-eastern of São Paulo State/ Brazil, which houses the remaining portions of Atlantic Semideciduous Forest. Previous studies revealed that the soils of these forest remnants were moderately contaminated by trace elements of anthropogenic origin (Dafré-Martinelli, 2015) and that their three most abundant native tree species differed in leaf accumulation levels of few toxic elements (Domingos et al., 2015). These results stimulated a further study on the subject, aiming to answer the following questions: 1) which native tree species has the greatest potential to accumulate major and trace elements?; 2) does the leaf accumulation level of potentially toxic elements (PTEs) vary between the edge and interior of the forest remnants, between dry and wet periods or due to local anthropogenic emission sources?; 3) which tree species, among the most abundant in the forest remnants, would better discriminate the PTEs variation?

Material and methods

Description of study area and forest remnants

This study was conducted in an area of the Campinas Metropolitan Region (CMR), which consists of 20 cities, covering an area of 3.8 thousand km² and inhabited by 2.8 million people. Since the urban expansion during the 1970's, the region has become highly industrialized. In addition, soil and groundwater contamination with toxic chemicals, such as organochlorine pesticides and chemical elements (mainly Al, Cr, Fe, Mn and Pb) was verified. Blood contamination of residents with As and Pb, was also verified as a result of twenty years (from 1975 to 1995) of activities of a pesticide factory (Suassuna, 2001). Presently, 13,290 chemical, agro-chemical and petrochemical industries are established in the entire region, among other segments (http://www.campinas.sp.gov.br), including the largest petroleum refinery of the country that demarcates the central point of the study area (Fig.1). In addition, the region has intense agricultural activities, mainly sugarcane, one important economic activity of the region at the present time.

The predominant climate in the CMR is Cwa (humid subtropical zone with dry winter and hot summer), according to Koeppen's classification (Alvares et al., 2013). It is characterized by a hot and rainy season between October and March and a dry season between April and September, as shown in the climate diagram included in Moura et al. (2014). The winds predominantly originate from the southeast (Fig. 1). The concentration of atmospheric pollutants in the CMR is influenced by these seasonal weather conditions. The particulate matter, which is the main input matrix of PTEs in the forest remnants, is present in higher concentrations during dry periods (autumn and winter) and in lower concentrations during wet periods (spring and summer) (more details are available at Domingos et al., 2015 and Moura et al., 2014). Dafré-Martinelli (2015), characterizing the atmospheric deposition of PTEs in the most urbanized and industrialized areas of the study region, found a moderate enrichment of Cu, Pb and Zn in rainwater and of Cu in PM_{10} and in this same matrix a highly enrichment of Mo.

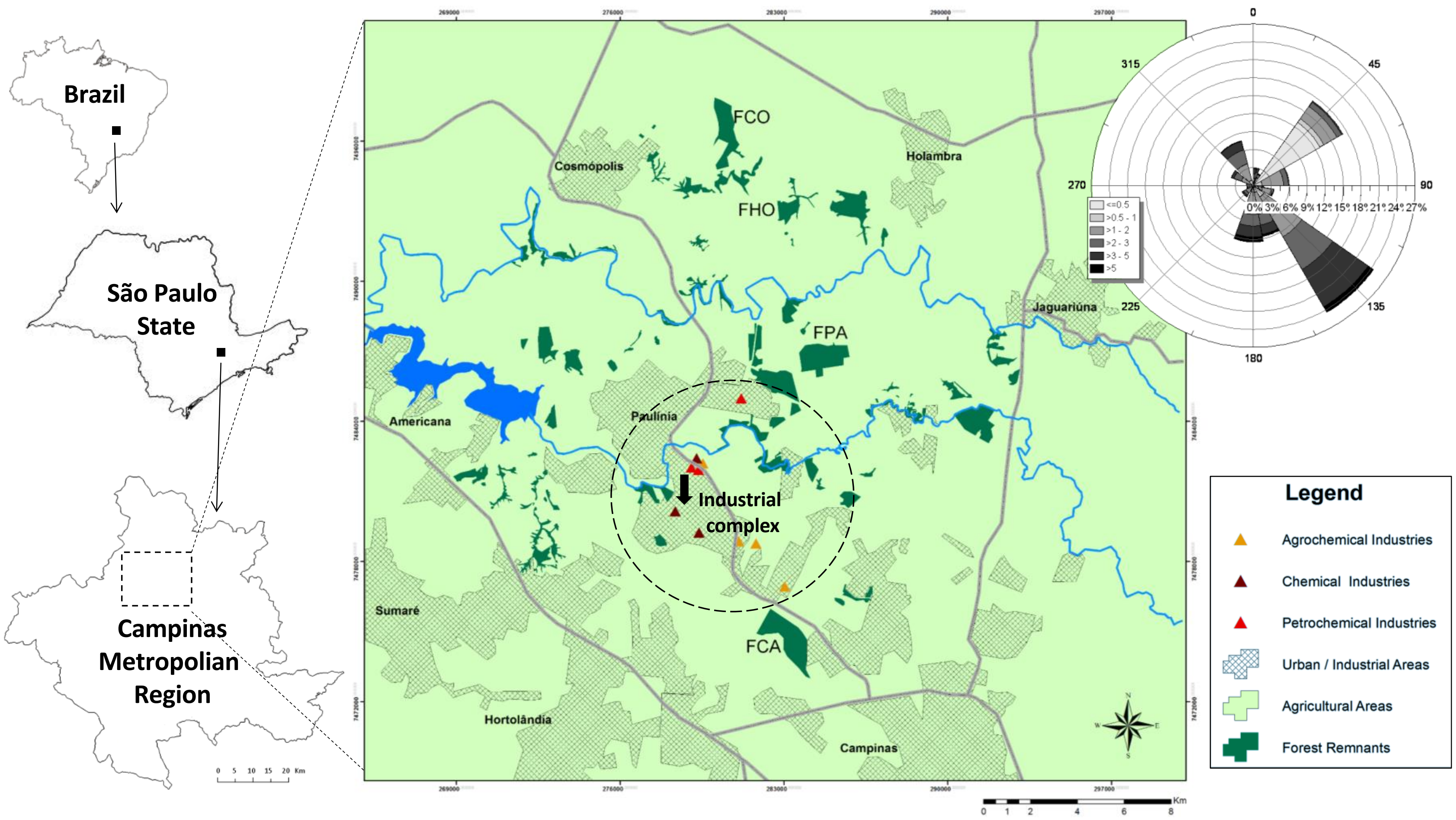


Figure 1 (modified from Domingos et al., 2015). Localization of the forest remnants studied and the prevailing winds at the Campinas Metropolitan Region (data of winds obtained from the air quality monitoring station of Environmental Company of São Paulo State (CETESB) installed in Paulínia city (22°46'18.60"S; 47° 9'15.09"W; location indicated by arrow symbol) and downloaded from www.cetesb.sp.org.br).

The regional native vegetation is classified as Semideciduous Forest, characterized by a number of tree species that partially lose their leaves during the dry season (Oliveira-Filho and Fontes, 2000) and has been fragmented in small and isolated “forest islands”. This forest is a subtype of the Atlantic Forest domain, which is characterized by high plant diversity and is the most threatened Biome in the country, as a consequence of increasing land use by man (Freitas et al., 2010; Lira et al., 2012).

Leaf samples of native tree species were collected in four forest remnants located in Campinas, Cosmópolis, Holambra and Paulínia (named as FCA, FCO, FHO and FPA respectively). FCA and FPA are located closer to emission sources of particulate matter, among them urban areas, intense vehicular traffic and petrochemical/chemical/agrochemical industries, than FCO and FHO. All forests are surrounded by agricultural land, mainly dedicated to sugarcane cultivation (Fig. 1; Table 1). The soils of the selected forest remnants are classified as Oxisols (Lopes et al., 2015), according USDA Soil Taxonomy, and were originated from different parental materials and have different textures (Table 1).

Table 1. Description of the forest remnants selected in the Campinas Metropolitan Region (CMR), São Paulo, Brazil.

Parameters	**FCA**	**FCO**	**FHO**	**FPA**
Cities	Campinas	Cosmópolis	Holambra	Paulínia
Latitude	22°49'17''S	22°37'22''S	22°39'21''S	22°42'47''S
Longitude	47°06'41''W	47°08'06''W	47°06'36''W	47°05'52''W
Size (ha)	235	120	35	190
Land use on surrounding	agricultural (sugarcane), urban	agricultural (sugarcane)	agricultural (citrus/ sugarcane)	agricultural (sugarcane), industrial
Distance to industrial area[1]	10	12	8	3.5
Soil texture [2]	clay	sandy clay loam	sandy clay loam	sandy clay loam
Parental material [2]	diabase	sandstone, diabase, siltstone	sandstone	diabase, sandstone
Tree density (N ind ha^{-1})[3]	1150	1575	1200	1256
Basal area (m^2 ha^{-1}) [3]	60.9	51.9	57.6	41.6

[1] Distance (km) calculated from the center of the remnant to the major petrochemical industry of CMR (indicated by white arrow in Fig. 1).

[2] Data obtained from Lopes et al. (2015).

[3] Phytosociological data estimated considering the six most abundant tree species, according Domingos et al. (2015).

Tree species and sampling approach

Astronium graveolens Jacq., *Croton floribundus* (L.) Spreng. and *Piptadenia gonoacantha* (Mart.) J.F. Macbr were three of the most abundant native tree species in the studied forest remnants, among other species represented by trees with a circumference at breast height (CBH) ≥ 30 cm. The distinct leaf morphologies and classification in different successional stages were additional criteria for their selection for this study (Domingos et al., 2015).

Fully expanded leaf samples and with similar age from four to five branches fully exposed to sun of four to six trees per species were collected during the 2011 and 2012 dry seasons (Aug/ Sep) and during the 2012 and 2013 wet seasons (Jan/ Feb) at four distinct locations of each forest remnant. Two of them were at the forest edge and the other two were within the forest (between 100 and 200 m from the edge), here referred as interior. After collection, the leaf samples were combined to one sample per species for each location, so that four composed samples were collected per species, forest remnant (two at the edge and two in the interior) and for each seasonal sampling campaign.

As expected, dry and wet seasons, regardless of the year, were distinguished by rainfall amount, air temperature and PM_{10} levels. Therefore, the sampling campaigns performed in the dry seasons (2011 and 2012) or in the wet seasons (2012 and 2013) were considered as replicates. The total amount of rainfall and mean temperature and PM_{10} concentrations registered during the 2011 dry season were 42 mm, 22ºC and 50 µg m^{-3} respectively. During the 2012 dry season, rainfall, mean temperature and PM_{10} concentrations were 36 mm, 22 ºC, 46 µg m^{-3}. The 2012 wet season was characterized by 460 mm, 26ºC and 24 µg m^{-3} and the 2013 wet season by 340 mm, 25ºC and 22 µg m^{-3}, as shown in Figure 2.

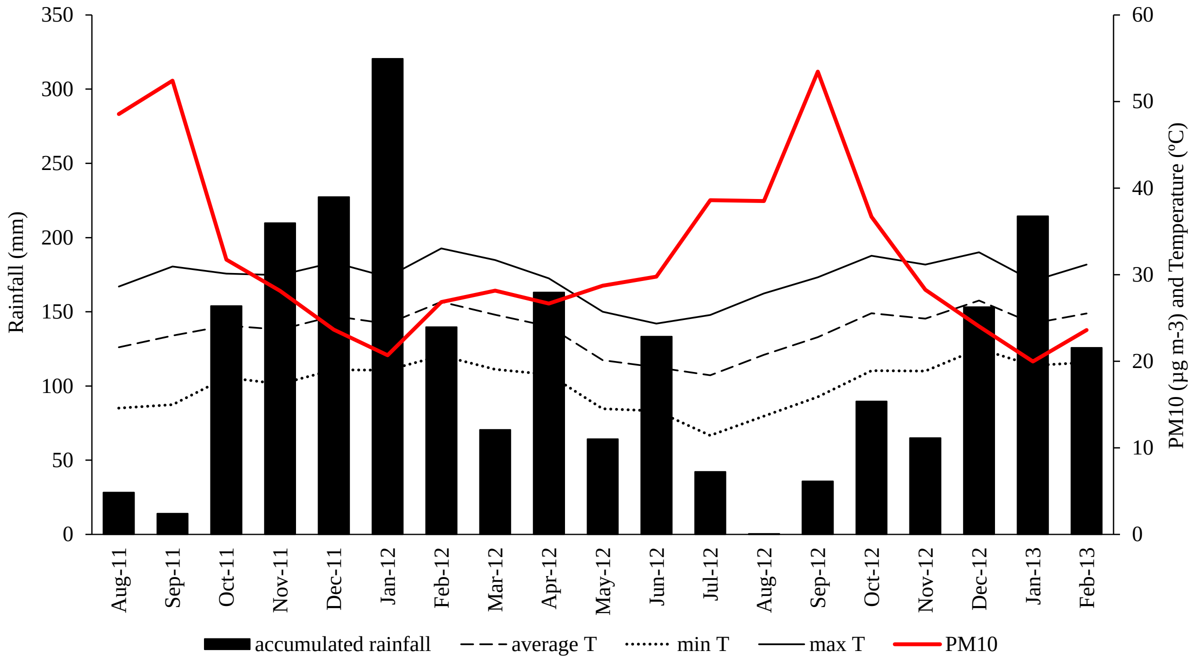


Figure 2. Monthly accumulated rainfall, minimum, mean and maximum temperatures from August 2011 to February 2013, obtained by Centre of Meteorological Research and Climate Applied to Agriculture (CEPAGRI) located in Campinas city (22°49'07"S; 47º03'43"W). Monthly mean PM_{10} concentrations from August 2011 to February 2013, obtained from the air quality monitoring station of Environmental Company of São Paulo State (CETESB), located in Paulínia city (22°46'18.60"S; 47° 9'15.09"W).

Chemical analysis

The unwashed leaves were dried under 40ºC to a constant weight, milled in an agate ball mill and stored in polypropylene vials. The concentrations of major and trace elements (Ag, Al, As, Au, B, Ba, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, Hg, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, Sb, Se, Sr, Te, Th, Ti, Tl, U, V, W and Zn) were determined at the AcmeLabs Laboratory (Vancouver, Canada), using inductively coupled plasma mass spectrometry (ICP MS) as analytical technique (PerkinElmer NEXION 300 ICP-MS), after digestion with *acqua regia* (HNO3:HCl, 1:1). The accuracy and precision of the analyses were verified by processing blanks and analyzing certified reference materials (http://acmelab.com/services/quality-control/).

The concentrations of rare-earth elements Ce, La, Lu, Nd, Sc, Sm, Tb and Yb were determined only in samples from the forest remnants located in the most urbanized and industrialized areas of the study region (Paulínia/FPA and Campinas/FCA; Fig.1), during the 2011 dry season and the 2012 wet season, by instrumental neutron activation analysis (INAA). The irradiation of samples and reference materials were carried out for 8 hours at a thermal neutron flux of 10^{12} n cm^{-2} s^{-1} at the nuclear research reactor IEA-R1, at the Instituto de Pesquisas Energéticas e Nucleares (IPEN), São Paulo. The measurements of the induced gamma-ray activity were carried out using a GX20190 hyperpure Ge detector. The multichannel analyzer was an 8192 channel Canberra S-100 plug-in-card in a PC computer. The resolution (FWHM) of the system was 1.90 keV for the 1332 keV gamma-ray of 60Co. The gamma-ray spectra were processed by using an in-house gamma-ray software, VISPECT. To evaluate the accuracy and precision of the data, two biological reference materials were analyzed: Orchard Leaves (NIST SRM 1571), and Apple Leaves (NIST SRM 1515). The results showed good accuracy (relative errors to certified values < 5% for most of elements) and good precision (relative standard deviations < 15%).

Data presentation and Statistics

All elemental concentrations were expressed on a leaf dry weight basis. First, the concentrations of all studied elements analyzed in leaves of *A. graveolens*, *C. floribundus* and *P. gonoacantha* were presented as mean, median, minimum and maximum values. The nonparametric Kruskal-Wallis One Way Analysis of Variance (ANOVA) on Ranks, followed by Dunn's method ($p < 0.05$) when necessary, was applied, using SIGMA PLOT (Version 11.0), to compare element concentrations among tree species.

Further statistical analyses were performed for the potentially toxic elements (PTEs) (Ba, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Sb, Se, Sr and Zn), as defined by Birani et al. (2015) and Davis et al. (1978).

A principal component analysis (PCA), using PCORD (Version 6.0), was applied to the variance/covariance matrix of data of the three species, collected in all forest remnants during the studied seasons (n= 183), in order to have a general graphic representation of element accumulation level in each species. All variables were $\log_{10}$ transformed to normalize the data. A Pearson correlation was performed with the variables and axis 1 and 2.

The nonparametric Mann–Whitney rank sum test ($p < 0.05$), using SIGMA PLOT (Version 11.0), was used to compare leaf concentrations of PTEs of all tree species, collected at the edge and in the interior of all forest remnants during the studied seasons.

Analyses of variance (Two-way ANOVA), followed by multiple comparison analysis (Holm-Sidak test), using SIGMA PLOT (Version 11.0), were performed to highlight significant differences in element concentrations among forest remnants (factor 1) and sampling seasons (factor 2). Interactions of both factors were tested. Data were transformed by rank or $\log_{10}$ when necessary to satisfy assumptions of normality and equal variances. Non-parametric ANOVA and Mann-Whitney rank sum test, as described before, were applied when normal distribution (Sb in all species, Se in *C. floribundus* and *A. graveolens*, Cd and Co in *A. graveolens*) or equal variances (Cr and Se in *A. graveolens* and *P. gonoacantha*, Ni in *C. floribundus* and *P. gonoacantha*, and Mo in *P. gonoacantha*) were not reached.

Forward stepwise discriminant function analysis was applied separately on the standardized dataset of each species, using STATISTICA (Version 7.1), in order to further investigate which species would indicate more appropriately the differences among forest remnants (groups) concerning the potential impact of PTEs (independent variables) and which elements would be the most relevant for such supposedly spatial discrimination. The highest classification function coefficients were used to determine to which group each element most likely belonged. Plots were also constructed with data of each species using the first two roots resulting from the standardized canonical coefficients proposed by the discriminant function analyses, to visualize which species would be more adequate to discriminate the spatial variations, by means of group centroids. The percentage of cases correctly classified in each group also helped to achieve this last goal.

Results

The concentrations of Ag, Al, As, Bi, Ga, Te, Th, Tl, U, V and W in leaves of all species were below the detection limits in most analyzed samples (the LD of these elements, in mg kg^{-1}, were 0.002; 100; 0.1; 0.02; 0.1; 0.02; 0.01; 0.02; 0.01; 2 and 0.1 respectively). So, they were excluded from further analyses.

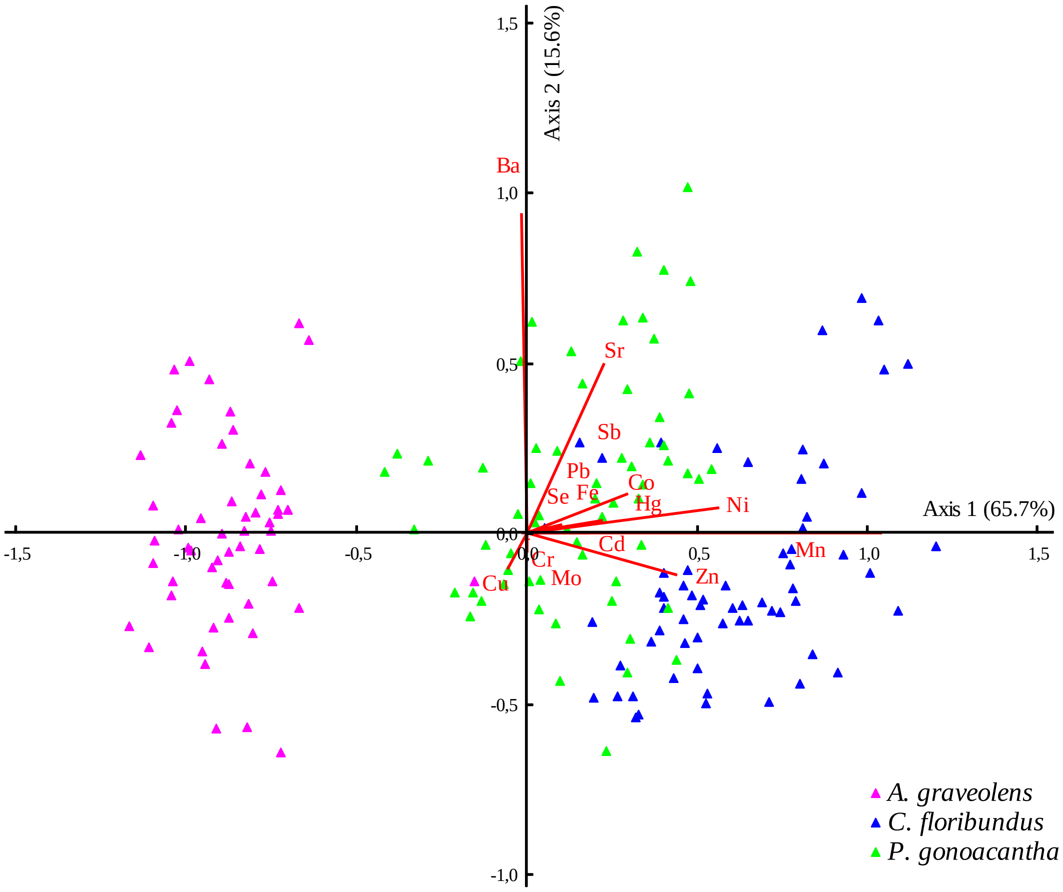
The leaf concentrations of Au, Cr, Lu, Sc and Se were similar in the three species. The highest contents of K, Cd, Co, Mg, Mo, Mn, P, Ti and Zn were observed in leaves of *C. floribundus*. *A. graveolens* and *P. gonoacantha* presented the highest leaf concentrations of B and Na, respectively. Leaves of *P. gonoacantha* and *C. floribundus* showed higher contents of Ca, Fe, Hg, Ni, Pb, S, Sb, Sm and Sr than observed in the leaves of *A. graveolens*. This last species and *C. floribundus* presented higher leaf contents of Cu than *P. gonoacantha*, which in turn accumulated more Ba, as well as *A. graveolens*, than *C. floribundus*. P. *gonoacantha* showed higher concentrations of the rare-earth elements than the other studied species (Table 2).

Table 2. Mean, median, minimum and maximum concentrations of all studied elements in leaves of *A. graveolens* (n=56), *C. floribundus* (n=64) and *P. gonoacantha* (n=63) collected during the dry and wet seasons in four forest remnants of Campinas Metropolitan Region (CMR).

		A.graveolens				***C. floribundus***				***P.gonoacantha***			
		Mean	**Med**	**Min**	**Max**	**Mean**	**Med**	**Min**	**Max**	**Mean**	**Med**	**Min**	**Max**
%	**Ca**	0.82	0.73 **b**	0.38	1.48	1.06	1.04 **a**	0.59	2.02	1.14	1.1 **a**	0.46	2.18
	Fe	0.02	0.02 **b**	0.01	0.06	0.04	0.03 **a**	0.01	0.25	0.03	0.03 **a**	0.01	0.22
	K	0.99	1 **b**	0.68	1.40	1.49	1.41 **a**	0.82	2.96	0.72	0.72 **c**	0.43	1.02
	Mg	0.29	0.29 **b**	0.19	0.43	0.39	0.38 **a**	0.19	0.63	0.26	0.26 **c**	0.19	0.38
	Mn	0.004	0.004 **c**	0.002	0.02	0.11	0.1 **a**	0.03	0.33	0.04	0.04 **b**	0.01	0.08
	P	0.16	0.16 **b**	0.12	0.23	0.19	0.18 **a**	0.12	0.33	0.12	0.12 **c**	0.08	0.17
	S	0.21	0.2 **b**	0.16	0.27	0.29	0.29 **a**	0.20	0.38	0.27	0.27 **a**	0.21	0.35
mg kg^{-1}	**B**	37.1	37 **a**	20.0	62	20.4	19.5 **c**	13.0	32	28.1	27 **b**	12.0	49
	Ba	36	33 **a**	6.0	101	25	18 **b**	9.6	105	42	31 **a**	5.9	176
	Cd	0.014	0.01 **b**	0.01	0.05	0.03	0.02 **a**	0.01	0.11	0.02	0.02 **b**	0.01	0.03
	Ce	1.21	0.79 **b**	0.26	2.89	1.85	1.82 **ab**	0.87	3.28	2.71	2.39 **a**	0.97	6.2
	Co	0.06	0.05 **c**	0.02	0.14	0.54	0.29 **a**	0.07	3.9	0.2	0.17 **b**	0.05	1.69
	Cr	1.8	1.9 **a**	0.8	2.7	1.8	1.8 **a**	0.7	3.4	1.8	1.8 **a**	0.8	3.2
	Cu	13	12 **a**	7.1	49	11	11 **a**	7.0	19.3	8.6	6.7 **b**	4.1	93.5
	La	0.99	0.73 **b**	0.24	2.60	1.37	1.27 **ab**	0.58	2.37	1.97	1.85 **a**	0.68	4.8
	Mo	0.15	0.11 **b**	0.03	1.1	0.22	0.18 **a**	0.04	1.1	0.1	0.1 **c**	0.03	0.3
	Na	24	20 **b**	10	50	24	20 **b**	10	60	37	30 **a**	10	180
	Nd	0.87	0.7 **b**	0.16	2.39	1.33	1.1 **ab**	0.36	3.24	1.6	1.1 **a**	0.7	3.82
	Ni	0.45	0.4 **b**	0.2	0.9	1.52	1.3 **a**	0.6	4.1	1.3	1.1 **a**	0.5	5.2
	Pb	0.31	0.26 **b**	0.1	0.73	0.45	0.37 **a**	0.04	1.88	0.4	0.38 **a**	0.15	1.24
	Sb	0.03	0.03 **b**	0.02	0.09	0.04	0.03 **a**	0.02	0.15	0.04	0.03 **a**	0.02	0.1
	Sc	0.06	0.05 **a**	0.01	0.17	0.12	0.11 **a**	0.01	0.52	0.08	0.06 **a**	0.01	0.36
	Se	0.17	0.15 **a**	0.1	0.4	0.19	0.2 **a**	0.1	0.4	0.2	0.2 **a**	0.1	0.4
	Sm	0.09	0.08 **b**	0.01	0.31	0.17	0.15 **a**	0.04	0.40	0.21	0.19 **a**	0.03	0.47
	Sr	76	68 **b**	34	149	108	91 **a**	45	305	122	102 **a**	51	301
	Ti	7.8	8 **b**	2	19	12	9.5 **a**	2	48	8.8	7 **b**	2	35
	Zn	12	11 **c**	8.1	20.7	24	22 **a**	15	39	15	14.6 **b**	9.6	43.1
µg kg^{-1}	**Au**	1.2	0.9 **a**	0.2	9.9	1.4	1.05 **a**	0.2	5.5	1.5	1.1 **a**	0.2	7
	Hg	27	28 **b**	10	59	46	37 **a**	13	120	35	34 **a**	9	66
	Lu	4.1	3.5 **a**	0.7	13	7.6	7.2 **a**	1.5	21	5.4	4.5 **a**	0.6	15
	Tb	11	9.2 **b**	2	31	20	19 **ab**	3	50	31	31 **a**	6	68
	Yb	24	21 **b**	5	72	49	47 **a**	4	123	34	30 **ab**	4	88

Different letters indicate significant differences ($p < 0.05$) among species, according Dunn’s Method.

The PCA summarized 81.3 % of the total variability of the data in the first and significant ($p < 0.001$) two axes (Fig. 3). The strongest correlation with axis 1 and 2 was observed for Mn ($r = 0.998$) and Ba ($r = 0.948$), respectively. PCA revealed an evident separation of tree species mainly by variables strongly correlated with axis 1. Samples of *A. graveolens* were grouped in the negative side of axis 1 and were only related with concentrations of Ba and Cu, as observed in Table 2. All samples of *C. floribundus* were located in the positive side of axis 1 and at least 70% of them were in the negative side of axis 2. Most of the samples of *P. gonoacantha* were also observed in the positive side of axis 1, wherein some of them are mixed with samples of *C. floribundus*, showing that both species present similar concentrations of some elements (for example, Fe, Ni and Hg) as observed in Table 2.



	Ba	Cd	Cr	Co	Cu	Fe	Mn	Mo	Ni	Pb	Sb	Sr	Zn
Axis 1	-0.127	0.415	0.006	0.534	-0.236	0.253	0.998	0.04	0.734	0.32	0.271	0.466	0.651
Axis 2	0.948	-0.034	-0.092	0.332	-0.322	0.075	-0.029	-0.242	0.27	0.155	0.177	0.692	-0.349

Figure 3. Principal component analysis (PCA) of PTEs determined in leaves of *A. graveolens* (n=56), *C. floribundus* (n=64) and *P. gonoacantha* (n=63), sampled in the four forest remnants of Campinas Metropolitan Region (CMR) during the dry and wet seasons. The table shows the correlation coefficients of each element to axis 1 and 2.

All species showed higher leaf concentrations of Mo at the edge than in the interior of the remnants. *A. graveolens* was the only species that also presented higher leaf contents of Cu and Ba in leaves collected at the edge. Higher leaf contents of Ni occurred in samples of *C. floribundus* collected within the forests. Leaves of *P. gonoacantha* sampled at the forest edge accumulated more Fe than leaves collected inside the forest. *C. floribundus* and *P. gonoacantha* leaves presented higher content of Mn and Sr in their leaves collected in the interior of remnants (Table 3).

Table 3. Median contents of PTEs, in mg kg^{-1}, found in leaves of *A. graveolens* (n=56), *C. floribundus* (n=64) and *P. gonoacantha* (n=63) collected at the edge and in the interior of all forest remnants during the dry and wet seasons.

	A. graveolens		***C. floribundus***		***P. gonoacantha***	
	Edge	**Interior**	**Edge**	**Interior**	**Edge**	**Interior**
Ba	34.0*	24.8	18.7	16.5	31.8	30.7
Cd	0.01	0.01	0.02	0.02	0.02	0.02
Co	0.06	0.05	0.44	0.25	0.23	0.13
Cr	1.85	1.90	1.70	1.80	1.90	1.80
Cu	13.0*	11.8	11.0	10.9	6.4	7.0
Fe	185	170	290	230	290*	240
Hg	0.02	0.03	0.03	0.04	0.03	0.04
Mn	33	40	727	1400*	302	466*
Mo	0.19*	0.08	0.23*	0.13	0.09*	0.06
Ni	0.40	0.40	1.00	1.55*	1.05	1.10
Pb	0.25	0.27	0.34	0.40	0.42	0.36
Sb	0.03	0.02	0.03	0.03	0.04	0.03
Se	0.20	0.10	0.20	0.10	0.20	0.20
Sr	67.1	67.7	71.8	115*	85.5	140*
Zn	12.5	10.9	21.4	25.7	14.5	15.1

Significant higher values ($p < 0.05$) observed in the edge or interior of remnants for each species were indicated with asterisks (*).

As indicated by two way ANOVA, interactions between seasons and forest remnants were not significant for the PTEs analyzed in leaves of *A. graveolens*, *C. floribundus* and *P. gonoacantha* sampled in FCA, FCO, FHO and FPA, during dry seasons (2011/2012) and wet seasons (2012/2013), except for the leaf contents of Zn in *P. gonoacantha*. The leaf contents of Cd, Cr, Mn and Mo in *A. graveolens*, Cd and Mo in *C. floribundus* and Cr and Mo in *P. gonoacantha* did not vary among seasons and forest remnants. Samples collected in FCO and FPA contained more Zn during the wet season than during dry season (Table 4).

All species showed higher leaf concentrations of Co, Fe, Hg, Pb, Sb and Se during the dry season. *A. graveolens* and *C. floribundus* presented higher leaf concentrations of Zn during the wet season. Higher contents of Ni in leaf samples of *A. graveolens* and *P. gonoacantha* and of Mn in leaf samples of *C. floribundus* and *P. gonoacantha* were measured during dry seasons. *P. gonoacantha* also showed higher values of Ba and Sr in the dry season and *C. floribundus* showed higher content of Cr during the dry season and of Cu during the wet season (Table 4).

In both seasons, spatial variations were not observed in the leaf contents of Mo and Se in all species, Cd, Hg, Mn and Pb in *A. graveolens* and *C. floribundus*, Cr in *A. graveolens* and *P. gonoacantha*, Ni in *A. graveolens*, Cu in *C. floribundus* and Zn in *P. gonoacantha*. Distinct spatial patterns were identified for the other elements, depending on the species. For example, the leaves of *A. graveolens* collected in the FCA forest were more concentrated in Fe, Sb and Zn than at least two other forest remnants. Similar results were obtained for *C. floribundus* concerning Co, Fe, Ni and Zn and for *P. gonoacantha* concerning Ba, Cd, Co, Fe, Hg and Sb. The plants of all species from the FHO forest were also more concentrated in Sr and Ba than observed in at least two other forest remnants (Table 4).

Table 4. Mean concentrations of PTEs, in mg kg^{-1}, in leaves of *A. graveolens*, *C. floribundus* and *P. gonoacantha* collected in forest remnants of Campinas (FCA), Cosmópolis (FCO), Holambra (FHO) and Paulínia (FPA) during the dry (2011/2012) and the wet (2012/2013) seasons.

		Dry season				**Wet season**				**p1**	**p2**	**p3**
		FCA	**FCO**	**FHO**	**FPA**	**FCA**	**FCO**	**FHO**	**FPA**			
A. graveolens	**Ba**	46.4 **ab**	32.1 **b**	66.0 **a**	18.7 **c**	40.0 **ab**	25.2 **b**	51.2 **a**	16.5 **c**	<0.001	0.111	0.988
	Cd	0.016 **a**	0.015 **a**	0.013 **a**	0.011 **a**	0.013 **a**	0.023 **a**	0.012 **a**	0.013 **a**	0.194	0.831	—
	Co	0.10 **a***	0.07 **ab***	0.07 **ab***	0.05 **b***	0.06 **a**	0.03 **ab**	0.03 **ab**	0.03 **b**	0.003	<0.001	—
	Cr	1.85 **a**	1.97 **a**	1.85 **a**	1.88 **a**	1.43 **a**	1.95 **a**	1.48 **a**	1.86 **a**	0.393	0.151	—
	Cu	11.9 **ab**	14.1 **a**	11.0 **b**	12.1 **ab**	13.2 **ab**	14.6 **a**	15.2 **b**	10.7 **ab**	0.008	0.464	0.322
	Fe	306 **a***	343 **ab***	242 **bc***	193 **c***	165 **a**	130 **ab**	102 **bc**	105 **c**	<0.001	<0.001	0.113
	Hg	0.038 **a***	0.039 **a***	0.038 **a***	0.034 **a***	0.018 **a**	0.015 **a**	0.015 **a**	0.019 **a**	0.954	<0.001	0.417
	Mn	42.6 **a**	42.8 **a**	40.5 **a**	35.3 **a**	39.0 **a**	35.0 **a**	33.5 **a**	52.6 **a**	0.884	0.365	0.599
	Mo	0.12 **a**	0.16 **a**	0.09 **a**	0.12 **a**	0.15 **a**	0.37 **a**	0.15 **a**	0.10 **a**	0.254	0.310	0.849
	Ni	0.56 **a***	0.42 **a***	0.48 **a***	0.60 **a***	0.39 **a**	0.35 **a**	0.38 **a**	0.39 **a**	0.082	<0.001	0.347
	Pb	0.45 **a***	0.36 **a***	0.40 **a***	0.41 **a***	0.24 **a**	0.20 **a**	0.17 **a**	0.21 **a**	0.347	<0.001	0.839
	Sb	0.05 **a***	0.03 **b***	0.03 **b***	0.03 **b***	0.03 **a**	0.02 **b**	0.02 **b**	0.02 **b**	0.019	<0.001	—
	Se	0.24 **a***	0.23 **a***	0.18 **a***	0.20 **a***	0.15 **a**	0.10 **a**	0.10 **a**	0.16 **a**	0.344	<0.001	—
	Sr	73.6 **bc**	85.4 **ab**	111 **a**	59.2 **c**	59.0 **bc**	87.8 **ab**	91.1 **a**	58.4 **c**	<0.001	0.192	0.553
	Zn	13.3 **a**	9.9 **b**	10.7 **ab**	9.9 **b**	14.6 **a***	12.1 **b***	13.2 **ab***	12.0 **b***	<0.001	<0.001	0.799
C. floribundus	**Ba**	28.6 **b**	19.4 **b**	47.7 **a**	14.1 **c**	21.5 **b**	17.0 **b**	40.3 **a**	14.9 **c**	<0.001	0.065	0.456
	Cd	0.04 **a**	0.02 **a**	0.03 **a**	0.02 **a**	0.03 **a**	0.05 **a**	0.03 **a**	0.02 **a**	0.204	0.875	0.567
	Co	0.67 **b***	0.33 **c***	1.71 **a***	0.23 **c***	0.48 **b**	0.11 **c**	0.66 **a**	0.13 **c**	<0.001	<0.001	0.214
	Cr	2.16 **ab***	2.39 **a***	1.55 **b***	1.95 **ab***	1.48 **ab**	1.73 **a**	1.31 **b**	1.66 **ab**	0.020	0.001	0.621
	Cu	11.1 **a**	10.2 **a**	9.2 **a**	9.6 **a**	13.3 **a***	11.8 **a***	12.1 **a***	11.1 **a***	0.062	<0.001	0.727
	Fe	576 **a***	929 **a***	344 **b***	330 **b***	244 **a**	198 **a**	124 **b**	126 **b**	<0.001	<0.001	0.361
	Hg	0.062 **a***	0.079 **a***	0.062 **a***	0.061 **a***	0.028 **a**	0.026 **a**	0.023 **a**	0.02 **a**	0.596	<0.001	0.862
	Mn	1179 **a***	1317 **a***	1596 **a***	1316 **a***	725 **a**	967 **a**	1116 **a**	962 **a**	0.053	0.010	0.995
	Mo	0.21 **a**	0.18 **a**	0.18 **a**	0.17 **a**	0.24 **a**	0.26 **a**	0.34 **a**	0.19 **a**	0.330	0.399	0.946
	Ni	1.80 **a**	1.24 **b**	1.93 **ab**	1.16 **b**	1.70 **a**	1.01 **b**	2.04 **ab**	1.28 **b**	0.008	0.518	—
	Pb	0.69 **a***	0.76 **a***	0.51 **a***	0.50 **a***	0.28 **a**	0.29 **a**	0.23 **a**	0.30 **a**	0.497	<0.001	0.509
	Sb	0.079 **a***	0.049 **a***	0.040 **a***	0.043 **a***	0.031 **a**	0.024 **a**	0.028 **a**	0.025 **a**	0.098	<0.001	—
	Se	0.20 **a***	0.26 **a***	0.21 **a***	0.24 **a***	0.18 **a**	0.14 **a**	0.10 **a**	0.18 **a**	0.364	<0.001	—
	Sr	79 **c**	120 **ab**	165 **a**	90 **bc**	62 **c**	120 **ab**	140 **a**	84 **bc**	<0.001	0.314	0.892
	Zn	24.9 **a**	19.5 **b**	17.9 **b**	18.2 **b**	30.5 **a***	28.2 **b***	26.3 **b***	26.3 **b***	<0.001	<0.001	0.699
P. gonoacantha	**Ba**	37.9 **b***	27.5 **c***	88.6 **a***	30.5 **c***	31.2 **b**	17.8 **c**	80.7 **a**	20.0 **c**	<0.001	0.013	0.552
	Cd	0.021 **a**	0.019 **a**	0.018 **b**	0.014 **b**	0.016 **a**	0.021 **a**	0.011 **b**	0.014 **b**	0.024	0.189	0.282
	Co	0.33 **a***	0.23 **b***	0.55 **a***	0.16 **b***	0.19 **a**	0.07 **b**	0.28 **a**	0.07 **b**	<0.001	<0.001	0.586
	Cr	2.06 **a**	2.16 **a**	1.58 **a**	1.74 **a**	1.63 **a**	1.85 **a**	1.45 **a**	1.86 **a**	0.224	0.351	—
	Cu	7.58 **a**	5.81 **b**	10.51 **ab**	6.42 **ab**	7.82 **a**	5.75 **b**	16.92 **ab**	7.34 **ab**	0.002	0.643	0.471
	Fe	550 **a***	759 **ab***	281 **b***	283 **b***	296 **a**	161 **ab**	148 **b**	144 **b**	0.004	<0.001	0.605
	Hg	0.053 **a***	0.046 **c***	0.043 **bc***	0.050 **ab***	0.026 **a**	0.017 **c**	0.022 **bc**	0.026 **ab**	0.003	<0.001	0.341
	Mn	316 **b***	594 **a***	459 **a***	529 **a***	198 **b**	384 **a**	317 **a**	458 **a**	<0.001	<0.001	0.547
	Mo	0.11 **a**	0.08 **a**	0.06 **a**	0.07 **a**	0.08 **a**	0.10 **a**	0.11 **a**	0.11 **a**	0.347	0.448	—
	Ni	1.28 **ab***	1.08 **b***	2.28 **a***	1.14 **ab***	1.10 **ab**	0.71 **b**	1.51 **a**	1.10 **ab**	0.009	0.025	—
	Pb	0.74 **a***	0.57 **ab***	0.48 **b***	0.51 **ab***	0.37 **a**	0.26 **ab**	0.23 **b**	0.34 **ab**	0.008	<0.001	0.415
	Sb	0.07 **a***	0.04 **b***	0.04 **b***	0.04 **ab***	0.04 **a**	0.02 **b**	0.03 **b**	0.03 **ab**	0.004	<0.001	—
	Se	0.21 **a***	0.26 **a***	0.19 **a***	0.23 **a***	0.18 **a**	0.16 **a**	0.13 **a**	0.23 **a**	0.083	0.014	—
	Sr	118 **b***	158 **ab***	201 **a***	116 **b***	75 **b**	91 **ab**	137 **a**	82 **b**	0.001	<0.001	0.832
	Zn	15.5 **a**	12.5 **a**	14.2 **a**	12.6 **a**	15.8 **a**	16.4 **a***	18.4 **a**	16.7 **a***	0.215	<0.001	0.047

Distinct letters indicate significant differences ($p < 0.05$) among forest remnants for each species. * Indicates significantly higher values ($p < 0.05$) in one seasonal period compared to the other. **p1< 0.05:** indicates significant effects of factor 1 (forest remnants); **p2< 0.05:** indicates significant effects of factor 2 (seasons); **p3< 0.05:** indicate significant interactions between factor 1 and 2.

The discriminant function analysis summarized the spatial distribution of PTEs analyzed in each species. The discrimination among forest remnants was confirmed for *A. graveolens* (Wilk’s lambda = 0.041; p< 0.0000), *C. floribundus* (Wilk’s lambda = 0.065; p<

0.0000) and *P. gonoacantha* (Wilk's lambda = 0.040; $p<0.0000$). The function classification cross-validated correctly, on average, 87.5% of cases of *A. graveolens* (FCA:81.3%; FCO:100%; FHO:83.3% and FPA:87.5%), 84.4% of cases of *C. floribundus* (FCA:81.3%; FCO:87.5%; FHO:81.3% and FPA:87.5%) and 85.7% of cases of *P. gonoacantha* (FCA:93.8%; FCO:81.3%; FHO:86.7% and FPA:85.7%).

The first two discriminant functions (roots 1 and 2) for all tree species were highly significant ($p<0.001$). Discriminant plots for *A. graveolens*, *C. floribundus* and *P. gonoacantha* explained respectively 86% (root 1: 51%; root 2: 34.8%), 75% (root 1: 47.1%; root 2: 7.8%) and 94% (root 1: 73.1%; root 2: 20.9%) of total variability among forest remnants (Fig. 4).

The first discrimination function (Wilk's lambda = 0.041) for *A. graveolens* was mainly defined by leaf contents of Fe, Zn and Sb (with standardized canonical coefficients 1.269, -1.243 and -1.000, respectively). Ba (0.841 standardized canonical coefficient) was more strongly related with the second discriminant function (Wilk's lambda = 0.170). The first root extracted from data of *C. floribundus* samples (Wilk's lambda = 0.065) was rather related to Zn and Mo (with standardized canonical coefficients of -0.991 and 0.887, respectively). Sb and Ba (with standardized canonical coefficients of -0.609 and -0.535, respectively) seemed to be linked to second root (Wilk's lambda = 0.206). Leaf concentrations of Mn, Zn and Ba (with standardized canonical coefficients of -1.708, 1.511 and 1.240, respectively) in *P. gonoacantha* samples showed great association with the first root (Wilk's lambda = 0.040) and Cu (-1.116 standardized canonical coefficient) to the second root (Wilk's lambda = 0.265).

The percentage of group cases correctly classified and the group centroids (Fig. 4) clearly showed that *A. graveolens* better discriminated the samples of all the forest remnants. *C. floribundus* and *P. gonoacantha* could not distinguish the forest remnants of Cosmópolis (FCO) and Paulínia (FPA) so well as *A. graveolens.*

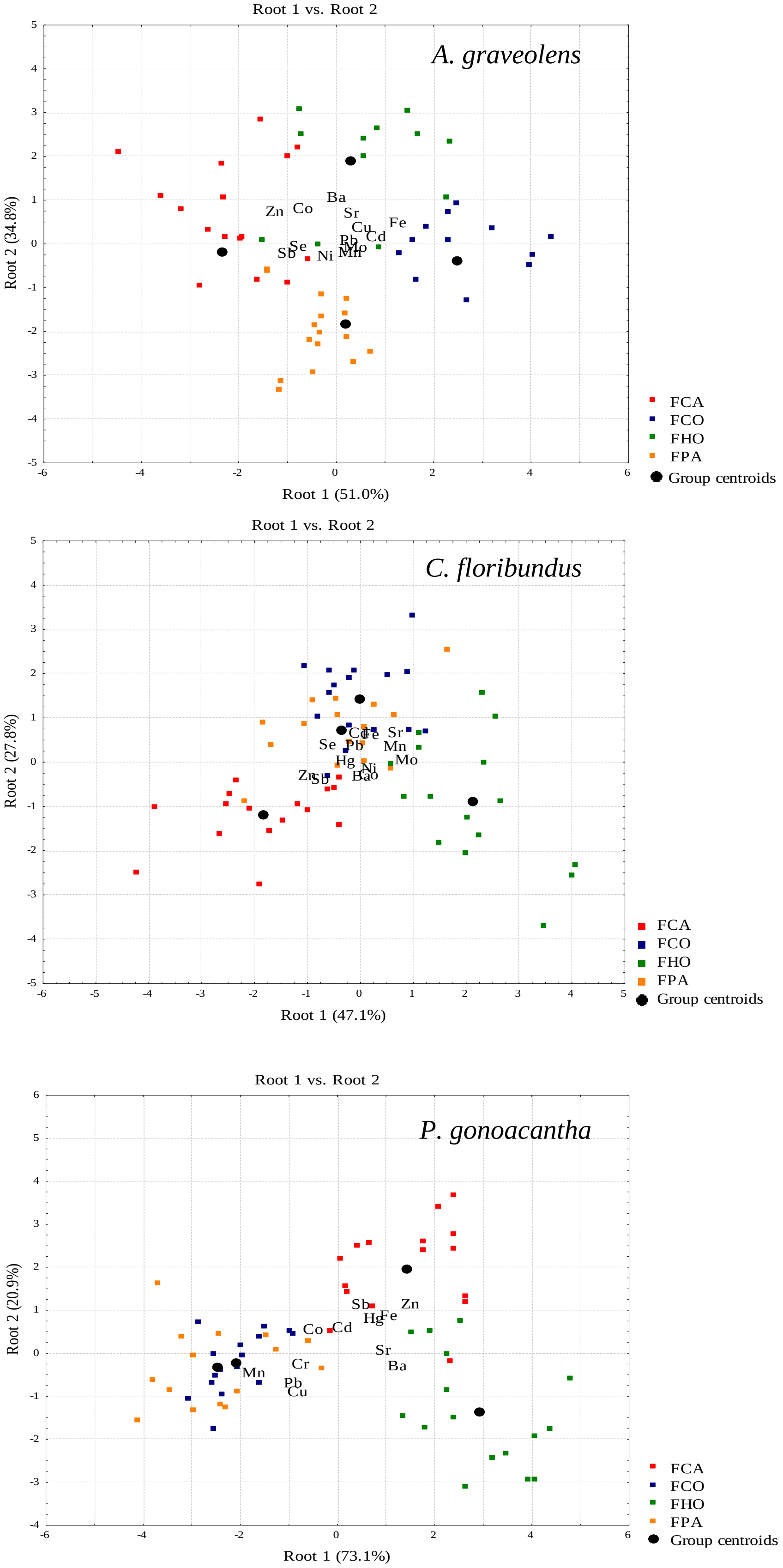


Figure 4. Graphic representation of discriminant analyses performed with the concentrations of PTEs in leaves of *A. graveolens*, *C. floribundus* and *P. gonoacantha* collected in the four forest remnants (FCA, FCO, FHO and FPA), during all the studied seasons. The canonical group centroids per species for each forest remnant were represented by black circles.

The classification function coefficients (Table 5) applied to the data of *A. graveolens* indicated that Campinas (FCA) was more discriminated by Co, Sb, Se and Zn than by the other elements, Cosmópolis (FCO) by Cd, Cu, Fe and Mo, Holambra (FHO) by Ba, Pb and Sr and Paulínia (FPA) by Mn and Ni. Considering *C. floribundus,* FCA was marked by Ba, Hg, Sb and Zn, FCO by Cd, Fe and Sr, FHO by Ba, Co, Mn, Mo and Sr and FPA by Ni, Pb, Se. Coefficients proposed with the data of *P. gonoacantha* showed that FCA was more related to Cd, Fe, Hg, Sb and Zn than to the other elements, FCO to Co and Cu, FHO to Ba and Sr and FPA to Co, Cr, Cu, Mn and Pb. In brief, based on data of all the species, FCA was strongly related to Sb and Zn and FHO with Ba and Sr, while at least two species showed that FCO was related with Cd, Cu and Fe and FPA with Mn, Ni and Pb.

Table 5. Classification function coefficients of the discriminant analysis for the PTEs in leaves of *A. graveolens*, *C. floribundus* and *P. gonoacantha* sampled in Campinas (FCA), Cosmópolis (FCO), Holambra (FHO) and Paulínia (FPA) during all the studied seasons.

	A. graveolens				*C. floribundus*				*P. gonoacantha*			
	FCA	**FCO**	**FHO**	**FPA**	**FCA**	**FCO**	**FHO**	**FPA**	**FCA**	**FCO**	**FHO**	**FPA**
Ba	0.247	-0.642	**2.752**	-1.829	**0.678**	-1.011	**0.645**	-0.312	1.192	-4.297	**6.576**	-3.703
Cd	-1.336	**2.975**	-0.396	-0.598	-0.239	**1.491**	-0.412	-0.840	**0.921**	0.227	-0.219	-0.991
Co	**2.389**	-1.651	0.218	-1.314	0.612	-0.073	**1.033**	-1.572	-0.057	**1.081**	-2.094	**1.142**
Cr	*not in model*				*not in model*				-2.044	1.554	-1.588	**2.216**
Cu	-1.172	**1.221**	0.880	-0.404	*not in model*				-3.375	**2.099**	-0.832	**2.249**
Fe	-2.270	**3.966**	0.804	-1.308	0.157	**3.805**	0.217	-4.179	**2.836**	-1.642	2.261	-3.685
Hg	*not in model*				**1.033**	0.737	-0.365	-1.405	**2.268**	-2.317	1.416	-1.457
Mn	-1.074	0.579	-0.259	**0.834**	-1.576	-0.386	**1.297**	0.665	-4.342	3.886	-4.748	**5.552**
Mo	-0.745	**1.626**	-0.866	0.175	-1.385	-0.202	**2.046**	-0.459	*not in model*			
Ni	-0.314	-1.042	-0.380	**1.380**	-0.147	-1.391	0.758	**0.780**	*not in model*			
Pb	-1.307	-0.089	**0.976**	0.642	-1.051	-2.922	-0.504	**4.477**	-3.120	1.769	-1.402	**2.936**
Sb	**2.740**	-1.766	-2.838	0.713	**2.463**	-0.188	-1.087	-1.189	**2.873**	-1.557	0.222	-1.640
Se	**1.588**	-1.821	-1.245	0.711	0.584	-0.727	-1.526	**1.669**	*not in model*			
Sr	-0.362	1.083	**1.215**	-1.362	-1.827	**1.493**	**1.258**	-0.924	1.132	-1.177	**3.329**	-3.503
Zn	**3.381**	-3.559	-0.006	-0.708	**2.819**	-0.027	-1.709	-1.082	**3.925**	-3.516	3.119	-3.763
Constant	-4.008	-5.591	-4.105	-3.518	-3.834	-3.090	-4.115	-3.048	-4.283	-3.982	-6.623	-4.892

The highest coefficients for element in each species are detached in **bold.**

Discussion

C. floribundus, followed by *P. gonoacantha*, showed a better capacity to accumulate the majority of the studied elements in their leaves than *A. graveolens*. Domingos et al. (2015) have already raised the hypothesis that *C. floribundus* is a good accumulator of Cu, Cd, Mn, Ni, S and Zn, attributing such a characteristic to the high content of epicuticular wax and dense layer of stellar trichomes on leaf surfaces. These leaf traits seem to facilitate the capture of particulate matter containing major and trace elements by *Croton* leaves, despite the lower leaf area and dry mass, compared to the dimensions of *A. graveolens* leaves (Domingos et al., 2015).

Tomašević et al. (2011), studying trace metal accumulation in unwashed tree leaves from an urban area of Belgrado, found similar concentrations of Cd (0.04 mg kg^{-1}), Cr (1.55 mg kg^{-1}), Cu (11 mg kg^{-1}), Fe (0.04 %) and Zn (19 mg kg^{-1}) in *Aesculus hippocastanum*, of Co (0.46 mg kg^{-1}) in *Betula pendula* and of Sr (89 mg kg^{-1}) in *Tilia cordata*, compared to those the average leaf contents of *C. floribundus* (Table 2). Guzmán-Morales et al. (2011) also observed similar concentrations of Cr (1.63 mg kg^{-1}), Cu (12.3 mg kg^{-1}) and Ni (1.81 mg kg^{-1}) in unwashed leaves of *Ficus benjamina*, in urban areas of Mexico, in relation to mean contents measured in leaves of *C. floribundus*. In contrast, *C. floribundus* showed lower mean leaf concentrations of Pb and Sb (0.45 and 0.04, in mg kg^{-1}, respectively) than observed in *F. benjamina* (Pb - 4.59 mg kg^{-1} and Sb -0.302 mg kg^{-1}) (Guzmán-Morales et al., 2011). Leaves of *A. hippocastanum* (Tomašević et al., 2011) also contained more Pb than *C. floribundus* leaves (5.93 mg kg^{-1}). Leaf contents of Mn observed in *C. floribundus* (0.11%) were distinguishably higher not only than *A. graveolens* and *P. gonoacantha*, but also than those found in *A. hippocastanum* (0.005%), *A. platanoides* (0.021%), *B. pendula* (0.005%) and *T. cordata* (0.004%) by Tomašević et al. (2011).

The mean leaf content of Mn in *C. floribundus* was also five times higher than the minimum critical level of toxicity (0.02%) according Krämer (2010). Although this element is essential to plants, participating in the structure of photosynthetic proteins and enzymes, Mn may alter enzymatic activity, absorption, translocation and the use of other mineral elements (Ca, Mg, Fe and P), when in excess in plant tissues. It may also cause oxidative stress, which can damage the photosynthetic apparatus, among other cell disturbances (Millaleo et al., 2010). However, Domingos et al. (2015) found that *C. floribundus* may tolerate stronger environmental oxidative stress than the other species, attributable to biochemical markers, such as high redox state of ascorbate and high content of glutathione. However, this species could not be considered a Mn hyperaccumulator, once the average concentrations measured in *C. floribundus* leaves were below 10,000 mg kg^{-1}, which was the limit concentration suggested by Krämer (2010) for defining a species as a Mn hyperaccumulator.

Higher element leaf content in plants collected at the remnant edges were expected, since they are more exposed to disturbances and agro-nutrient inputs than plants inside the forest (Bergkvist et al., 1989; Ishino et al., 2012; Pellissier et al., 2013). However, differences between forest edge and interior were not confirmed in our study for most of the trace elements analyzed in all three species, showing perhaps that the edge effect was deeper in the forest than initially expected. The edge influence depends on many variables, extending into the forest for just few meters or for several hundred meters (Pellissier et al., 2013). Only molybdenum (Mo) was found in higher levels in leaves of all species collected at the forest edge than in the interior (Table 3).

The higher leaf contents of most of the studied PTEs occurred during the dry season, coinciding with the low rainfall amounts and high particulate matter concentrations in the region. The rain may remove particulate matter deposited on the leaf surfaces (Przybysz et al., 2014), explaining the mentioned differences between the dry and wet seasons.

The three species growing in the forest remnant located in Paulínia (FPA), the closest remnant to the industrial complex, showed similar or lower leaf contents of most of PTEs than other farther remnants (Table 4), contrasting with the usual inverse association proved in many studies between leaf concentrations and distance from specific emission sources (Keane et al., 2001). Other anthropogenic sources of PTEs occur throughout the region, such as vehicular traffic and agricultural activities that may help to explain the absence of spatial variation in foliar content of several elements. In addition, although the prevailing yearly wind in the CMR is northwest (Fig. 1), the wind direction changes typically between east and south during the day, which may promote a mixture of air pollutants in the whole area, as discussed by Bulbovas et al. (2015).

A. graveolens showed to be the best species to indicate the PTE pollution variability, even considering its low accumulating capacity of most of the elements compared to the other tree species (Table 2; Fig. 3). The results of discriminant analyses applied on the dataset of such species, which were corroborated by those from analysis applied on the dataset of at least one of the other two tree species sampled, highlighted that the most urbanized forest (FCA) was distinguished from the others by elements usually related to traffic emissions (Zn and Sb) (Bosco et al., 2005; Celo and Dabek-Zlotorzynska 2010). The discriminant analyses also indicated that the forest close to the industrial area (FPA) was discriminated by elements, which may have been originated from oil burning, such as Mn and Ni (Calvo et al., 2013) and the forest located in the rural area (FCO) by Cd and Cu, which may have originated from agricultural activities (Nagajyoti et al., 2010; Nanos and Martin 2012) and (FHO) by Ba and Sr. Since this last forest is located in a low-traffic area, these elements may be of crustal origin according to Calvo et al. (2013). This assumption was reinforced by the high contents of Ba and Sr naturally found in the FHO soil (Dafré-Martinelli, 2015). Therefore, *A. graveolens* appeared to be an attractive candidate for biological monitoring of PTEs pollution in the remaining forest ecosystems from Campinas Metropolitan Region (CMR).

Conclusions

Leaves of *Croton floribundus*, followed by *P. gonoacantha*, showed higher ability of accumulating the majority of the elements analyzed, including macronutrients, rare-earths and PTEs than *A. graveolens* leaves. Leaf content of Mn in *C. floribundus* exceeded the leaf contents of tree species sampled in other studies. However, this species may not be considered as a Mn hyperaccumulator.

The edge effect was not evidenced for most of PTEs in all species, probably due to disturbances covering a larger area than 200 m from the edge of the forest. Higher concentrations of the most analyzed PTEs were measured during the dry season in all species. The distance of the forest remnant from industrial complex was not a relevant parameter for inferring associations between leaf accumulation and industrial emissions.

A. graveolens showed to be the most appropriate species to discriminate spatial variations on PTEs pollution in the region, even though the lowest leaf concentrations of most elements were observed in this species.

Acknowledgments

The authors thank *Programa SISBIOTA-BRASIL* (proc. CNPQ 563335/2010; proc. FAPESP 2010/52319-2) for financial support; CAPES for offering a Ph.D. scholarship to the first author; ARIES *“Mata de Santa Genebra”* and *“Matão de Cosmópolis”* and *Cooperativa Agropecuária Holambra* for permitting the collections in their properties.

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Capítulo 5

Discussão e conclusões gerais

Discussão Geral

Em toda a biosfera e em cada ecossistema existem inter-relações entre os teores de elementos traço no ar, solo e plantas. Em geral, a composição química das plantas reflete a composição química do meio em que vive (Greger, 2004; Voutsa et al., 1996).

Compostos metálicos solúveis e/ou metais ligados em complexos de troca na solução do solo estão disponíveis para a absorção radicular (Machender et al., 2011). Além disso, partículas finas e ultrafinas de material particulado (MP) enriquecidas com metais traço, podem ser incorporadas nas folhas através da cutícula ou abertura estomática, enquanto aglomerados maiores de partículas tendem a ficar presos na cera da superfície foliar (Schreck et al., 2012).

Portanto, neste capítulo, objetiva-se avaliar as possíveis rotas de entrada dos elementos químicos encontrados nas folhas das três espécies arbóreas, uma vez que até então avaliamos separadamente as concentrações dos elementos, principalmente dos metais traço ambientalmente relevantes nas amostras de solo, de água de chuva, deposição seca, MP_{10} e folhas das espécies arbóreas nativas coletadas nos fragmentos florestais da Região Metropolitana de Campinas. No entanto, vale salientar que somente as folhas das três espécies foram coletadas em todos os período de estudo (seco de 2011 e de 2012 e úmidos de 2012 e 2013). Além do mais, amostras de água de chuva, de deposição seca e MP_{10} foram coletadas somente nos fragmentos de Campinas (FCA) e Paulínia (FPA), de modo que esta análise comparativa será realizada apenas com dados provenientes destes locais.

Vale salientar, ainda, que as técnicas analíticas utilizadas e/ou limites de detecção dos métodos, nem sempre possibilitaram determinar as concentrações dos mesmos elementos nas diferentes matrizes estudadas. Dentre os elementos químicos, com maior relevância ambiental (Ba, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Sb, Se, Sr e Zn) observados nas folhas de plantas, somente Co, Hg, Sb e Se não foram determinados em outra matriz. A tabela 1 mostra as concentrações médias dos elementos traço determinados acima dos limites de detecção em pelo menos 4 das 7 matrizes avaliadas nos fragmentos da RMC durante o período de estudo.

Tabela 1. Concentrações médias de elementos - traço em folhas de *A. graveolens, C. floribundus* e *P. gonoacantha*, solos (totais e disponíveis), eventos de água de chuva, deposição seca e material particulado < 10 µg (MP$_{10}$), coletados nos fragmentos florestais de Campinas (FCA), Cosmópolis (FCO), Holambra (FHO) e Paulínia (FPA) em todo o período de estudo.

Local	Amostras	Ba	Cd	Cr	Cu	Fe	Mn	Mo	Ni	Pb	Sr	Zn
FCA	*A. graveolens*	43.2	0.01	1.64	12.5	236	40.8	0.1	0.5	0.3	66	14
	C.floribundus	25.0	0.03	1.82	12.2	410	952	0.2	1.8	0.5	71	28
	P. gonoacantha	34.5	0.02	1.84	7.7	423	257	0.1	1.2	0.6	97	16
	Solo (total)	84.8	—	47.8	109	—	—	3.0	23	15	21	50
	Solo (disponível)	—	—	—	6.0	—	74.7	—	0.4	2.0	—	3.1
	Água de chuva	71.1	—	16.8	24.1	312	76.5	9	9	13	7	166
	Deposição seca	4.0	0.03	0.78	1.4	140	11.7	0.03	0.4	1.0	1.7	8.6
	MP$_{10}$	—	—	0.002	0.1	0.7	0.01	0.1	0.001	—	0.01	0.03
FCO	*A. graveolens*	28.6	0.02	1.96	14.4	237	38.9	0.3	0.4	0.3	87	11
	C.floribundus	18.2	0.04	2.06	11.0	563	1142	0.2	1.1	0.5	120	24
	P. gonoacantha	22.7	0.02	2.01	5.8	460	489	0.1	0.9	0.4	124	14
	Solo (total)	53.9	—	43.4	6.9	—	—	1.9	6.1	6.1	12	16
	Solo (disponível)	—	—	—	0.6	—	44.1	—	—	—	—	0.9
	Água de chuva	—	—	—	—	—	—	—	—	—	—	—
	Deposição seca	—	—	—	—	—	—	—	—	—	—	—
	MP$_{10}$	—	—	—	—	—	—	—	—	—	—	—
FHO	*A. graveolens*	58.6	0.01	1.67	13.1	172	37.0	0.1	0.4	0.3	101	12
	C.floribundus	44.0	0.03	1.43	10.7	234	1356	0.3	2.0	0.4	152	22
	P. gonoacantha	84.7	0.01	1.51	13.7	214	388	0.1	1.9	0.4	169	16
	Solo (total)	234	—	51.8	6.4	—	—	2.0	4.3	7.6	27	15
	Solo (disponível)	—	—	—	1.3	—	82.97	—	0.4	—	—	1.7
	Água de chuva	—	—	—	—	—	—	—	—	—	—	—
	Deposição seca	—	—	—	—	—	—	—	—	—	—	—
	MP$_{10}$	—	—	—	—	—	—	—	—	—	—	—
FPA	*A. graveolens*	17.6	0.01	1.87	11.4	149	43.9	0.1	0.5	0.3	59	11
	C.floribundus	14.5	0.02	1.81	10.4	228	1139	0.2	1.2	0.4	87	22
	P. gonoacantha	24.9	0.01	1.81	6.9	209	491	0.1	1.1	0.4	98	15
	Solo (total)	47.4	—	69.4	7.5	—	—	1.8	4.7	8.0	12	15
	Solo (disponível)	—	—	—	0.6	—	32.3	—	—	—	—	0.9
	Água de chuva	35.3	—	4.15	18.8	520	58.0	14	0.3	3	4	202
	Deposição seca	1.3	0.05	0.76	0.9	304	3.8	0.02	0.4	0.7	1.5	16.1
	MP$_{10}$	—	—	0.02	0.1	1.9	0.02	0.5	0.1	—	0.01	0.3

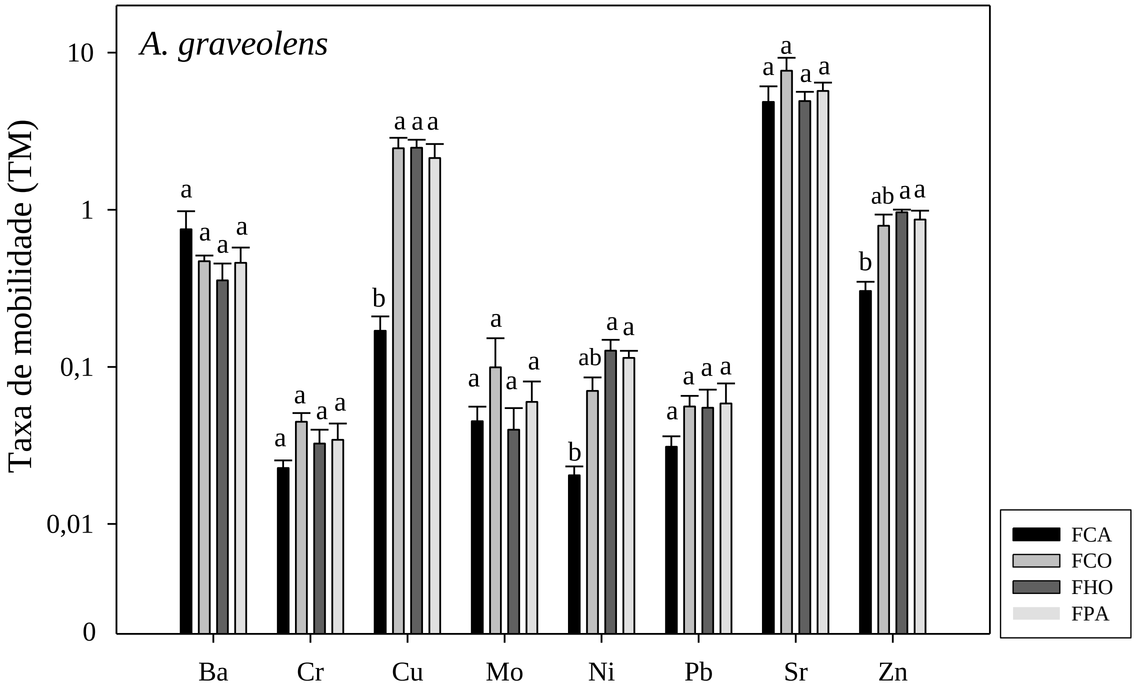
Concentrações determinadas em folhas e solo estão em mg kg^{-1}, água de chuva em µg m^{-2}, deposição seca em mg m^{-2} e MP$_{10}$ em µg m^{-3}.

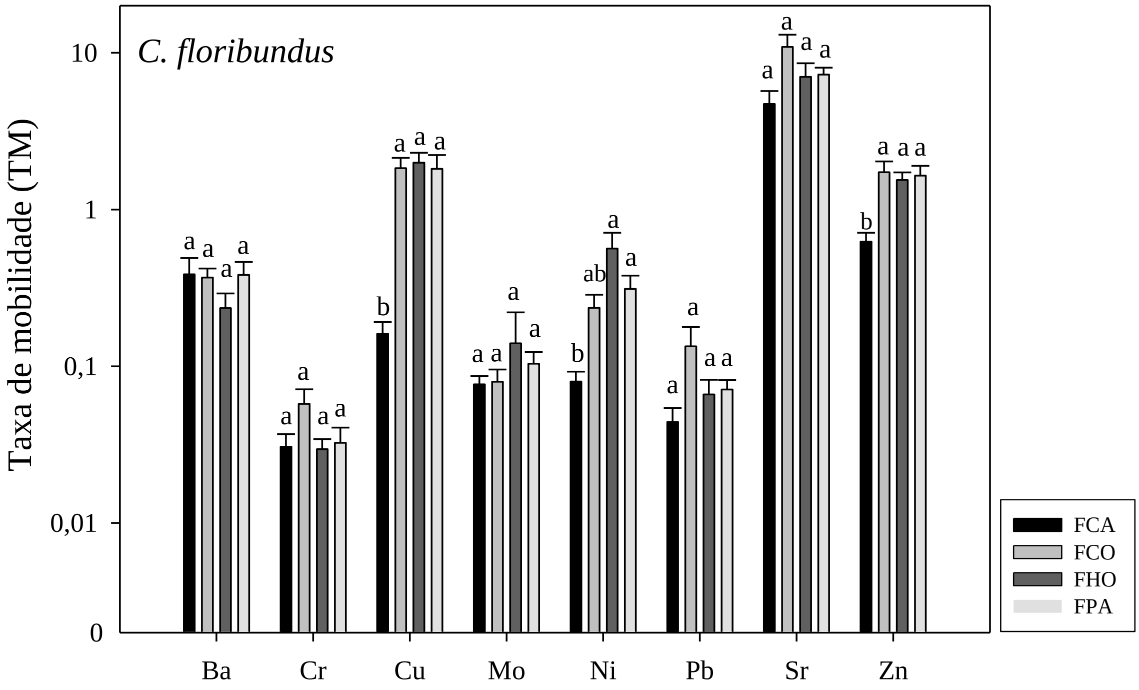
A fim de avaliar a taxa de mobilidade (adotando a terminologia proposta por Mingorance et al., 2007) de Ba, Cr, Cu, Mo, Ni, Pb, Sr e Zn do solo para as folhas das três espécies nos quatro fragmentos florestais, nos períodos seco/2011 e úmido/2012, foi calculada a razão entre concentrações foliares e concentração total obtidas no solo (Fig. 1). Este índice tem sido amplamente utilizado (De França et al., 2007; Kleckerová and Dočekalová 2014; Mingorance et al., 2007; Moreno-Jiménez et al., 2009; Zhou et al., 2013) para compreender as características de absorção dos elementos químicos pelas plantas.

Uma análise de variância não paramétrica, seguida do teste de Dunn ($p < 0{,}05$) feita para cada espécie, indicou diferenças na taxa de mobilidade (TM) dos elementos traço entre os fragmentos florestais avaliados.

A TM de Ba, Cr, Mo, Pb não variou entre os fragmentos para as três espécies. A menor TM de Cu ocorreu no fragmento de Campinas (FCA) para todas as espécies. A TM de Ni também variou da mesma forma para todas as espécies, indicando que no fragmento de Campinas (FCA) a mobilidade deste elemento do solo para as plantas foi menor do que em Holambra (FHO) e Paulínia (FPA). Para *C. floribundus* e *P. gonoacantha,* a TM de Zn foi menor em FCA do que nos outros fragmentos, já para *A. graveolens* a TM de Zn foi menor em FCA, quando comparada à TM obtida em FHO e FPA.

A baixa taxa de mobilidade (TM) de Cu, Ni e Zn encontrada em Campinas, demonstra que as altas concentrações totais destes elementos no solo deste fragmento (capítulo 3), nem sempre proporcionaram maiores concentrações foliares destes elementos nas espécies arbóreas estudadas (capítulo 4).


A. graveolens
Taxa de mobilidade (TM)
10
1
0,1
0,01
0
Ba
Cr
Cu
Mo
Ni
Pb
Sr
Zn
FCA
FCO
FHO
FPA


C. floribundus
Taxa de mobilidade (TM)
10
1
0,1
0,01
0
Ba
Cr
Cu
Mo
Ni
Pb
Sr
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FCA
FCO
FHO
FPA

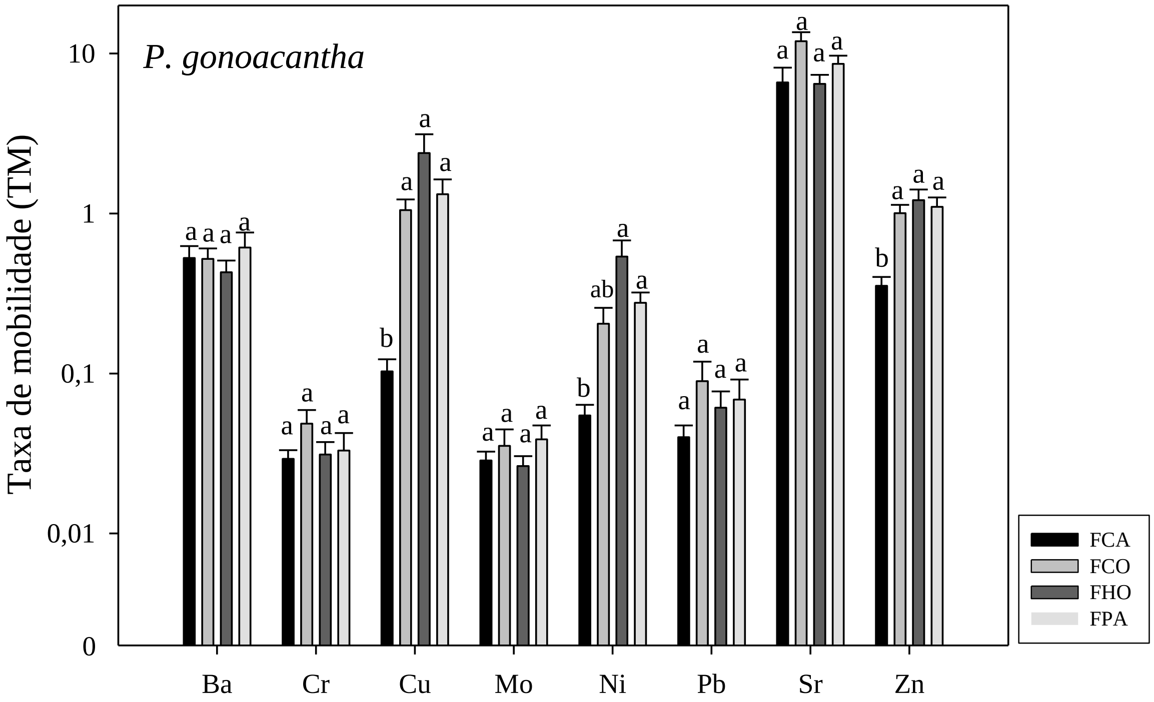

Figura 1. Taxas médias de mobilidade (TM) e respectivos erros padrão de elementos traço entre solo e folhas de *A.graveolens, C. floribundus* e *P. gonoacantha* coletados no período seco de 2011 e úmido de 2012 nos fragmentos de Campinas (FCA), Cosmópolis (FCO), Holambra (FHO) e Paulínia (FPA). Letras diferentes apontam diferenças significativas ($p < 0,05$) entre os fragmentos florestais, para um mesmo elemento. Escala do eixo y foi ajustada para $\log_{10}$ pasra facilitar a visualização de todas as TM médias.

Lembrando que os teores totais de metais traço no solo não estão totalmente disponíveis para a absorção das plantas, a taxa de mobilidade é apenas um indicativo potencial de absorção radicular (Chojnacka et al., 2005). Por esta razão, correlações de Spearman entre os teores totais de Ba, Cr, Cu, Mo, Ni, Pb, Sr, Zn e disponíveis de Cu, Ni, Pb, Zn no solo e as correspondentes concentrações foliares nas três espécies foram realizadas (Tabela 2).

Correlações positivas e significativas foram encontradas entre: a) concentrações totais de Ba no solo e nas folhas das três espécies avaliadas; b) concentrações totais e disponíveis de Cu no solo e nas folhas de *C. floribundus* e *P. gonoacantha*; c) concentrações de Ni total no solo e nas folhas de *C. floribundus*. Na literatura, em muitos casos, não existe uma dependência linear entre concentrações totais e foliares dos metais traço (Chojnaka et al 2005).

Tabela 2. Coeficientes de correlação de Spearman entre concentrações totais e disponíveis dos elementos traço no solo e nas folhas de *A. graveolens, C. floribundus* e *P. gonoacantha*. (n=32).

		A. graveolens	*C. floribundus*	*P. gonoacantha*
Concentrações totais	**Ba**	0,733**	0,522*	0,648**
	Cr	-0,262	0,027	0,016
	Cu	0,207	0,398*	0,359*
	Mo	0,160	0,192	0,328
	Ni	0,048	0,377*	0,211
	Pb	0,173	-0,032	0,188
	Sr	0,303	-0,075	-0,061
	Zn	0,297	0,217	0,161
Concentrações disponíveis	**Cu**	0,242	0,468*	0,380*
	Ni	0,285	0,469	0,396
	Mn	-0,056	0,284	-0,057
	Pb	0,190	-0,190	-0,238
	Zn	0,313	0,141	0,221

*p < 0,01; **p < 0,001

No entanto, a falta de relações significativas e positivas entre os teores disponíveis de Mn, Ni, Pb e Zn no solo e suas respectivas concentrações foliares pode indicar outra via de entrada destes elementos nas plantas, como, por exemplo, a deposição foliar (seca e/ou úmida). Maisto et al. (2004), estudando os teores de Cd, Cu, Cr e Pb em folhas de *Quercus ilex* L. e no solo (concentrações totais e disponíveis) em áreas urbanas e remotas da Itália, encontraram relações significativas apenas entre os teores disponíveis de Cu no solo e concentrações foliares, concluindo que o acúmulo foliar de Cd, Cr e Pb foi devido essencialmente à deposição atmosférica e absorção foliar.

Foram realizadas, assim, análises multiexploratórias de cluster, por meio do método de Ward como regra de separação de grupos e correlação de Pearson (1 – r) como métrica para determinação das distâncias entre grupos, com as concentrações médias, para todos os períodos de estudo, de Cr, Cu, Mo, Ni, Sr e Zn em todas as matrizes avaliadas: água de chuva (µg m^{-2}), deposição seca (µg m^{-2}), MP_{10} (µg m^{-3}), solo (concentrações totais em mg kg^{-1}) e folhas de *A. graveolens, C. floribundus* e *P. gonoacantha* (mg kg^{-1}) nos fragmentos de Campinas (FCA) e Paulínia (FPA) (Fig.2).

No fragmento de Campinas (Fig. 2 a, b, c) na distância 1 e no fragmento de Paulínia (Fig. 2 d, e, f) na distância 0,25, foi possível observar que MP_{10} e deposição seca foram as matrizes que mais esteviram associadas às espécies estudadas, indicando que as fonte principais destes elementos nas folhas foram a deposição atmosférica seca e material particulado até 10 µg. O solo, que sempre esteve no mesmo agrupamento das espécies, do MP_{10} e da deposição seca, parece ser uma fonte mais importante destes elementos para determinar as concentrações foliares do que a água de chuva.

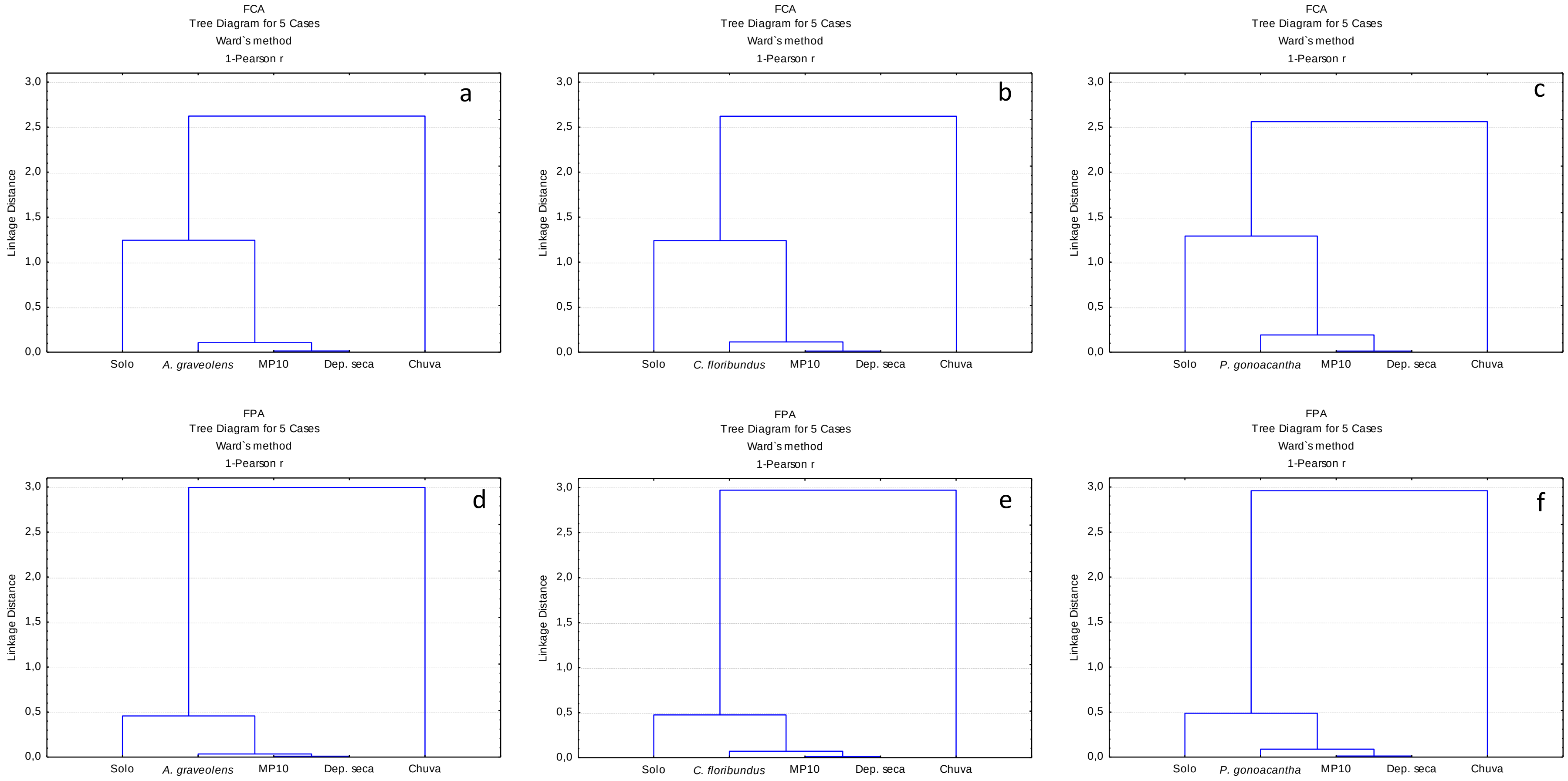

Figura 2. Dendogramas de 5 matrizes agrupadas (água de chuva, deposição seca, MP10, solo e folhas) em função das concentrações médias de Cr, Cu, Mo, Ni, Sr e Zn de todos os períodos avaliados. a, b, c. agrupamentos propostos para o fragmento de Campinas (FCA); d, e, f. agrupamentos propostos para o fragmento de Paulínia (FPA) . Método de agrupamento de Ward e distância entre grupos medida por correlação de Pearson (1 - r).

Conclusões gerais

Houve importante contribuição de fontes naturais em Campinas (CA) e Paulínia (PA) para a deposição seca e úmida registrada pela abundância de Al e Fe nestas amostras. O MP_{10}, através de elementos marcadores, também indicou a contribuição de fontes veiculares-industriais (Cr e Zn) e agrícolas (Cu, K, Mg, S) na região. A variação sazonal dos teores dos elementos químicos estudados na deposição seca e úmida não foi claramente definida. O fluxo de deposição seca foi preponderante sobre o da deposição úmida para a maioria dos elementos em CA e PA. A água de chuva, em CA e PA, mostrou-se moderadamente enriquecida por Cu, Pb e Zn. O MP_{10} teve enriquecimento moderado de Cu e alto enriquecimento de Mo, em ambas as áreas.

Não houve variação sazonal na concentração dos elementos traço avaliados no solo e a variação espacial foi influenciada não só pela diversidade de fontes antropogênicas destes elementos como pelas rochas matrizes que originaram os solos da região. A proximidade do complexo industrial não influenciou os teores de elementos traço nos solos dos fragmentos. O solo do fragmento de Campinas, um dos mais distantes do complexo industrial, apresentou maiores teores totais e disponíveis da maioria dos elementos avaliados, no entanto as concentrações foliares dos mesmos, nas três espécies arbóreas estudadas, indicaram que a deposição seca e o MP_{10} demonstraram ser fontes mais importantes do que o solo. A disponibilidade dos elementos traço no solo da região foi influenciada não somente pelo seu teor total mas também pelo pH, teor de matéria orgânica e de argila, capacidade de troca catiônica (CTC) e presença de óxidos de Al, Fe e Mn.

Folhas de *Croton floribundus,* seguido de *P.gonoacantha*, demonstraram maior habilidade em acumular a maioria dos elementos analisados do que as folhas de *A. graveolens.* Durante a estação seca, foram obtidos os maiores teores foliares de grande parte dos elementos avaliados em todas as espécies, possibilitando o uso das três em estudos de biomonitoramento para este fim. A proximidade do complexo industrial também não influenciou as concentrações foliares dos elementos químicos avaliados nos diferentes fragmentos florestais. *A. graveolens* demonstrou ser a espécie mais indicada para discriminar a variação espacial de elementos traço ambientalmente importantes, na Região Metropolitana de Campinas. O fragmento de Campinas esteve muito associado a elementos marcadores de fontes veiculares (Sb e Zn) e Holambra a elementos crustais (Ba e Sr).

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