UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
INSTITUTO DE BIOCIÊNCIAS – RIO CLARO

PROGRAMA DE PÓS-GRADUAÇÃO EM EM CIÊNCIAS BIOLÓGICAS
(BIOLOGIA VEGETAL)

Acúmulo de alumínio em espécies tolerantes: distribuição do Al na planta e relação com o solo

Luá Taibo Timpone

Rio Claro – SP
2023

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
INSTITUTO DE BIOCIÊNCIAS – RIO CLARO

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS
(BIOLOGIA VEGETAL)

Acúmulo de alumínio em espécies tolerantes: distribuição do Al na planta e relação com o solo

Luá Taibo Timpone

Orientador: Prof. Dr. Gustavo Habermann

Tese apresentada ao Instituto de Biociências do Câmpus de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Doutor em Ciências Biológicas (Biologia Vegetal).

Rio Claro – SP
2023

Timpone, Luá Taibo
T586a Acúmulo de alumínio em espécies tolerantes: distribuição do Al na planta e relação com o solo / Luá Taibo Timpone. -- Rio Claro, 2023
78 p. : il., tabs., fotos, mapas

Tese (doutorado) - Universidade Estadual Paulista (Unesp), Instituto de Biociências, Rio Claro
Orientador: Gustavo Habermann

1. Plantas acumuladoras de alumínio. 2. Plantas não acumuladoras de alumínio. 3. Espécies lenhosas do Cerrado. 4. Solo ácido. 5. Miconia spp.. I. Título.

Sistema de geração automática de fichas catalográficas da Unesp. Biblioteca do Instituto de Biociências, Rio Claro. Dados fornecidos pelo autor(a).

Essa ficha não pode ser modificada.

CERTIFICADO DE APROVAÇÃO

TÍTULO DA TESE: **Acúmulo de alumínio em espécies tolerantes: distribuição do Al na planta e relação com o solo**

AUTORA: LUÁ TAIBO TIMPONE

ORIENTADOR: GUSTAVO HABERMANN

Aprovada como parte das exigências para obtenção do Título de Doutora em Ciências Biológicas (Biologia Vegetal), área: Biologia Vegetal pela Comissão Examinadora:

Prof. Dr. GUSTAVO HABERMANN (Participaçao Virtual)
Departamento de Biodiversidade / Instituto de Biociencias de Rio Claro Unesp

Prof. Dr. DAVI RODRIGO ROSSATTO (Participaçao Virtual)
Departamento de Biologia Aplicada a Agropecuaria / UNESP - FCAV Jaboticabal

Prof. Dr. LUIZ ALFREDO RODRIGUES PEREIRA (Participaçao Virtual)
Departamento de Botânica / Universidade de Brasília

Profª. Drª. ANNA CAROLINA GRESSLER BRESSAN (Participaçao Virtual)
Departamento de Ecologia / Universidade de São Paulo - Instituto de Biociências

Profª. Drª. SÓNIA MARINA PINTO NUNES DA SILVA (Participaçao Virtual)
Departamento de Química / Universidade de Aveiro

Rio Claro, 28 de setembro de 2023

É com imensa satisfação que eu dedico esse trabalho aos meus antepassados e principalmente ao meu pai e minha mãe, que sempre prezaram pela minha educação.

AGRADECIMENTOS

O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior- Brasil (CAPES) - código de financiamento 001.

Agradeço à Universidade Estadual Paulista “Júlio de Mesquita Filho - UNESP” e, em especial, ao Departamento Biodiversidade do Instituto de Biociências do Câmpus de Rio Claro, pelo apoio e infraestrutura disponibilizados.

Agradeço ao Programa de Pós-graduação em Ciências Biológicas, principalmente ao Conselho da Pós-graduação, por todo apoio e suporte financeiro.

Um agradecimento especial ao meu Prof. Dr. Gustavo, que com toda sua paciência e dedicação me ensinou o que é ciência e, principalmente, o que é fazer pesquisa. Nesses quatro anos de parceria foram muitos campos, conversas, conselhos e críticas, momentos com os quais eu pude aprender muito sobre ciência e sobre a vida.

Agradeço aos meus colegas de laboratório Anna, Marina Gavassi, Giselle, Brenda, Matheus pelas críticas e sugestões ao longo desses quatro anos. À Marina Zaia e ao Matheus, em especial, pela ajuda nas saídas de campo e na realização de experimentos.

Agradeço à toda minha família Taibo e Timpone, que sempre me apoiaram nesse caminho e especialmente ao meu pai Adalberto e minha mãe Mônica, que sempre priorizaram pela minha educação e pelo meu ensino.

Agradeço ao meu companheiro Murilo e à minha fiel cãopanheira Kali, por todos esses anos, de muito amor, ao meu lado.

A todos aqueles que de alguma forma contribuíram e participaram da realização desse projeto.

“O cientista não é o homem que fornece as verdadeiras respostas; é quem faz as verdadeiras perguntas”

Claude Lévi-Strauss

RESUMO

O alumínio (Al) é um dos principais constituintes do solo, principalmente na forma de alumino-silicatos. Em solos ácidos (pH < 5,0), o Al é solubilizado em outras formas, incluindo o Al^{3+}, tornando-se tóxico às plantas sensíveis. Porém, inúmeras espécies de plantas nativas desenvolveram mecanismos de resistência à toxicidade ao Al, como a complexação do Al com ácidos orgânicos (tolerância externa), ou ainda, a absorção e acúmulo de Al em suas folhas sem danos aparentes (tolerância interna). As espécies que armazenam no mínimo 1.000 mg de Al kg^{-1} de folha seca são classificadas como acumuladoras de Al e pertencem a poucas famílias, como Melastomataceae, Rubiaceae, Vochysiaceae. Apesar das raízes serem o primeiro órgão de contato das plantas com o Al, nos estudos de campo, as avaliações de Al são geralmente limitadas às suas folhas. Ademais, no Brasil, o Al trocável dos solos ácidos e distróficos não ocorre apenas no Cerrado, mas também na região Amazônica e na Mata Atlântica. Portanto, buscando ampliar os limites dos estudos realizados anteriormente, nosso objetivo consistiu em verificar a capacidade das espécies nativas em direcionar o Al para outros órgãos além de suas folhas. Assim testamos as seguintes hipóteses: (1) se as espécies de plantas nativas do Cerrado que não acumulam Al em suas folhas podem fazê-lo em suas raízes e (2) se espécies acumuladoras de Al apresentam outros sítios de acúmulo que não suas folhas. Além disso, procuramos espécies acumuladoras de Al do gênero *Miconia* na Mata Atlântica e (3) testamos se o acúmulo de Al em suas folhas está relacionado à disponibilidade de Al no solo. Sendo assim, para testar H1 investigamos a presença de Al nas raízes e folhas de duas espécies acumuladoras *Vochysia tucanorum* e *Qualea grandiflora* (Vochysiaceae) e duas espécies de não acumuladoras *Xylopia aromatica* (Annonaceae) e *Caryocar brasiliense* (Caryocaraceae), nativas do Cerrado. A partir das análises de concentração do Al nesses órgãos, verificamos que as plantas não acumuladoras apresentam mínimas concentrações de Al nas folhas e raízes. Além disso, para testar H2 elegemos *Miconia albicans* (Sw) Triana, uma espécie acumuladora de Al do Cerrado, para avaliar a distribuição do Al por toda a planta. A partir da concentração de Al nas diferentes estruturas vegetativas (folha, raiz, casca, caule) e reprodutivas (inflorescência, racemo, frutos), encontramos que em *M. albicans*, o acúmulo de Al não está restrito a alguns tecidos/órgãos, sendo maior nos órgãos senescentes, que poderia ser uma maneira de eliminar Al da planta. E por fim, para testar H3 investigamos se existe associação entre a concentração do Al nas folhas de plantas acumuladoras e a disponibilidade de Al no solo, fizemos coletas de folhas do gênero *Miconia* e de amostras solo, em 11 diferentes gradientes altitudinais, da Mata Atlântica. Encontramos 27 espécies de *Miconia spp.* que podem ser consideradas

acumuladoras. Além disso, as análises de saturação por Al no solo (m%) evidenciaram que apesar do m% aumentar com as altitudes, as espécies de *Miconia spp*. acumuladoras encontradas não respondem à variação da saturação do Al no solo.

Palavras-chave: *Miconia spp*., espécie não acumuladora de Al, solo ácido e Cerrado.

ABSTRACT

Aluminum (Al) is one of the main constituents of soil, mainly in the form of aluminosilicates. In acidic soils (pH < 5.0), Al is solubilized in other forms, including $Al^{3+,}$ becoming toxic to sensitive plants. However, several native plant species have developed mechanisms of resistance to Al toxicity, such as the complexation of Al with organic acids (external tolerance), or the absorption and accumulation of Al in their leaves without apparent damage (internal tolerance). Species that store at least 1000 mg of Al kg^{-1} of dry leaves are important as Al accumulators and belong to a few families, such as Melastomataceae, Rubiaceae, Vochysiaceae. Although roots are the plants' first contact organ with Al, in field studies, Al assessments are generally limited to their leaves. Furthermore, in Brazil, the exchange of acidic and dystrophic soils does not only occur in the Cerrado, but also in the Amazon region and the Atlantic Forest. Therefore, searching to expand the limits of previous studies, our objective is to verify the ability of native species to direct Al to organs other than their leaves. Therefore, we tested the following hypotheses: (1) whether plant species native to the Cerrado that do not accumulate Al in their leaves can do in their roots and (2) whether species that accumulate Al have other accumulation sites than their leaves. Furthermore, we searched for Al-accumulating species of the genus *Miconia* in the Atlantic Forest and (3) tested whether Al accumulation in their leaves is related to Al availability in the soil. Therefore, to test H1 we investigated the presence of Al in the roots and leaves of two accumulated species *Vochysia tucanorum* and *Qualea grandiflora* (Vochysiaceae) and two non-accumulated species *Xylopia aromatica* (Annonaceae) and *Caryocar brasiliense* (Caryocaraceae), native to the Cerrado. From the concentration analyzes of all these organs, we found that the non-accumulated plants had minimum concentrations of all leaves and roots. Furthermore, to test H2 we *chose Miconia albicans* (Sw) Triana, an Al-accumulating species from the Cerrado, to evaluate the distribution of Al throughout the plant. Based on the concentration of Al in the different vegetative (leaf, root, bark, stem) and reproductive (inflorescence, raceme, fruits) structures found in *M. albicans*, the accumulation of Al is not restricted to some tissues/organs, being greater in senescent organs, which could be a way to eliminate Al from the plant. And finally, to test H3, we investigated whether there is an association between the concentration of Al in the leaves of accumulated plants and the availability of Al in the soil. We collected leaves of the genus *Miconia* and soil samples, in 11 different altitudinal gradients, from the Mata Atlantic. I found 27 species of *Miconia spp.* that can be considered hoarded. Furthermore, analyzes of Al

saturation in the soil (m%) showed that despite the increase in m% with altitude, *Miconia spp*. Accumulators found did not fluctuate with variation in Al saturation in the soil.

Keywords: *Miconia spp*., species non-Al-accumulating, acid soil, Brazilian savanna.

SUMÁRIO

CAPÍTULO 3: Aluminum-accumulating *Miconia* species across an elevation gradient in the Tropical Atlantic Rain Forest in Brazil

INTRODUÇÃO GERAL

O alumínio (Al) é terceiro elemento mais abundante da crosta terrestre, representando cerca 8% da sua composição (Totten and MacKenzie 2003, Kochian et al. 2004). Devido a sua alta afinidade com o oxigênio, o Al é encontrado na natureza principalmente na forma de óxidos e aluminosilicatos, conservando-se em um estado insolúvel e não tóxico (Kochian 1995; Totten and MacKenzie 2003). Porém, em solos ácidos (pH < 5,5) essas estruturas são dissociadas em diversas formas monoméricas, sendo a catiônica trivalente (Al^{3+}) a mais fitotóxica (Brunner and Sperisen 2013, Bojórquez-Quintal et al. 2017).

Solos ácidos abrangem cerca de um terço da área terrestre livre de gelo, dos quais 15% são ocupados por terras agricultáveis, correspondendo a 40% dos solos agricultáveis do globo (vonUexküll and Mutert, 1995). E essa acidez, frequentemente, apresenta desafios para o crescimento e desenvolvimento das plantas cultivadas, uma vez que interfere no funcionamento das células radiculares e, consequentemente, prejudica a exploração do solo, absorção de água e nutrientes e produtividade (Kochian 1995; Vitorello et al. 2005; Horst et al. 2010; Banhos et al. 2016; Singh et al. 2017).

Por outro lado, 67% desses solos ácidos estendem-se por dois cinturões globais principais (von Uexküll and Mutert 1995; Brunner and Sperisen 2013). Um cinturão ao norte (região boreal) e outro cinturão tropical meridional, sendo ambas regiões cobertas por densas florestas e bosques, com uma a alta biodiversidade e biomassa (von Uexküll and Mutert 1995; Brunner and Sperisen 2013). Assim sendo, as plantas lenhosas nativas não apresentam sintomas aparentes de estresse ao Al, pois desenvolveram diferentes mecanismos de resistências (Haridasan, 2008; Ryan et al., 2011; Brunner and Sperisen, 2013). Um desses mecanismos é a tolerância externa que consiste na exsudação de ácidos orgânicos (*eg*. ácido cítrico, málico, succínico e oxálico) pelas raízes, que formam complexos estáveis com o Al na rizosfera, limitando a absorção simplástica (não acumuladoras de Al) (Ma et al., 2001; Ryan et al., 2011; Brunner and Sperisen, 2013). O outro é o mecanismo de tolerância interna, o qual consiste na tolerância intrínseca ao Al, ou seja, elas absorvem e acumulam o Al em grandes quantidades em seus órgãos e tecidos, sem danos aparentes ao crescimento vegetal e ao metabolismo (acumuladoras de Al) (Haridasan 1982; Jansen et al. 2002; Souza et al. 2015; Silva et al. 2016). Segundo Chenery (1948), espécies acumuladoras de Al são aquelas que mostram pelo menos 1000 mg Al por kg de folhas secas.

Nos trópicos, mais especificamente na vegetação do Cerrado na América do Sul, solos ácidos com alta saturação de alumínio (m% > 77%) abrigam espécies acumuladoras e não

acumuladoras de Al (Haridasan 1982; Souza et al. 2015a). Os representantes lenhosos acumuladores de Al pertencem a algumas famílias de Melastomataceae, Rubiaceae e Vochysiaceae, sendo que o restante da comunidade vegetal são de não acumuladoras de Al (Haridasan, 1982; Haridasan et al., 1986; Souza et al. 2015). Portanto, levando em consideração as concentrações foliares de Al, esses grupos de espécies distintas ocorrendo simultaneamente no Cerrado possuem uma distribuição bimodal das concentrações de Al (Metali, et al.2012). Como por exemplo, em *Caryocar brasiliense* Camb., uma espécie não Al-acumuladora, que apresenta 330 mg Al kg^{-1} folha seca, enquanto a *Qualea grandiflora* Mart., uma espécie Al-acumuladora, que exibe cerca de 5160 mg Al kg^{-1} folha seca (Haridasan 1982).

Contudo, como as raízes são o primeiro órgão de contato das espécies vegetais com o solo ácido e as primeiras a mostrar sintomas de toxicidade (Kochian 1995), vê-se a necessidade de mais investigações dos mecanismos de resistência ao Al pelo viés radicular. Um estudo com *Rudgea viburnoides* (Cham.) Benth, uma espécie hiperacumudora de Al do Cerrado, registrou o acúmulo de Al tanto em suas folhas quanto nas suas raízes (Malta et al. 2016), porém não há registros de estudos de campo sobre a presença do Al nas raízes de plantas nativas não acumuladoras de Al. Portanto, a comparação da concentração de Al nas raízes de espécies acumuladoras e não acumuladoras pode levar a uma melhor compreensão, de como as raízes das espécies nativas de plantas conseguem lidar com o estresse ao Al edáfico.

As folhas são o principal dreno para as plantas acumuladoras de Al (Watanabe et al. 1998). Por isso, há décadas muito se investigou apenas o Al foliar das espécies nativas, desde sua concentração até sua compartimentalização a nível celular (Haridasan, 1982; Souza et al. 2015; Bressan et al. 2016 Guilherme Pereira et al. 2018). No entanto, em *Miconia albicans* (Sw.) Triana não apenas suas folhas podem acumular até 8600 mg de Al kg^{-1} de folhas secas, mas também suas sementes contêm grandes concentrações de Al, cerca de 6900 mg de Al kg^{-1} (Haridasan 2008; Bressan et al., 2016). Além disso, a análise anatômica de folhas maduras de *M. albicans*, coradas com cromo azurol, mostrou uma coloração positiva ao Al em todos os elementos do floema (Haridasan, 1982; Bressan et al., 2016). Dessa forma, a alta concentração de Al nas sementes e a reação positiva dos elementos do floema são evidências do transporte de Al pelo floema, porém pouco se conhece sobre os locais dessas realocações do Al pelo corpo vegetal. Portanto, a medição direta do teor de Al na casca dos troncos, flores e fruto podem fornecer evidências úteis sobre o transporte e acúmulo de Al na planta.

Nas folhas de *Miconia albicans*, *M. fallax* DC., *M. pohliana* Cogn. crescendo em solos do Cerrado ácidos já foi relatado um acúmulo de 3.100 até 8.600 mg de Al kg^{-1} de folhas secas (Bressan et al., 2016). Jansen (2002) revisou a distribuição dos representantes da Tribo

Miconiae com relação ao acúmulo ou não de Al, encontrando que de 384 espécimes 314 eram potencialmente acumuladores, assim, o acúmulo de Al "parece", ser uma característica primitiva dentro da família Melstomataceae.

Miconia Ruiz & Pav. da família Melastomataceae, é um grande gênero com cerca de 1057 espécies, amplamente distribuído ao longo da América tropical (Goldenberg 2008; Goldenberg et al. 2013). No Brasil, são encontradas 274 espécies, ocorrendo, em sua maioria, em florestas tropicais úmidas das terras baixas e savanas, preferencialmente sobre solos ácidos (Ruokolainen et al. 1997; Goldenberg et al. 2013). Como solos ácidos com baixa disponibilidade de nutrientes e alto m% ocorrem tanto no Cerrado e Amazônia quanto na Mata Atlântica (Haridasan 2008; Martins et al, 2015), então, é possível que existam espécies acumuladoras de Al na Mata Atlântica, sobretudo espécies do gênero *Miconia*. Além disso, para realizar a comparação dos níveis de Al em plantas provenientes de diferentes localidades, é imprescindível levar em consideração a incorporação de fatores ecológicos, tais como a acidez e a saturação do Al no solo (Jansen, 2004).

Tendo em vista os fatos apresentados, nosso objetivo foi verificar a capacidade das espécies nativas de direcionar o Al para outros órgãos/tecidos. Assim testamos as seguintes hipóteses: (1) se as espécies de plantas nativas que não acumulam Al em suas folhas podem fazê-lo em suas raízes e (2) se espécies acumuladoras de Al apresentam outros sítios de acúmulo que não suas folhas. Além disso, procuramos espécies acumuladoras de Al do gênero *Miconia* na Mata Atlântica e (3) testamos se o acúmulo de Al em suas folhas está relacionado à disponibilidade de Al no solo. Nessa perspectiva, no primeiro capítulo dessa Tese procuramos avaliar se espécies não acumuladoras de Al podem acumulá-lo suas raízes. Para isso, coletamos folhas e raízes de duas espécies não acumuladoras (*Caryocar brasiliense* Camb. – Caryocaraceae – e *Xylopia aromatica* (Lam.) Mart. - Annonaceae), e duas espécies acumuladoras de Al (*Qualea grandiflora* Mart. e *Vochysia tucanorum* Mart. – ambas Vochysiaceae) e comparamos a concentração de Al entre essas espécies. A partir das coletas das amostras de solo determinamos suas propriedades químicas e físicas. No segundo capítulo, nós investigamos a diferença no acúmulo do Al entre os diferentes órgãos e tecidos de *Miconia albicans*, uma espécie acumuladora de Al. Para isso, nós coletamos folha (broto, duas fases de folhas jovens e senescentes), caule, casca, raiz, inflorescência (racemo, broto, flor) e fruto (duas fases de fruto inicial, fruto imaturo e maduro). As folhas maduras foram, ainda, separadas em limbo e nervura (principal e secundária). Todos esses materiais vegetais coletados foram avaliados quanto a concentração do Al. No terceiro e último capítulo, coletamos em um gradiente latitudinal amostras de solo e de folhas de espécies de *Miconia spp.* da Mata Atlântica

para investigar se existem espécies acumuladoras de Al na Mata Atlântica. Além disso, procuramos avaliar se a concentração foliar de Al está relacionada a disponibilidade de Al no solo.

Referências bibliográficas

Banhos OFAA, Carvalho BM de O, da Veiga EB, Bressan ACG, Tanaka FAO, Habermann G. 2016. **Aluminum-induced decrease in CO2 assimilation in "Rangpur" lime is associated with low stomatal conductance rather than low photochemical performances**. Sci Hortic 205:133–140

Bojórquez-Quintal E, Escalante-Magaña C, Echevarría-Machado I, Martínez-Estévez M. 2017. **Aluminum, a friend or a foe of higher plants in acid soils**. *Frontiers in Plant Science*, 8: 1767

Bressan ACG, Coan AI, Habermann G. 2016. **X-ray spectra in SEM and staining with chrome azurol S show Al deposits in leaf tissues of Al-accumulating and non-accumulating plants from the cerrado**. Plant Soil 404: 293–306

Brunner I, Sperisen C. 2013. **Aluminum exclusion and aluminum tolerance in woody plants**. Front Plant Sci 4: 1–12

Chenery EM. 1948. **Aluminium in the plant world. part I: general survey in dicotyledons**. Kew Bull 1948:173–183

Goldenberg R, Almeda F, Caddah MK, Martins AB, Meirelles J, Michelangeli FA, Weiss M. 2013. **Nomenclator botanicus for the Neotropical genus Miconia (Melastomataceae: Miconieae)**. Phytotaxa 106(1): 1–171.

Goldenberg R, Penneys DS, Almeda F, Judd WS, Michelangeli FA. 2008. **Phylogeny of *Miconia* (Melastomataceae): patterns of stamen diversification in a megadiverse neotropical genus**. Int J Plant Sci, 169: 963-979

Guilherme Pereira C, Clode PL, Oliveira RS, Lambers H. 2018. **Eudicots from severely phosphorus-impoverished environments preferentially allocate phosphorus to their mesophyll**. New Phytol 218:959–973

Haridasan M. 2008. **Nutritional adaptations of native plants of the cerrado biome in acid soils**. Braz J Plant Physiol 20: 183–195

Haridasan M, Paviani TI, Schiavini I. 1986. **Localization of aluminium in the leaves of some aluminium-accumulating species**. Plant Soil 94: 435–437

Horst WJ, Wang Y, Eticha D. 2010. **The role of the root apoplast in aluminium-induced inhibition of root elongation and in aluminium resistance of plants: a review**. Ann Bot 106:185–197

Jansen S, Watanabe T, Smets E (2002). **Aluminium accumulation in leaves of 127 species in Melastomataceae, with comments on the order Myrtales**. Ann Bot 90: 53-64

Jansen, S., Watanabe, T., Dessein, S., Robbrecht, E., Smets, E. 2004. **The evolution of aluminium accumulation in angiosperms.** In: Hemsley, A.R., Poole, I. (Eds.), The Evolution of Plant Physiology. Linnean Society Symposium Series 21. Elsevier Academic Press, London, pp. 467–479.

Kochian LV. 1995. **Cellular mechanisms of aluminum toxicity and resistance in plants**. Annu Rev Plant Physiol Plant Mol Biol 46: 237–260

Kochian LV, Hoekenga OA, and Piñeros MA. 2004. **How do crop plants tolerate acid soils? Mechanisms of aluminum tolerance and phosphorous efficiency**. Annu. Rev. Plant Biol. 55, 459–493.

Ma JF, Ryan PR, Delhaize E. 2001. **Aluminium tolerance in plants and the complexing role of organic acids**. Trends Plant Sci 6: 273–278

Malta PG, Arcanjo-Silva S, Ribeiro C, Campos NV, Alves-Azevedo A. 2016. ***Rudgea viburnoides* (Rubiaceae) overcomes the low soil fertility of the Brazilian Cerrado and hyperaccumulates aluminum in cell walls and chloroplast**. Plant Soil 408: 369–384

Martins SC, Neto ES, de Cássia Piccolo M, Almeida DQ, de Camargo PB, do Carmo JB, Martinelli, LA. 2015. **Soil texture and chemical characteristics along an elevation range in the coastal Atlantic Forest of Southeast Brazil**. Geoderma Reg 5: 106-116

Metali F, Salim KA, Burslem DFRP. 2012. **Evidence of foliar aluminium accumulation in local, regional and global datasets of wild plants**. New Phytol 193:637–649

Ruokolainen, K., Linna, A., & Tuomisto, H. 1997. **Use of Melastomataceae and Pteridophytes for Revealing Phytogeographical Patterns in Amazonian Rain Forests**. *Journal of Tropical Ecology*, *13*(2), 243–256.

Ryan PR, Tyerman SD, Sasaki T, Furuichi T, Yamamoto Y, Zhang WH, Delhaize E. 2011. **The identification of aluminium-resistance genes provides opportunities for enhancing crop production on acid soils**. J Exp Bot 62: 9–20

Silva RV, de Jesus DL, Silva BVL, Miranda BEC, Gondim JPE. 2016. **First report of *Ditylenchus gallaeformans* in *Miconia albicans* from the Brazilian Cerrado, State of Goiás**. Semina: Cienc Agr 37: 729-736

Singh S, Tripathi DK, Singh S, Sharmac S, Dubeyb NK, Chauhana DK, Vaculíke M. 2017. **Toxicity of aluminium on various levels of plant cells and organism: A review**. Env Exp Bot 137: 177–193

Souza MC, Bueno PC, Morellato LPC, Habermann G. 2015. **Ecological strategies of Al-accumulating and non-accumulating functional groups from the cerrado sensu stricto**. An Acad Bras Ciênc 87: 813-823

Totten E, MacKenzie DS. 2003. **Handbook of Aluminum** – Volume 1: Physical Metallurgy and Processes, Marcel Dekker Inc., New York

Vitorello VA, Capaldi FR, Stefanuto VA. 2005. **Recent advances in aluminum toxicity and resistance in higher plants**. Braz J Plant Physiol 17: 129–143

von Uexküll, H.R., Mutert, E. 1995). **Global extent, development and economic impact of acid soils**. Plant Soil 171:1–15

Watanabe T, Osaki M, and Tadano T. 1998. **Effects of nitrogen source and aluminum on growth of tropical tree seedlings adapted to low pH soils**. Soil Sci. Plant Nutr. 44, 655–666

CAPÍTULO 1

Do aluminum (Al)-accumulating species from the Brazilian savanna accumulate Al in the roots?

Marina Zaia[1], Luá Taibo Timpone[2], Gustavo Habermann[3*]

*[1]Curso de Graduação em Ecologia, Departamento de Biodiversidade, Instituto de Biociências, Universidade Estadual Paulista, UNESP, Av. 24-A, 1515; 13506-900, Rio Claro, SP, Brazil; [2]Programa de Pós-Graduação em Biologia Vegetal, Departamento de Biodiversidade, Instituto de Biociências, Universidade Estadual Paulista, UNESP, Av. 24-A, 1515; 13506-900, Rio Claro, SP, Brazil; [3]Departamento de Biodiversidade, Instituto de Biociências, Universidade Estadual Paulista, UNESP, Av. 24-A, 1515; 13506-900, Rio Claro, SP, Brazil. E-mail: gustavo.habermann@unesp.br; Tel: +0055 19 3526-4210 (*Corresponding author)*

Zaia M., Timpone L.T., Habermann G. (2022) **Do aluminum (Al)-accumulating species from the Brazilian savanna accumulate Al in the roots?** *Trees*, 36, 1677–1685.

Abstract

Edaphic aluminum (Al) is toxic to nearly all plant species. Al-accumulating species, however, can accumulate more than 1000 mg Al kg^{-1} dry leaves without showing toxicity symptoms. The Brazilian savanna, known as 'Cerrado', is home to a woody plant community composed of few species from four families of Al-accumulating plants and the rest, non-accumulating ones. The leaves are the target organs when searching for Al roles in these plants, although the roots are the first organ to get in contact with Al. Thus, the roots are being neglected in field studies with accumulating and non-accumulating plants. We expected that Al-accumulating species also accumulate Al in their roots, while non-accumulating ones, that accumulate less than 1000 mg Al kg^{-1} dry leaves, avoid this metal in their roots. *Vochysia tucanorum* and *Qualea grandiflora* (Vochysiaceae) showed more than 1000 mg Al kg^{-1} dry leaves, and the former accumulated 6.8-times more Al in its leaves than the latter. *Xylopia aromatica* (Annonaceae) and *Caryocar brasiliense* (Caryocaraceae) showed approximately 100 mg Al kg^{-1} dry leaves, being considered non-accumulating species. In the roots, *V. tucanorum* accumulated 7.8-times more Al than *Q. grandiflora*, which showed 748 ± 191 mg Al kg^{-1} dry roots. *Xylopia aromatica* and *C. brasiliense* accumulated, approximately, 50 and 250 mg Al kg^{-1} dry roots, respectively. While Al-accumulating species are tolerant, showing high Al concentration in their leaves and varying concentrations in their roots, non-accumulating species from the Cerrado are 'avoiders' and retain low Al concentrations in their roots and leaves.

Keywords: Aluminum; Cerrado; Cerrado woody species; non-accumulating plants.

1 Introduction

Aluminum (Al) is toxic to most plant species (Kochian, 1995; Singh et al. 2017). Nevertheless, some plants are intriguing and display high leaf Al concentration without exhibiting toxicity symptoms. Indeed, plants showing at least 1000 mg Al per kg dry leaves can be considered Al-accumulating species (Chenery, 1948b; Jansen et al. 2002a). These species occur worldwide in 45 families, and are mainly found in Melastomataceae, Rubiaceae, Symplocaceae, Theaceae and Vochysiaceae that naturally grow on acidic soils (pH < 5.0) with high Al saturation (m% > 60–70 %). These soils occupy approximately 30 % of the world's ice-free land (von Uexküll and Mutert 1995; Brunner and Sperisen 2013) and are commonly found in tropical areas subjected to high rainfall (Jansen et al. 2002a).

In Al-accumulating species, Al is transported from roots to shoots where it is accumulated in the leaves, especially in the phloem, collenchyma of the midrib, epidermal cells, and spongy parenchyma (Haridasan et al. 1986; Bressan et al. 2016; Malta et al. 2016; Guilherme Pereira et al. 2018). Up to 66,100 mg Al kg^{-1} dry leaves may be found in these plants (Chenery 1948a; Jansen et al. 2002b). Therefore, most studies focus on leaves as target organs when searching for Al and its effects or possible metabolic roles. Nevertheless, the roots are the first organ to get in contact with dystrophic soils. Despite existing studies reporting Al concentration in their leaves and roots (Mazorra et al. 1987— one species; Olivares et al. 2010— two species; Schmitt et al. 2016— four species), these are uncommon due to the difficulty to collect roots in the field. In addition, there is also a lack of root material in herbaria, which could be used for chemical analyses.

Aluminum-accumulating species (and non-accumulating ones) can be found in global, regional, and local floras, but all based on foliar Al concentrations (Metali et al. 2012). In the Australian-New Guinea flora, 80 species were considered leaf Al-accumulators (Webb 1954). Sub-Saharan Africa also presents about 450 million ha of acidic and Al-rich soils (Zingore et al. 2015), but no studies reporting root Al concentration in native woody species from African savannas are available. The Brazilian savanna, broadly known as 'Cerrado' vegetation in South America is comprised of woody species that can be classified into Al-accumulators and non-accumulators (Haridasan 1982; Souza et al. 2015a). In this vegetation, Al-accumulating plants belong to few species from Melastomataceae, Rubiaceae, Symplocaceae and Vochysiaceae, and non-accumulating ones are, essentially, the rest of the woody plant community (Souza et al. 2015a). Interestingly, as far as we are aware, there is just one study reporting Al concentration in the roots and leaves of an Al-accumulating Rubiaceae species from the Cerrado (Malta et al.

2016). Moreover, no studies are available about Al concentration in the roots comparing Al-accumulating and non-accumulating species from the Cerrado. Thus, it is expected that non-accumulating plants from this vegetation avoid Al uptake and will show low Al concentration in their roots either. In the case of Al-accumulating species from this vegetation, it may be expected that these species from Vochysiaceae, which accumulate more than 5000 mg Al kg^{-1} dry leaves (Haridasan 1982; Bressan et al. 2016; Nogueira et al. 2019) will also show more than 1000 mg Al kg^{-1} in their roots. This premise comes from the evidence that *Miconia albicans* (Sw.) Triana, an Al-accumulator from the Cerrado distributes Al among different organs (Timpone and Habermann 2022), and *Rudgea virbunoides* (Cham.) Benth., an Al-accumulating Rubiaceae species, accumulates more than 8000 mg kg^{-1} dry root (Malta et al. 2016).

In the present study, we elected two non-accumulating and two Al-accumulating woody species from a cerrado *sensu stricto* (*s. str.*) — a savanna-like vegetation — remnant. The first group of plants was *Caryocar brasiliense* Camb. (Caryocaraceae) and *Xylopia aromatica* (Lam.) Mart. (Annonaceae) that are common woody species from this vegetation. The second was *Qualea grandiflora* Mart. and *Vochysia tucanorum* Mart., Vochysiaceae Al-accumulating species also frequently occurring in the Cerrado. We measured soil chemical properties, including m%, and collected leaves and roots to compare Al concentration between these species. Here we expected to highlight root Al accumulation by these plants, something that is little studied.

2 Material and methods

2.1 Experimental site

This field study was conducted in a cerrado *s. str.* remnant (260 ha) on São José da Conquista farm (22°13′ S 47° 53′W; 610 m of altitude), in the municipality of Itirapina, São Paulo state, southeastern, Brazil. In this region, the climate can be characterized as Cwa (Köppen, 1948), showing a warm and wet season (Oct–Mar) and a cold and dry season (Apr–Sep) with mean monthly temperature of 32 ºC and 18 ºC, respectively, and annual rainfall of 1512 mm (Souza et al. 2015b). The local vegetation is comprised of small trees (8–10 m in height) and shrubs (2–4 m in height).

2.2 Plant material

We used two Al-accumulating species from Vochysiaceae and two non-accumulating species from Caryocaraceae and Annonaceae, predominant families from the Cerrado woody vegetation. *Qualea grandiflora* Mart. (Vochysiaceae) is a brevideciduous tree (Souza et al.

2015a) that can reach 6–8 m in height and accumulates 4,000–6,000 mg Al kg^{-1} dry leaves (Haridasan, 1982; Nogueira et al. 2019). *Vochysia tucanorum* Mart. (Vochysiaceae) is an evergreen tree that can reach 5–6 m in height and accumulates up to 20,930 mg Al kg^{-1} dry leaves (Haridasan and Araújo, 1988).

Caryocar brasiliense Camb. (Caryocaraceae) and *Xylopia aromatica* (Lam.) Mart. (Annonaceae) were elected as representatives from Al non-accumulating species. *C. brasiliense*, a deciduous tree (Souza et al. 2015a), commonly named 'pequi' in Portuguese, is a tree with approximately 10 m in height and shows between 170 and 330 mg Al kg^{-1} dry leaves (Haridasan, 1982; Haridasan and Araújo, 1988). *X. aromatica* is an evergreen tree (Souza et al. 2015a) that shows up to 8 m in height, with monopodial growth behavior and horizontal branches. It accumulates approximately 150 mg Al kg^{-1} dry leaves (Haridasan and Araújo, 1988), and it is the most occurrent Annonaceae species in the cerrado *s. str.* (Miranda-Melo et al. 2007).

2.3 Experimental design

Five soil samples were randomly collected in the study area, close to the plant species, at 20–40 cm of depth, where nutrients are most available for native plant species (Wigley et al. 2013). Physical and fertility parameters, especially m%, were measured.

Five adult individuals from each plant species were randomly chosen in the area for leaf and root sampling in order to assess the Al concentration at the peak of the warm and wet season (Nov, 2019). The plant canopies were divided into four quadrants (N, S, E and W), where 5–10 healthy, mature sun-exposed green leaves were collected, depending on the leaf size of each species (e.g., *X. aromatica* and *V. tucanorum* show a 7–10-cm long leaf, while *Q. grandiflora* and *C. brasiliense* exhibit a 15–20-cm long leaf). From the same individuals, root sampling was also performed. For this, we dug up close to the main trunk until reaching typical root branching (0.40–0.60 m of depth), where woody root samples (1.5 ± 0.5 cm in diameter) were collected.

2.4 Soil sampling

Soil samples were collected using an auger, digging up to 20–40 depth range. The samples were bagged, identified, and sent to the soil science laboratory (Instituto Agronômico de Campinas, IAC, Campinas, Brazil). Chemical analyses, including soil pH (in $CaCl_2$), cation exchange capacity (CEC) and m% was performed following the method by van Raij et al. (2001). Physical analysis (percentage of sand, silt, and clay) was done according to protocols described by (Embrapa 1997).

2.5 Al concentration in leaves and roots

Leaf and root samples were washed three times in deionized water to remove dust and dirt. The root samples were dismembered, shattered, and put separately into paper bags. Leaf and root samples were, then, oven-dried at 60 ºC until constant mass and sent to the Plant Nutrition Laboratory at Instituto Agronômico de Campinas (IAC) for the Al concentration analysis. Samples were ground and digested in a solution of sulfuric:nitric:percloric acids (1:10:2, v/v/v). After this, Al concentration was determined by the atomic absorption spectrophotometric method (Sarruge and Haag 1974), using an inductively coupled plasma optical emission spectrometer (Optima 8300; Perkin Elmer, Billerica, MA, USA), and Al was expressed as mg kg^{-1} dry mass.

2.6 Data analysis

Aluminum concentration measured in leaf and root samples was assessed using five replicates (adult individuals). A one-way analysis of variance (ANOVA) was run to test differences in Al concentration in roots and leaves. For each organ (leaves and roots), mean values were compared by Tukey test ($p < 0.05$) to check differences between species.

In addition, we collected, from the literature, leaf and root Al concentrations of accumulating and non-accumulating woody species occurring in other vegetations worldwide to check any further pattern within a broader systematic context.

3 Results and discussion

The study area showed a sandy soil texture, exhibiting approximately 12.5-times more sand than clay (Table 1). As expected, the soil pH represented an acidic soil (pH = 3.8), with low CEC and even lower V%, which resulted in Al saturation (m%) above 80% (Table 2). Phosphorous and micronutrients contents were also low, representative of a Cerrado soil (Table 2). Originally, the Cerrado vegetation occupies 11 Brazilian states and two million km^2 (Pinheiro and Monteiro, 2010) and, consequently, the soil types and their physical and chemical characteristics could vary considerably (Ratter et al. 2003). Nevertheless, the Cerrado vegetation usually grows on oxysols that are sandy, acidic (pH < 5.0) and show m% between 63 and 85% (Haridasan and Araújo, 1988; Souza et al. 2015b; Bressan et al. 2016; Nogueira et al. 2019), falling within the same characteristics described in the present study (Table 2).

In general, leaves of Al-accumulating plants were more coriaceous (Fig. 1H and 1P) in relation to non-accumulating species (Fig. 1D and 1L). Both accumulating and non-accumulating species exhibited branched roots found at 30–40 cm of depth (Fig. 1).

In the leaves, Al concentration differed significantly between Al-accumulating species. *V. tucanorum* showed 6.8-times more Al than *Q. grandiflora* (Fig. 2), and these two species accumulated more than 1000 mg Al kg^{-1} dry leaves, falling within the criterium of Chenery (1948b) to be considered an Al-accumulating species. Indeed, in a field study, *V. tucanorum* accumulated 20,930 while *Q. grandiflora*, 5,414 mg Al kg^{-1} dry leaves, attesting the higher capacity of the former to accumulate this metal in their leaves (Haridasan and Araújo, 1988). One of the pioneer studies about Al-accumulating species from the Cerrado showed that *Q. grandiflora* accumulates 5,160 ± 930 mg Al kg^{-1} dry leaves (Haridasan, 1982). Recent field studies also describe *Q. grandiflora* accumulating between 3,800 and 6,000 mg Al kg^{-1} dry leaves (Bressan et al. 2016; Nogueira et al. 2019). Therefore, our results corroborate the Al accumulation range exhibited by both species occurring in the field reported in the literature. When our results are compared with values shown by Al-accumulating woody species from other vegetations around the world (Table 3), the leaf Al concentration range is similar, indicating that leaf accumulation in the Cerrado follows a conserved pattern among species worldwide. Like *Symplocos* species occurring in Asia, exhibiting more than 20,000 mg Al kg^{-1} dry leaves (Table3), in the present study, *V. tucanorum* showed 24,915 mg kg^{-1}, pointing out a large capacity of these species to accumulate Al in their leaves. *V. tucanorum* seems to be highly dependent on Al, as under its absence this plant shows leaf chlorosis (Souza et al. 2017), leaf shedding, root necrosis and plant death (Bressan et al. 2021). Nevertheless, the higher capacity of *V. tucanorum* to accumulate Al in relation to *Q. grandiflora* (Fig. 2) remains unclear.

In contrast, *X. aromatica* and *C. brasiliense* showed similar leaf Al concentration, and these species accumulated approximately 100 mg Al kg^{-1} dry leaves (Fig. 2), falling out of the criterion used by Chenery (1948b). *C. brasiliense* was reported to accumulate 330 ± 140 (Haridasan, 1982) and 170 mg Al kg^{-1} dry leaves (Haridasan and Araújo, 1988), and *X. aromatica*, 150 mg Al kg^{-1} dry leaves (Haridasan and Araújo, 1988). Our results, therefore, also show the same leaf Al concentration range for these two species like the few data reported in previous studies for Cerrado woody species and for non-accumulating species occurring in other vegetations (Table 3).

In the roots, *V. tucanorum* was the one that most accumulated Al, being 7.8-times higher than *Q. grandiflora*, which showed root Al concentration similar to *C. brasiliense* (Fig. 3). Among non-accumulating species, *X. aromatica* was the one that least accumulated this metal in their roots but did not show different values from *C. brasiliense* (Fig. 3). Rare field studies reported Al accumulation in the roots of Cerrado woody species. *Rudgea virbunoides* (Rubiaceae) from the Cerrado, was found to accumulate between 5,530 ± 580 and 13,680 ±

1,970 mg Al kg^{-1} dry roots, for a plant that accumulates approximately 13,000 mg Al kg^{-1} dry leaves (Malta et al. 2016). *Pterogastra divaricata* (Bompl.) Naudin tribe and *Pterolepis trichotoma* (Rottb.) Cogn. (Melastomataceae) growing in a palm-swamp community in Venezuela showed 12,660 ± 2,320 and 17,500 ± 2,520 mg Al kg^{-1} dry roots, respectively, and 12,230 ± 870 and 17,830 ± 1,200 mg Al kg^{-1} dry leaves, respectively (Olivares et al. 2010). Recently, Symplocaceae species growing in Central Sulawesi, Indonesia, were found to accumulate between 3,000 and 11,000 mg Al kg^{-1} dry roots and between 7,000 and 23,000 mg Al kg^{-1} dry leaves (Schmitt et al. 2016). In the case of the Al non-accumulating species, *X. aromatica*, growing on an Al-rich soil in a swamp-palm community at Orinoco Llanos in Venezuela, it showed 758 ± 509 mg Al kg^{-1} dry roots and 716 ± 118 mg Al kg^{-1} dry leaves (Mazorra et al. 1987). Thus, it seems that species that accumulate less than 1000 mg Al kg^{-1} dry leaves also accumulate this range in their roots, and those that show large Al concentration in their leaves tend to exhibit, at least, more than 1000 mg Al kg^{-1} dry roots, and this is confirmed for Al-accumulating species occurring in other vegetations worldwide (Table 3). The reason why these plants accumulate Al in their roots could be due to recent evidence that Al may be distributed among different plant organs (Timpone and Habermann, 2022), but more studies are welcome in this area.

Notwithstanding, in the present study, *Q. grandiflora* showed 748 ± 191 mg Al kg^{-1} dry roots, despite exhibiting 3,625 ± 712 mg Al kg^{-1} dry leaves (Fig. 3), thus, being considered an Al-accumulating species (Haridasan, 1982; Bressan et al. 2016). The present study is limited for having analyzed leaves and roots of only two Al-accumulating species from the Cerrado, from a universe of few species from four families, and it seems that Chenery criterium (Chenery, 1948b) can be accepted when leaves '*only*' are analyzed. Here we show that roots of *Q. grandiflora*, an Al-accumulating species, can accumulate less than 1000 mg Al kg^{-1}, and it deserves further investigation. Al non-accumulating species, here represented by *C. brasiliense* and *X. aromatica*, accumulate less than 1000 mg Al kg^{-1} in both leaves and roots, reiterating that these species belong to the largest Cerrado woody community, composed of Al non-accumulating species. Thus, while Al-accumulating species (*Q. grandiflora* and *V. tucanorum*) are tolerant, accumulating high Al concentrations in their leaves and varying values in their roots, non-accumulating ones (*C. brasiliense* and *X. aromatica*) could be classified as 'avoiders' because these species retain low Al concentration in their roots and leaves. More studies with these 'avoiders', considering, for example, Al exclusion mechanisms or root microbiome are welcome as prospects.

Author contribution statement Conceived the idea and designed the experiment: MZ, LTT and GH. Performed the experiment: MZ and LTT. Analyzed data: MZ, LTT and GH. Provided reagents, instruments, material, and analytical tools: GH. Wrote the manuscript: LTT and GH.

Acknowledgements: We acknowledge the Brazilian National Council for Scientific and Technological Development (CNPq) for a Scientific Initiation scholarship for M. Zaia and a research fellowship granted to G. Habermann (Grant 307431/2020-7). We extend the acknowledgments to Coordination for Improvement of Graduate Personnel (Capes)
for a PhD scholarship granted to Luá T. Timpone. We thank MSc. Matheus A. Nogueira for field assistance.

4 References

Bressan ACG, Coan AI, Habermann G. 2016. **X-ray spectra in SEM and staining with chrome azurol S show Al deposits in leaf tissues of Al-accumulating and non-accumulating plants from the cerrado**. Plant Soil 404:293–306

Bressan ACG, Bittencourt BMOC, Silva GS, Habermann G. 2021. **Could the absence of aluminum (Al) impair the development of an Al-accumulating woody species from Brazilian savanna?** Theor Exp Plant Physiol 33:281–292

Brunner I, Sperisen C. 2013. **Aluminum exclusion and aluminum tolerance in woody plants**. Front Plant Sci 4:1–12

Chenery EM. 1948a. **Aluminium in plants and its relation to plant pigments**. Ann Bot 12:121–136

Chenery EM. 1948b. **Aluminium in the plant world**. part I: general survey in dicotyledons. Kew Bull 1948:173–183

Empresa Brasileira de Pesquisa Agropecuária (Embrapa). 1997. **Manual for methods of soil analysis**, 2nd edn. Embrapa, Rio de Janeiro

Guilherme Pereira C, Clode PL, Oliveira RS, Lambers H. 2018. **Eudicots from severely phosphorus-impoverished environments preferentially allocate phosphorus to their mesophyll**. New Phytol 218:959–973

Haridasan M. 1982. **Aluminium accumulation by some Cerrado native species of central Brazil**. Plant Soil 65:265–273

Haridasan M, Paviani TI, Schiavini I. 1986. **Localization of aluminium in the leaves of some aluminium-accumulating species**. Plant Soil 94:435–437

Haridasan M, Araújo GM. 1988. **Aluminium-accumulating species in two forest communities in the Cerrado region of Central Brazil**. For Ecol Manag 24:15–26

Jansen S, Broadley MR, Robbrecht E, Smets E. 2002a. **Aluminum hyperaccumulation in angiosperms: A review of its phylogenetic significance**. The Bot Rev 68:235–269

Jansen S, Watanabe T, Smets E. 2002b. **Aluminium accumulation in leaves of 127 species in Melastomataceae, with comments on the order Myrtales**. Ann Bot 90:53–64

Kochian LV. 1995. **Cellular mechanisms of aluminum toxicity and resistance in plants**. Ann Rev Plant Physiol Plant Mol Biol 46:237–260

Köppen W. 1948. **Climatología con un estudio de los climas de la Tierra**. Mexico: Ed. Fondo de Cultura Económica-Pánuco

Malta PG, Silva AS, Ribeiro C, Campos NV, Azevedo AA. 2016. ***Rudgea viburnoides* (Rubiaceae) overcomes the low soi fertility of the Brazilian Cerrado and hyperaccumulates aluminum in cell walls and chloroplasts**. Plant Soil 408:369–384

Mazorra MA, Jose JJS, Montes R, Miragaya JG, Haridasan M. 1987. **Aluminium concentration in the biomass of native species of the Morichals (swamp palm community) at the Orinoco Llanos, Venezuela**. Plant Soil 102:275–277

Metali F, Salim KA, Burslem DFRP. 2012. **Evidence of foliar aluminium accumulation in local, regional and global datasets of wild plants**. New Phytol 193:637–649

Miranda-Melo AA, Martins FR, dos Santos FAM. 2007. **Estrutura populacional de *Xylopia aromatica* (Lam.) Mart. e de *Roupala montana* Aubl. em fragmentos de Cerrado no Estado de São Paulo**. Rev Bras Bot 30:501–507

Nogueira MA, Bressan ACG, Pinheiro MHO, Habermann G. 2019. **Aluminum-accumulating Vochysiaceae species growing on a calcareous soil in Brazil**. Plant Soil 437:313–326

Olivares E, Colonnello G, Peña E, Rodriguez L. 2010. **Aluminum accumulation in nineteen Melastomataceae species from three contrasting plant formations in acid soils**. J Plant Nutr Soil Sci 173:453–460

Pinheiro MHO, Monteiro R. 2010. **Contribution to the discussions on the origin of the cerrado biome: Brazilian savanna**. Braz J Biol 70:95–102

Ratter J, Bridgewater S, Ribeiro JF. 2003. **Analysis of the floristic composition of the Brazilian Cerrado vegetation**. III: comparison of the woody vegetation of 376 areas. Edinb J Bot 60:57–109

Samantaray S, Rout GR, Das P. 2001. **Heavy metal and nutrient concentration in soil and plants growing on metalliferous chromite minespoil**. Environ Tech 22:1147–1154

Sarruge JR, Haag HP. 1974. **Análises Químicas em plantas. Escola Superior de Agricultura Luiz de Queiróz, Piracicaba** [In Portuguese]

Schmitt M, Boras S, Tjoa A, Watanabe T, Jansen S. 2016. **Aluminum accumulation and intra-tree distribution patterns in three *Arbor aluminosa* (Symplocos) species from Central Sulawesi**. PlosOne 11:e0149078

Singh S, Tripathi DK, Singh S, Sharma S, Dubey NK, Chauhan DK, Vaculík M. 2017. **Toxicity of aluminium on various levels of plant cells and organism: A review**. Environ Exp Bot 137:177–193

Souza MC, Bueno PCP, Morellato LPC, Habermann G. 2015a. **Ecological strategies of Al-accumulating and non-accumulating functional groups from the cerrado *sensu stricto***. Ann Acad Bras Ciên 87:813–823

Souza MC, Franco AC, Haridasan M, Rossatto DR, De Araújo JF, Morellato LPC, Habermann G. 2015b. **The length of the dry season may be associated with leaf scleromorphism in cerrado plants**. Ann Acad Bras Ciên 87:1691–1699

Souza MC, Habermann G, Amaral CL, Rosa AL, Pinheiro MHO, Costa FB. 2017. ***Vochysia tucanorum* Mart.: an aluminum-accumulating species evidencing calcifuge behavior**. Plant Soil 419:377–389

Timpone LT, Habermann G. 2022. **Is aluminum (Al) eliminated by senescent structures of *Miconia albicans*, an Al-accumulating species from Brazilian savanna?** Flora 289, 152036

Van Raij B, Andrade JC, Cantarella H, Quaggio JÁ. 2001. **Análise química para avaliação da fertilidade de solos tropicais**. Instituto Agronômico de Campinas (IAC), Campinas [In Portuguese]

von Uexküll HR, Mutert E. 1995. **Global extent, development and economic impact of acid soils**. Plant Soil 171:1–5

Webb LJ. 1954. **Aluminium accumulation in the Australian–New Guinea flora**. Austr J Bot 2:176–196

Wigley BJ, Coetsee C, Hartshorn AS, Bond WJ. 2013. **What do ecologists miss by not digging deep enough? Insights and methodological guidelines for assessing soil fertility status in ecological studies**. Acta Oecol 51:17–27

Zingore S, Mutegi J, Agesa B, Tamene L, Kihara J. 2015. **Soil degradation in sub-Saharan Africa and crop production options for soil rehabilitation**. Better Crops 99:24–26

Tables:

Table 1. Texture (sand, silt, and clay) and density of a cerrado *sensu stricto* soil from Itirapina-SP, southeastern Brazil.

Coarse sand	Fine sand	Total sand	Clay (in NaOH)	Natural clay (in H_2O)	Silt	Texture	Density
----------(g kg^{-1})----------							($g\ cm^{-3}$)
553 ± 75	363 ± 40	916 ± 50	73 ± 7	38 ± 3	11 ± 0.4	Sandy	1.43 ± 0.06[A]

Mean values (*n = 5*) ± standard deviation; [A]bulk density measured from a non-deformed soil sample.

Table 2. Macro- and micronutrient contents and fertility parameters of a cerrado *sensu stricto* soil from Itirapina-SP, southeastern Brazil.

pH	OM	P resin	Al^{3+}	$H^+ + Al^{3+}$	K^+	Ca^{2+}	Mg^{2+}	BS	CEC	V%	m%	B	Cu	Fe	Mn	Zn
($CaCl_2$)	(g dm^{-3})	(mg dm^{-3})	----------mmol charges dm^{-3}----------							(%)	(%)	----------mg dm^{-3}----------				
3.8±0.1	9.4±1.9	1.8 ± 0.4	15.8±2.6	41.6±5.7	0.4±0.2	2.4±0.9	1±0.0	3.8±1.0	45.4±5.3	8.6±2.8	80.4±6.8	0.27±0.0	0.28±0.0	55.2±17.2	1.4±0.6	0.1±0.0

BS = Base saturation ($K^+ + Ca^{2+} + Mg^{2+}$); CEC = $K^+ + Ca^{2+} + Mg^{2+} + H^+ + Al^{3+}$; V% = Fertility rate [$V = 100\ (K^+ + Ca^{2+} + Mg^{2+})\ CEC^{-1}$], %;
m% = (100 x Al^{3+})/(BS + Al^{3+})
Mean values (*n = 5*) ± standard deviation.

Table 3. Aluminum concentrations (mean ± S.D.) in roots and leaves of Al-accumulating and non-accumulating woody species from field studies worldwide.

Family	Plant species	Al strategy	Root Al concentration (mg kg^{-1})	Leaf Al concentration (mg kg^{-1})	References
Melastomataceae	*Miconia stephanthera Uie*	accumulator	6,752 ± 1,322	6,899 ± 1803	Mazorra et al. 1987
Escalloniaceae	*Polyosma celebica*	accumulator	10,046 ± 2,818	7,535 ± 1,528	Schmitt et al. 2016
Melastomataceae	*Pterogastra divaricata (Bompl.) Naudin*	accumulator	12,660 ± 2,320	12,230 ± 0,87	Olivares et al. 2010
Melastomataceae	*Pterolepis trichotoma (Rottb.) Cogn*	accumulator	17,500 ± 2,520	17,830 ± 1,200	Olivares et al. 2010
Rubiaceae	*Rudgea viburnoides (Cham.) Benth*	accumulator	8,466 ± 3,215	13,220 ± 1,688	Malta et al. 2016
Symplocaceae	*Symplocos ambangensis*	accumulator	11,164 ± 4,983	16,719 ± 1,120	Schmitt et al. 2016
Symplocaceae	*Symplocos odoratissima*	accumulator	3,244 ± 1,568	23,383 ± 9,593	Schmitt et al. 2016
Symplocaceae	*Symplocos ophirensis*	accumulator	11,380 ± 4,060	21,352 ± 2,802	Schmitt et al. 2016
Combretaceae	*Anogeissus latifolia*	non-accumulator	42.4 ± 0.7	59.6 ± 0.3	Samantaray et al. 2001
Malpighiaceae	*Byrsonima crassifolia* (L.) HBK	non-accumulator	1200	715	Mazorra et al. 1987
Rutaceae	*Chloroxylon swietenia*	non-accumulator	47.8 ± 0.8	68.4 ± 0.6	Samantaray et al. 2001
Dilleniaceae	*Curatella americana* L.	non-accumulator	807 ± 224	737 ± 297	Mazorra et al. 1987
Rubiaceae	*Haldina cordifolia*	non-accumulator	40.2 ± 0.8	66.6 ± 0.2	Samantaray et al. 2001
Lythraceae	*Lagerstroemia parviflora*	non-accumulator	36.2 ± 0.8	35.5 ± 0.4	Samantaray et al. 2001
Fagaceae	*Lithocarpus sp.*	non-accumulator	3,646 ± 1,826	176 ± 140	Schmitt et al. 2016
Onagraceae	*Ludwigia nervosa* (Poir) Hara	non-accumulator	920 ± 418	834 ± 653	Mazorra et al. 1987
Sapotaceae	*Madhuca longifolia*	non-accumulator	37.4 ± 0.5	41.0 ± 0.6	Samantaray et al. 2001

Arecaceae	*Mauritia flexuosa* L.f.	non-accumulator	1,795 ± 720	810 ± 233	Mazorra et al. 1987
Myrtaceae	*Syzygium sp.*	non-accumulator	986 ± 677	34 ± 13	Schmitt et al. 2016
Annonaceae	*Xylopia aromatica* (Lam) Mart	non-accumulator	758 ± 509	716 ± 118	Mazorra et al. 1987

Figure captions:

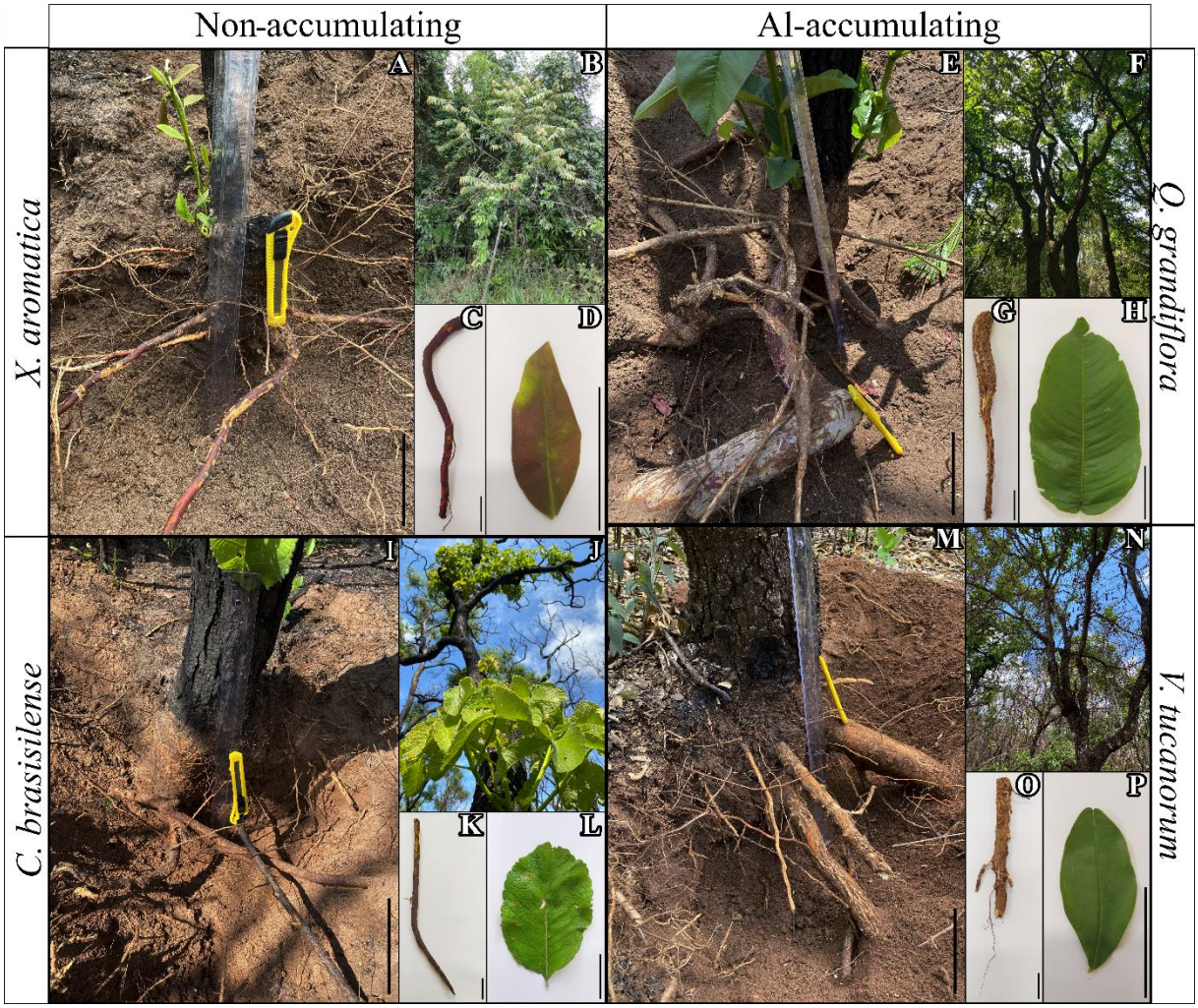

Fig. 1 Visual aspects of the roots (A, E, I, M), plant canopy (B, F, J, N) in the field, root segments (C, G, K, O) and leaves (D, H, L, P) in the laboratory of non-accumulating (A–D and I–L) and accumulating species (E–H and M–P) from a cerrado *sensu stricto* remnant in Itirapina, São Paulo state, southeastern Brazil. Scale bars: A, E, I, M = 10 cm; C, D, G, H, K, L, O, P = 5 cm.

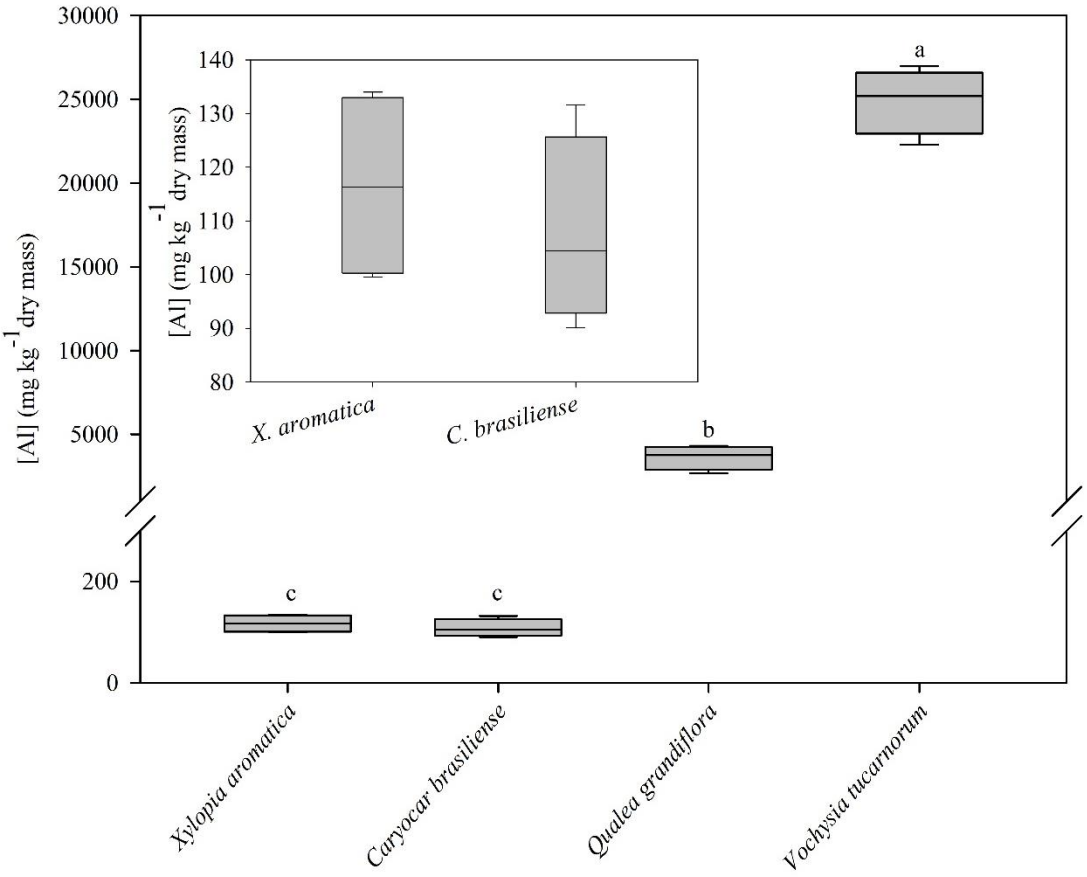

Fig. 2 Aluminum concentration in leaves of non-accumulating (*X. aromatica* and *C. brasiliense*) and accumulating species (*Q. grandiflora* and *V. tucanorum*) from a cerrado *sensu stricto* remnant in Itirapina, São Paulo state, southeastern Brazil. The box extends from the 25th to 75th percentiles, the continuous line within the box shows the median, and error bars represent 5th and 95th percentiles (n = 5 plants). Distinct letters indicate significant differences between species by Tukey test ($P < 0.05$). The small graph inside graph panel highlights values for *X. aromatica* and *C. brasiliense*.

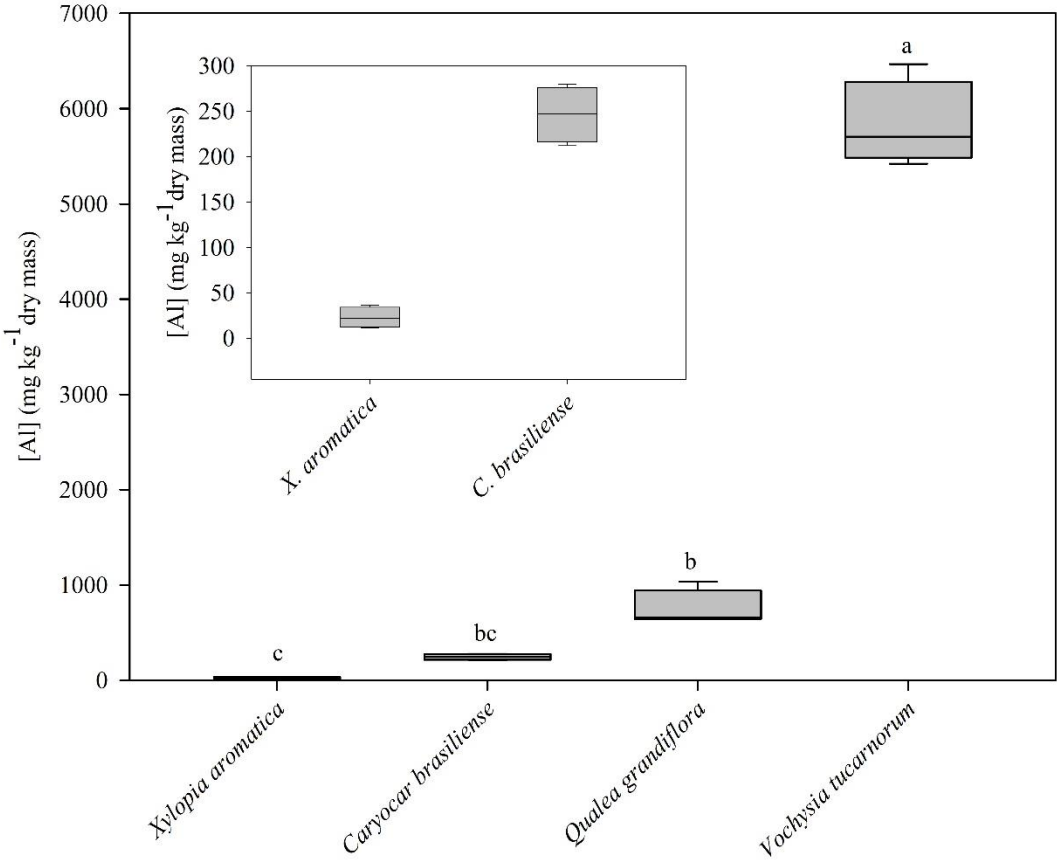

Fig. 3 Aluminum concentration in roots of non-accumulating (*X. aromatica* and *C. brasiliense*) and accumulating species (*Q. grandiflora* and *V. tucanorum*) from a cerrado *sensu stricto* remnant in Itirapina, São Paulo state, southeastern Brazil. The box extends from the 25th to 75th percentiles, the continuous line within the box shows the median, and error bars represent 5th and 95th percentiles (n = 5 plants). Distinct letters indicate significant differences between species by Tukey test ($P < 0.05$). The small graph inside graph panel highlights values for *X. aromatica* and *C. brasiliense*.

CAPÍTULO 2

Is aluminum (Al) eliminated by senescent structures of *Miconia albicans*, an Al-accumulating species from Brazilian savanna?

Luá Taibo Timpone[1], Gustavo Habermann[*2]

[1]*Programa de Pós-Graduação em Biologia Vegetal, Universidade Estadual Paulista (UNESP), Instituto de Biociências, Departamento de Biodiversidade, Av. 24-A, 1515, Rio Claro, SP, 13506-900, Brazil*

[2]*Universidade Estadual Paulista (UNESP), Instituto de Biociências, Departamento de Biodiversidade, Av. 24-A, 1515, Rio Claro, SP, 13506-900, Brazil. E-mail: gustavo.habermann@unesp.br; Tel: +0055 19 3526-4210*

[*]*Corresponding author*

Timpone LT, Habermann G. 2022. **Is aluminum (Al) eliminated by senescent structures of *Miconia albicans*, an Al-accumulating species from Brazilian savanna?** Flora 289, 152036

Abstract

Aluminum (Al)-accumulating plants are distributed worldwide. In the Cerrado vegetation in South America, these plants occur as few woody species from Melastomataceae, Rubiaceae, Syplocaceae and Vochysiaceae. Nevertheless, Al assessments are usually limited to their leaves. In this field study, we measured the Al concentration in different vegetative and reproductive structures of *Miconia albicans* (Sw.) Triana (Melastomataceae) with a special emphasis on changes of Al accumulation in senescent organs and tissues. We collected leaf (leaf bud, two young leaf phases and senescent leaf), wood, bark, root, inflorescence (raceme, flower bud and flower) and fruit (two initial fruit phases, unripe and ripe fruit) to evaluate the Al distribution within the whole plant. The mature leaf was separated into leaf blade and veins, and the former accumulated more Al in relation to leaf veins, following the same pattern observed for Vochysiaceae Al-accumulating plants. Senescent leaf and bark accumulate more Al than mature leaves, and as flowers develop into ripe fruits the Al concentration decreases. Aluminum accumulation is not limited to the leaves of *M. albicans*, and the greater Al accumulation in senescent leaves and bark suggests that this Al-accumulating species uses senescent leaves and bark to eliminate Al from the plant.

Key words: Al^{3+}; bark; Cerrado; inflorescence; Melastomataceae; root

1 Introduction

Plants that accumulate aluminum (Al) in their leaves are distributed worldwide but are most common in tropical areas with acidic soils (pH < 3–5) and high rainfall (Jansen et al., 2002). Acidic soils occupy, approximately, 30% of the world's ice-free land under cold and temperate humid climates (northern Canada, Europe, and Russia) and humid tropics (Southeast Asia, Pacific, part of Australia and New Zealand, sub-saharian Africa — part of the Central and East Africa, Southeast USA and Central and Northern South America) (von Uexküll and Mutert, 1995; Brunner and Sperisen, 2013).

Plants that accumulate Al belong to 45 families, and Melastomataceae, Rubiaceae, Symplocaceae, Theaceae and Vochysiaceae are those that are most studied (Jansen et al., 2002; Haridasan, 1982). Al-accumulating species may accumulate up to 23,000 mg Al kg^{-1} dry leaves (Haridasan and Araújo, 1988; Schmitt et al., 2016). Nevertheless, to be considered an Al-accumulating species, it must accumulate at least 1000 mg Al kg^{-1} dry leaves (Chenery, 1948; Jansen et al., 2002). Essentially, these plants have been identified by their capacity to accumulate Al in their leaves, although few studies have reported Al accumulation in barks (Masunaga et al., 1988; Schmitt et al., 2016), seeds (Haridasan, 2008), fruits (Pasta et al., 2019) and, rarely, roots (Malta et al., 2016; Schmitt et al., 2016).

Some of these studies mentioned above (Haridasan, 1982, 2008; Haridasan and Araújo, 1988; Pasta et al., 2019) have been conducted in the Cerrado vegetation in South America, broadly known as 'Brazilian savanna' that grows on acidic soils (pH < 5.0) with high Al saturation (m% > 70%) (Haridasan, 1982; Bressan et al., 2016). Grasslands, savanna-like, and forest physiognomies are found in this vegetation, which is subjected to seasonal drought (Apr–Sep) and wet and warm season (Oct–Mar) (Oliveira Filho and Ratter, 2002). Most woody plants from this vegetation are Al non-accumulating species and few Al-accumulating ones from Melastomataceae, Rubiaceae, Symplocaceae and Vochysiaceae (Haridasan, 1982; Souza et al., 2015a).

Studies with Al-accumulating woody species from the Cerrado unravel the Al distribution within the leaf. In *Qualea grandiflora* and *Q. parviflora* (Vochysiaceae), Al-accumulating species from this vegetation, more Al is found in the leaf blade than leaf veins (Nogueira et al., 2019), and this pattern seems to be related to the isolation of Al in epidermis so as not to interfere with photosynthetic leaf tissues (Guilherme Pereira et al., 2018). Thus, some studies have investigated a likely role for Al in the leaves of these plants, but less attention is given to the pattern of Al distribution among different organs other than leaves. In addition, mature leaves of Al-accumulating species from the Cerrado seem to accumulate more Al than

young leaves (Souza et al., 2015a), but the pattern of Al distribution between leaf phases and reproductive structures is even less known.

Using *Miconia albicans* (Sw.) Triana (Melastomataceae), an Al-accumulating shrub from a savanna-like physiognomy of the Cerrado vegetation, we collected its vegetative organs (leaf and root), tissues like wood and bark, and reproductive organs (inflorescence and fruit) to study the Al distribution within the whole plant. In addition, to confirm the assumption that Al-accumulating species accumulate more Al in the leaf blade than leaf vein, as in some Vochysiaceae species (Nogueira et al., 2019), we separated *M. albicans* leaves into blade and leaf veins to see if this pattern could repeat in this species. Leaves and reproductive organs were also collected during distinct phases so that we could observe the Al concentration from leaf buds until mature and senescent leaves. Inflorescence, including flower buds and raceme, until unripe and ripe fruit phases were also assessed. In this study, we tested the hypothesis that senescent leaf and bark contribute to Al elimination by the plant.

2 Material and methods

2.1 Plant material and experimental site

Adult plants of *Miconia albicans* (Sw.) Triana (Melastomataceae) naturally growing in a remnant of a savanna-like physiognomy called cerrado *sensu stricto* (*s. str.*) were used. It is an Al-accumulating species (Haridasan et al., 1986), with 3,000–8,600 mg Al kg^{-1} dry leaves (Bressan et al., 2016; Souza et al., 2018). This evergreen species (Souza et al., 2015a) grows as a shrub (1.5–3 m in height) with canopy diameter of 1.5–3 m. Flowering occurs at the end of the dry season in the Cerrado vegetation (April to September), and its unripe fruits are purplish, becoming green when ripe (Camargo et al., 2015).

The study was conducted in a cerrado *s. str.* remnant in the municipality of Itirapina, Southern São Paulo state, Brazil. This region shows a Cwa (Köppen, 1948) climate type, with a wet and warm (October – March; mean monthly temperature of 32 ºC) and a dry and cool (April – September; mean monthly temperature of 18 ºC) season, with annual rainfall of 1512 mm (Souza et al., 2015b). The experimental area (260 ha) was located on São José da Conquista farm (22° 13′ S, 47° 53′ W; 610 m asl.).

2.2 Experimental design

Soil samples (n = 5) were randomly collected in the area, at 20–30 cm of depth, where nutrient uptake for most native plant species occurs (Wigley et al., 2013). Physical and fertility parameters, especially soil Al saturation (m%), were measured.

Five adult plants were randomly selected in the area, from which healthy vegetative organs (leaf and root), secondary growing stem tissues (wood and bark) and reproductive organs (inflorescence and fruit) were collected to assess the Al concentration. The canopy was divided into four quadrants (N, S, E, W), where these organs and tissues were sampled. Leaf buds, two young leaf phases, mature leaves and senescent leaves were collected and categorized according to the leaf length (see results). A nylon® transparent net sack was placed around the plant canopy to save senescent leaves that shed from plants. Mature leaves were separated into whole leaf, leaf blade, primary and secondary leaf veins (using a scalpel) to measure the Al concentration. Roots were washed three times with deionized water to remove soil particles. Inflorescence was separated into flower bud, flower, and raceme. Fruits were divided into two young fruit phases, an unripe fruit phase, and a ripe fruit phase.

Collection of organs and tissues were performed throughout a 12-month period (2019/2020). Roots, wood, bark, leaf buds, the two young leaf phases and mature leaves were collected in the wet season (Oct–Mar). Senescent leaves were sampled from May to August. Reproductive organs were collected from the peak of the dry season (August) up to the middle of the wet season (December/January). Flower buds were collected before bloom (August), flowers and raceme in September, the two young fruit phases between October and November, and the unripe and ripe fruits, between November and January.

2.3 Soil sampling

Soil sampling spots were arranged in a zigzag on the experimental area, close to *M. albicans* individuals. The soil was collected with an auger until reaching 20–30 cm deep, and the samples were put in plastic bags, identified, and sent to the soil science laboratory (Instituto Agronômico de Campinas, Campinas, SP, Brazil). Chemical (fertility) analysis, including soil Al saturation (m%) and soil pH (in $CaCl_2$), was performed according to van Raij et al. (2001), described in English by Dantas and Batalha (2011). Physical analysis (percentage of sand, silt, and clay) was carried out following protocols described by Embrapa (1997).

2.4 Al concentration in plant material

Samples of vegetative organs, secondary growing stem tissues and reproductive organs were oven-dried at 60 ºC until constant mass and sent to the Plant Nutrition Laboratory at Instituto Agronômico de Campinas (IAC) for analysis of Al concentration. Samples were ground and digested in a solution of sulfuric:nitric:percloric acids (1:10:2, v/v/v). After

digestion, Al concentration was determined by the atomic absorption spectrophotometric method (Sarruge and Haag, 1974), and Al was expressed as mg kg^{-1} dry mass.

2.5 Data analysis

Aluminum concentration measured in vegetative organs, secondary growing stem tissues and reproductive organs was analyzed using five randomly selected replicates (plants), for each organ and tissue analyzed. A one-way analysis of variance (ANOVA) was run to test for differences in Al concentration between the different structures. Mean values were compared by Tukey test ($P < 0.05$) to test for differences between leaf buds, the two young leaf phases, mature leaf, and senescent leaf. The same procedure was used when comparing the Al concentration between leaf blade, primary and secondary leaf veins, and whole leaf, as well as between the reproductive organs (flower bud, flower, the two young fruit phases, unripe and ripe fruits).

Mean values of Al concentration shown by the main tree structures (flower bud, flower, raceme, mature fruit, leaf bud, mature and senescent leaf, wood, bark, and root) were highlighted in the whole plant scenario.

3 Results

As expected, the soil was acidic (pH ≈ 3.8) with high Al availability (m% > 80%), base saturation represented 8.6% of CEC, and P availability was also low (Table 1). This dystrophic soil showed a considerable amount of sand, being classified as a sandy soil (Table 2).

Six reproductive phases were identified, including flower bud (Fig. 1A), flower (Fig. 1B) (for details of the inflorescence and flower, see Fig. S1, Supplementary material), initial fruit with dry floral pieces, herein named ‘initial fruit 1’ (Fig. 1C), initial fruit with dry petals, herein named ‘initial fruit 2’ (Fig. 1D), purplish unripe fruit (Fig. 1E) and green ripe fruit (Fig. 1F). The raceme (Fig. 1L) bears the flowers (inflorescence) and fruit and was 7–18 cm in length. From leaf bud (Fig. 1G) to senescent leaf (Fig. 1K), we found three more leaf phases: ‘young leaf 1’ (4–7 cm in length) (Fig. 1H), young leaf 2 (7–10 cm in length) (Fig. 1I) and mature leaf (> 10 cm in length) (Fig. 1J). The trunk bark (Fig. 1M) easily separated from the wood (Fig. 1N). The main root was, approximately, 1–2 cm in diameter, but secondary branched roots were thinner (2–3 mm in diameter) (Fig. 1O).

The leaf bud, young leaf 1, young leaf 2 and mature leaves accumulated between 3,000 and 5,000 mg Al kg^{-1} dry mass, while senescent leaf accumulated two times more, approximately 8,000–10,000 mg Al kg^{-1} dry mass (Fig. 2). As expected, the leaf blade showed

more Al in relation to the primary and secondary leaf veins. The leaf blade, however, showed Al concentration similar to the whole leaf (Fig. 3). Therefore, Al accumulates over the leaf age, especially in the blade.

Among reproductive organs, the flower bud showed the highest Al concentration, being 2.1 times higher than initial fruit 1 and green ripe fruit (Fig. 4). Nevertheless, flower, initial fruit 2 and purplish unripe fruit showed intermediate Al concentration (Fig. 4).

Within all plant organs and tissues, the senescent leaf and bark showed the highest Al concentration, reaching a range of 6,000–8,000 mg Al kg^{-1} dry mass (Fig. 5). Mature leaf follows the Al accumulation rank, with 4,000–6,000 mg Al kg^{-1} dry mass, similar to flower bud and root. Leaf bud, flower and green ripe fruit accumulated between 2,000 and 4,000 mg Al kg^{-1} dry mass, and raceme and wood showed between 0 and 2,000 mg Al kg^{-1} dry mass (Fig. 5). Therefore, senescent leaves and bark accumulated the most Al, and raceme, green ripe fruit and wood, the least Al concentration.

4 Discussion

Our results suggest that (1) Al accumulation is not limited to the leaves of *M. albicans*; (2) bark and senescent leaves show the highest Al concentration among all analyzed plant structures; (3) despite being considered an Al-accumulator in the leaves, roots of *M. albicans* retain considerable Al concentration, and (4) as flowers develop into ripe fruits the Al concentration decreases. Therefore, our results suggest that leaves of *M. albicans* are not the only destination of this metal. We corroborate our hypothesis as *M. albicans* seems to use senescent leaves and bark to eliminate Al from the plant.

Most field studies using Al-accumulating plants from the Cerrado limit assessments of Al concentration to the leaves (Haridasan, 1982; Bressan et al., 2016; Nogueira et al., 2019). Even studies with *M. albicans* whose objective is the investigation of Al metabolism use leaves as the target to assess the Al concentration (Haridasan et al., 1986; Bressan et al., 2016; Souza et al., 2018). The magnitude of response (leaf Al concentration) of *M. albicans* in field studies conducted in the Cerrado includes 3,000–8,600 mg Al kg^{-1} dry leaf (Bressan et al., 2016) in the Southern periphery of the Cerrado (Itirapina and Mogi-Guaçu, São Paulo state, Brazil) and 6,640 ± 2,140 mg Al kg^{-1} dry leaf (Souza et al., 2018) in the central area of the Cerrado vegetation (Brasília, DF, Brazil). Therefore, the range of leaf Al concentration found in the present study is similar to other studies with this species in the Cerrado, which validates our results. Other studies have already found Al in seeds (Haridasan, 2008) and fruit (Pasta et al., 2019) of *M. albicans* and, by histochemical tests, in the main stem of this species (Milanez et

al., 2017), but as far as we are aware, the present study is the first with the objective of investigating Al accumulation in different organs and tissues of this species.

In *Qualea grandiflora* and *Q. parviflora* (Vochysiaceae), Al-accumulating woody species from this vegetation, the Al concentration in whole leaves and leaf blades is higher than that in leaf veins (Nogueira et al., 2019). Using quantitative cryo-scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS) (that quantifies tissue-specific element concentration), Guilherme Pereira et al. (2018) found that Al is accumulated in upper and lower epidermis and hypodermal cells of *Q. grandiflora* and *Q. parviflora* leaves. Our results show that *M. albicans* leaf blade accumulated 43% and 30% more Al than the primary and secondary leaf veins, respectively (Fig. 3). Thus, more Al observed in leaf blade in relation to leaf vein in these Vochysiaceae species (Nogueira et al., 2019) and in *M. albicans* (Fig. 3) could be because the leaf blade also contains the upper and lower epidermis and hypodermis that accumulate more of this metal. According to Guilherme Pereira et al. (2018), more Al in upper and lower epidermis and hypodermal cells in the leaf is associated to the avoidance of this metal in the mesophyll, where Al could make phosphorus unavailable, a nutrient useful for photosynthesis in this tissue. In addition, more Al in upper and lower epidermis and hypodermal cells in the leaf could also serve as defense against herbivores and pathogens. Nevertheless, studies using alternative methodologies to test these hypotheses is unavailable, and this remains to be studied.

Studies about leaf Al concentration in response to leaf phases, mainly in the Cerrado vegetation, are rare. A field study grouping Al-accumulating species (*Miconia albicans*, *M. rubiginosa*, *M. fallax* and *Q. grandiflora*) from the Cerrado showed that their mature leaves had 3.14-times more Al than their young leaves (Souza et al., 2015a). Our results, with just one of the species used by Souza et al. (2015a), show similar leaf Al concentration between leaf buds, young leaves (1 and 2) and mature leaves (Fig. 2), possibly suggesting that leaf Al concentration in response to leaf phases might be species dependent. Those authors collected the young leaves in the dry season and mature leaves in the wet season, while we concentrated the collection of leaf buds, young leaf 1 and 2 and mature leaves in the wet season (see Material and Methods), isolating the seasonal effect. Those authors did not measure Al concentration in senescent leaves; however, there are studies about macro- and micronutrient leaf cycling in Cerrado areas (Nardoto et al., 2006; Alves et al., 2018), but not involving Al recycling. Al-accumulating *Symplocos* species (Symplocaceae) from Central Sulawesi, Indonesia, showed higher Al concentration in old leaves than younger developmental leaf phases (Schmitt et al., 2016). *Memecylon laurinum* and *Pternandra caerulescens* (Melastomataceae), Al-accumulating plants from a tropical rain forest in west Sumatra, Indonesia, were found to accumulate 22,630 and

6,520 mg Al kg^{-1} dry bark samples, and the former accumulates more Al in the bark than in the leaves (Masunaga et al., 1998). In the present study, we found 1.6-times more Al in senescent leaves when compared to mature leaves (Fig. 2). Fruit, bark, and leaf, all shed from plants, but the bark and senescent leaves showed the highest Al concentration among all plant organs and tissues (Fig. 5). Aluminum recycling would be unlikely in Al-accumulating species like *M. albicans* because recycling is the reutilization of compounds whose immediate physiological function contributes to growth (Chapin et al., 1990) and no physiological function(s) has been ascribed to Al in Al-accumulating species. In addition, Al recycling evidence is difficult to get (Al isotopes, for example, is impossible to use due to its half-life of more than 1000 years). Therefore, this topic deserves further studies using alternative methods.

Roots are particularly important in the Cerrado because woody species from this vegetation can escape the long dry season due to their long roots reaching deep and moist soil layers (Rawishter et al., 1948; Franco, 1998; Habermann and Bressan, 2011). But despite this ecophysiological importance, rare field studies (Malta et al., 2016) have assessed root Al accumulation in Cerrado plants. Root Al concentration is more easily found in studies with Cerrado plants cultivated in potted soil (Souza et al., 2017) or in nutrient solution (Banhos et al., 2016; Bressan et al., 2020, 2021). *Rudgea virbunoides* (Rubiaceae), an Al accumulator from Cerrado areas, shows between 5,530 and 13,680 mg Al kg^{-1} dry mass in roots, and SEM-EDS probe points out the Al can be mostly found in periderm and cortical parenchyma root cells, but with the shoot:root Al concentration higher than 1.99 (Malta et al., 2016). A field study conducted in a palm-swamp community in Venezuela showed that *Pterogastra divaricata* (Bonpl.) Naudin tribe and *Pterolepis trichotoma* (Rottb.) Cogn. (Melastomataceae) accumulated 12,660 and 17,500 mg Al kg^{-1} dry mass in their roots, respectively (Olivares et al., 2010). More recently, roots of Symplocaceae species were found to exhibit between 3,000 and 11,000 mg Al kg^{-1} dry mass in Central Sulawesi, Indonesia (Schmitt et al., 2016). In the present study, the roots showed between 2,272 and 6,773 mg Al kg^{-1} dry mass, and this value indicates an organ that accumulates less Al than bark and leaves (mature and senescent) of *M. albicans* (Fig. 5). Therefore, our results demonstrate that despite being considered an Al-accumulator in the leaves, roots of *M. albicans* retain considerable Al concentration and reiterate that their leaves are not the only destination of this metal. In *M. albicans*, as well in all Al-accumulating species from the Cerrado, roots are the first organ to get in contact with edaphic Al, and our results suggest that roots also participate as an Al-accumulating organ within the plant.

Studies evidencing Al in flowers of Cerrado woody plants are not available, and perhaps this is the first study evidencing Al in flower buds and flowers. The present study, however,

cannot point out why the Al accumulation decreased from flower buds until the ripening of fruits (Fig. 4). Nevertheless, the Al binds with the matrix pectic net in primary cell walls (Wehr et al., 2010; Bressan et al., 2016) and, when the Al is measured in leaves of Al-accumulating Cerrado species, this element is found in tissues with high pectin concentration and low lignification (Haridasan et al., 1986; Bressan et al., 2016). Flower pieces (petals and sepals) of plants in general show high pectin concentration and low lignification (Kumar et al., 2008; Choon and Ding, 2017), and there is evidence showing that the action of pectin methylesterase (PME) leads to free pectic carboxylic groups in the cell wall, allowing normal cell elongation (Horst et al., 2010). In *Etlingera elatior* (Jack) R.M. Smith) (Zingiberaceae) (torch ginger), as the inflorescence develops and flowers open, cellulase and PME hydrolyze and induce cell wall loosening eventually exposing free pectic carboxylic groups to Al^{3+} (Choon and Ding, 2017). Then, it is possible that expected high pectin concentration of flower tissues could explain the high Al accumulation in flower buds and flowers observed here. The present study, however, has not measured the biochemical composition of these organs and more studies in this regard are needed.

5 Conclusion

In this field study, we demonstrate that Al accumulation is not limited to the leaves of *M. albicans*, and root, bark, and wood also accumulate this element. During the reproductive period, flower buds and flowers accumulate most Al, but its concentration decreases as fruits develop. Our results confirm that Al concentration in whole leaves and leaf blades is higher than that in leaf veins of *M. albicans*, the same response pattern already found for Al-accumulating Vochysiaceae species. Among all evaluated organs and tissues, senescent leaves and barks showed the highest Al concentration, and this suggest that although this species shows huge Al concentration in its leaves without showing toxicity symptoms, Al elimination through senescent leaves and bark could be a strategy to cope with acidic soils with high Al saturation found in the Cerrado.

Acknowledgments

We acknowledge the Coordination for Improvement of Graduate Personnel (Capes) for a PhD scholarship granted to Luá T. Timpone and the Brazilian National Council for Scientific and Technological Development (CNPq) for a research fellowship granted to G. Habermann (Grant 307431/2020-7). We thank Marina Zaia and Matheus A. Nogueira for field and laboratory assistance.

6 References

Alves, V.N., Torres, J.L.R., Lana, R.M.Q., Pinheiro, M.H.O., 2018. **Nutrient cycling between soil and leaf litter in the Cerrado (Brazilian savanna) on eutrophic and dystrophic Neosols**. Acta Bot. Bras. 32, 169–179.

Banhos, O.F.A.A., Souza, M.C., Habermann, G., 2016. **High aluminum availability may affect *Styrax camporum*, an Al non-accumulating species from the Brazilian savanna**. Theor. Exp. Plant Physiol. 28, 321–332.

Bressan, A.C.G., Coan, A.I., Habermann, G., 2016. **X-ray spectra in SEM and staining with chrome azurol S show Al deposits in leaf tissues of Al-accumulating and non-accumulating plants from the cerrado**. Plant Soil 404, 293–306.

Bressan, A.C.G., Schwab, G.S., Banhos, O.F.A.A., Tanaka, F.A.O., Habermann, G., 2020. **Physiological, anatomical and ultrastructural effects of aluminum on *Styrax camporum*, a native Cerrado woody species**. J. Plant Res. 133, 625–637.

Bressan, A.C.G., Bittencourt, B.M.O.C., Silva, G.S., Habermann, G., 2021. **Could the absence of aluminum (Al) impair the development of an Al-accumulating woody species from Brazilian savanna?** Theor. Exp. Plant Physiol. 33, 281–292.

Brunner, I., Sperisen, C., 2013. **Aluminum exclusion and aluminum tolerance in woody plants**. Front. Plant Sci. 4, 1–12.

Camargo, M.G.G., Schaefer, H.M., Habermann, G., Cazetta, E., Soares, N.C., Morellato, L.P.C., 2015. **Bicolored display of *Miconia albicans* fruits: Evaluating visual and physiological functions of fruit colors**. Am. J. Bot. 102, 1453–1461.

Chapin III, F.S., Schulze, E.D., Mooney, H.A., 1990. **The ecology and economics of storage in plants**. Ann. Rev. Ecol. Syst., 21, 423–447.

Chenery, E.M., 1948. **Aluminium in the plant world. Part I: General survey in dicotyledons**. Kew Bull 1948, 173–183.

Choon, S.Y., Ding, P., 2017. **Developmental changes in cellular structure and cell wall metabolism of torch ginger (*Etlingera elatior* (Jack) R.M. Smith) inflorescence**. Curr. Plant Biol., 9–10, 3–10.

Dantas, V.L., Batalha, M.A., 2011. **Vegetation structure: fine scale relationships with soil in a cerrado site**. Flora 206, 341–346.

Embrapa (Empresa Brasileira de Pesquisa Agropecuária), 1997. **Manual for methods of soil analyses**, Second ed. Embrapa, Rio de Janeiro.

Franco, A.C., 1998. **Seasonal patterns of gas exchange, water relations and growth of *Roupala montana*, an evergreen savanna species**. Plant Ecol. 136, 69–76.

Guilherme Pereira, C., Clode, P.L., Oliveira, R.S., Lambers, H., 2018. **Eudicots from severely phosphorus-impoverished environments preferentially allocate phosphorus to their mesophyll**. New Phytol. 218, 959–973.

Habermann, G., Bressan, A.C.G., 2011. **Root, shoot and leaf traits of the congeneric *Styrax*** species may explain their distribution patterns in the cerrado *sensu lato* areas in Brazil. Funct. Plant Biol. 38, 209–218.

Haridasan, M., 1982. **Aluminium accumulation by some Cerrado native species of central Brazil**. Plant Soil 65, 265–273.

Haridasan, M., 2008. **Nutritional adaptations of native plants of the cerrado biome in acid soils**. Braz. J. Plant Physiol. 20, 183–195.

Haridasan, M., Araújo, G.M., 1988. **Aluminium-accumulating species in two forest communities in the Cerrado region of Central Brazil**. For. Ecol. Manag. 24, 15–26.

Haridasan, M., Paviani, T.I., Schiavini, I., 1986. **Localization of aluminium in the leaves of some aluminium-accumulating species**. Plant Soil 94, 435–437.

Horst, W.J., Wang, Y., Eticha, D., 2010. **The role of the root apoplast in aluminium-induced inhibition of root elongation and in aluminium resistance of plants: A review**. Ann. Bot. 106, 187–197.

Jansen, S., Broadley, M.R., Robbrecht, E., Smets, E., 2002. **Aluminum hyperaccumulation in angiosperms: A review of its phylogenetic significance**. The Bot. Rev. 68, 235–269.

Köppen, W., 1948**. Climatología con un estudio de los climas de la Tierra**. Ed. Fondo de Cultura Económica-Pánuco, Mexico.

Kumar, N., Srivastava, G.C., Dixit, K., 2008. **Flower bud opening and senescence in roses (*Rosa hybrida* L.)**. Plant Growth Regul. 55, 81–99.

Malta, P.G., Silva, A.S., Ribeiro, C., Campos, N.V., Azevedo, A.A., 2016. ***Rudgea viburnoides* (Rubiaceae) overcomes the low soil fertility of the Brazilian Cerrado and hyperaccumulates aluminum in cell walls and chloroplasts**. Plant Soil 408, 369–384.

Masunaga, T., Kubota, D., Hotta, M., Wakatsuki, T., 1998. **Mineral composition of leaves and bark in aluminum accumulators in a tropical rain forest in Indonesia**. Soil Sci. Plant Nutr. 44, 347–358.

Milanez, C.R.D., Marcati, C.R., Machado, S.R., 2017. **Trabeculae and Al-accumulation in the wood cells of Melastomataceae species from Brazilian savana**. Botany 95, 501–530.

Nardoto, G.B., Bustamante, M.M.C., Pinto, A.S., Klink, C.A., 2006. **Nutrient use efficiency at ecosystem and species level in savanna areas of Central Brazil and impacts of fire**. J. Trop. Ecol. 22, 191–201.

Nogueira, M.A., Bressan, A.C.G., Pinheiro, M.H.O., Habermann, G., 2019**. Aluminum-accumulating Vochysiaceae species growing on a calcareous soil in Brazil**. Plant Soil 437, 313–326.

Olivares, E., Colonnello, G., Peña, E., Rodriguez, L., 2010. **Aluminum accumulation in nineteen Melastomataceae species from three contrasting plant formations in acid soils**. J. Plant Nutr. Soil Sci. 173, 453–460.

Oliveira Filho, A.T., Ratter, J.A., 2002**. Vegetation physiognomies and woody flora of the cerrado biome.** In: Oliveira PS, Marquis RJ (Eds.), The Cerrados of Brazil, pp. 91–120. Columbia University Press, New York.

Pasta, P.C., Durigan, G., Moraes, I.C.F., Ribeiro, L.F., Haminiu, C.W.I., Branco, I.G., 2019. **Physicochemical properties, antioxidant potential and mineral content of *Miconia albicans* (Sw.) Triana: A fruit with high aluminium content**. Braz. J. Bot. 42, 209–216.

Rawistscher, F., 1948. **The water economy of the campos cerrados in the Southern of Brazil**. J. Ecol. 36, 237–268.

Sarruge, J.R., Haag, H.P., 1974. **Análises Químicas em plantas**. Escola Superior de Agricultura Luiz de Queiróz, Piracicaba.

Schmitt, M., Boras, S., Tjoa, A., Watanabe, T., Jansen, S., 2016. **Aluminum accumulation and intra-tree distribution patterns in three *Arbor aluminosa* (Symplocos) species from Central Sulawesi**. PlosOne 11, e0149078.

Souza, M.C., Bueno, P.C.P., Morellato, L.P.C., Habermann, G., 2015a. **Ecological strategies of Al-accumulating and non-accumulating functional groups from the cerrado *sensu stricto***. Ann. Acad. Bras. Ciên. 87, 813–823.

Souza, M.C., Franco, A.C., Haridasan, M., Rossatto, D.R., De Araújo, J.F., Morellato, L.P.C., Habermann, G., 2015b. **The length of the dry season may be associated with leaf scleromorphism in cerrado plants**. Ann. Acad. Bras. Ciên. 87, 1691–1699.

Souza, M.C., Habermann, G., Amaral, C.L., Rosa, A.L., Pinheiro, M.H.O., Costa, F.B., 2017. ***Vochysia tucanorum* Mart.: An aluminum-accumulating species evidencing calcifuge behavior**. Plant Soil 419, 377–389.

Souza, M.C., Rosa, A.L., Poschenrieder, C., Tolrà, R., Da Costa, F.B., 2018. **Fingerprinting metabolomics in tropical mistletoes: A case study with facultative aluminum-accumulating species**. Photochem. Lett. 25, 90–94.

Van Raij, B., Andrade, J.C., Cantarella, H., Quaggio, J.A., 2001. **Análise química para avaliação da fertilidade de solos tropicais**. Instituto Agronômico de Campinas (IAC), Campinas.

von Uexküll, H.R., Mutert, E., 1995**. Global extent, development and economic impact of acid soils**. In: Date, R.A., et al. (Eds) Plant soil interactions at low pH, pp. 5–19. Kluwer Academic Publishers, Dordrecht.

Wehr, J.B., Blamey, F.P.C., Hanna, J.V., Kopittke, P.M., Kerven, G.L., Menzies, N.W., 2010. **Hydrolysis and speciation of Al bound to pectin and plant cell wall material and its reaction with the dye chrome azurol S**. J. Agric. Food Chem. 58, 5553–5560.

Wigley, B.J., Coetsee, C., Hartshorn, A.S., Bond, W.J., 2013. **What do ecologists miss by not digging deep enough? Insights and methodological guidelines for assessing soil fertility status in ecological studies**. Acta Oecol. 51, 17–27.

Tables:

Table 1. Macro- and micronutrient contents and fertility parameters of a cerrado *sensu stricto* soil from Itirapina-SP, southeastern Brazil.

pH	OM	P resin	Al^{3+}	$H^+ + Al^{3+}$	K^+	Ca^{2+}	Mg^{2+}	BS	CEC	V%	m%	B	Cu	Fe	Mn	Zn
($CaCl_2$)	(g dm^{-3})	(mg dm^{-3})	----------mmol charges dm^{-3}----------							(%)	(%)	----------mg dm^{-3}----------				
3.8±0.1	9.4±1.9	1.8 ± 0.4	15.8±2.6	41.6±5.7	0.4±0.2	2.4±0.9	1±0.0	3.8±1.0	45.4±5.3	8.6±2.8	80.4±6.8	0.27±0.0	0.28±0.0	55.2±17.2	1.4±0.6	0.1±0.0

OM = Organic matter; BS = Base saturation ($K^+ + Ca^{2+} + Mg^{2+}$); CEC = $K^+ + Ca^{2+} + Mg^{2+} + H^+ + Al^{3+}$; V% = Fertility rate [$V = 100 (K^+ + Ca^{2+} + Mg^{2+}) CEC^{-1}$], %; Soil Al saturation (m%) = $(100 \times Al^{3+})/(BS + Al^{3+})$
Mean values (*n = 5*) ± standard deviation.

Table 2. Texture (sand, silt, and clay) and density of a cerrado *sensu stricto* soil from Itirapina-SP, southeastern Brazil.

Coarse sand	Fine sand	Total sand	Clay (in NaOH)	Natural clay (in H_2O)	Silt	Texture	Density
----------(g kg^{-1})----------							(g cm^{-3})
553 ± 75	363 ± 40	916 ± 50	73 ± 7	38 ± 3	11 ± 0.4	Sandy	1.43 ± 0.06[A]

Mean values (*n = 5*) ± standard deviation; [A]bulk density measured from a non-deformed soil sample.

Figure captions:

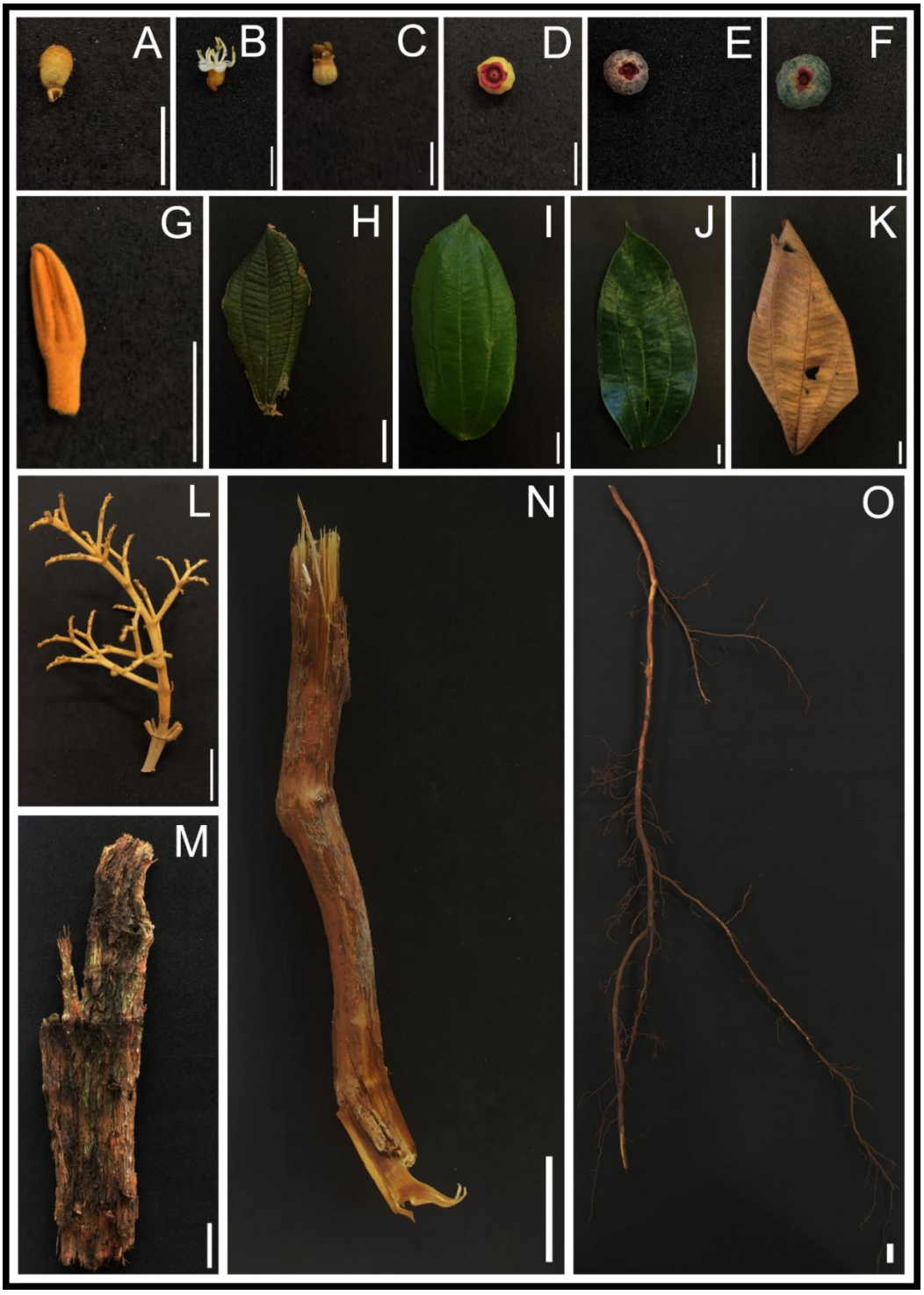

Fig. 1 Morphological aspects of reproductive (A–F and L) and vegetative (G–K and M–O) structures of *Miconia albicans* (Sw.) Triana (Melastomataceae). Reproductive structures: A = flower bud; B = flower; C = initial fruit 1; D = initial fruit 2; E = purplish unripe fruit; F = green ripe fruit; L = raceme. Vegetative structures: G = leaf bud; H = young leaf 1; I = young leaf 2; J = mature leaf; K = senescent leaf; M = trunk bark; N = wood; O = root. Scale bars: A–F = 0.5 cm; G–K = 1 cm; L–O = 5 cm.

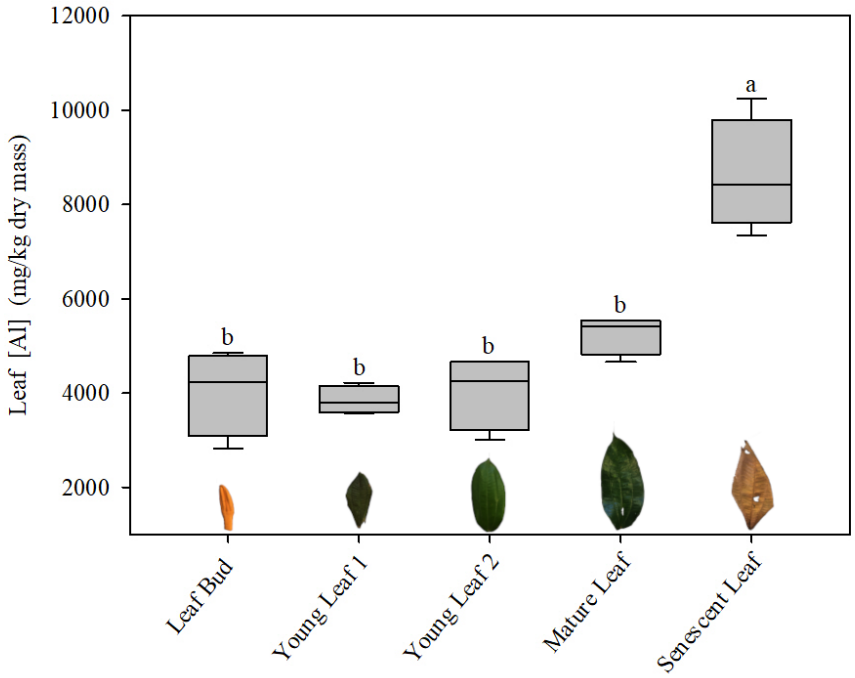

Fig. 2 Aluminum (Al) concentration in leaf bud, young leaf 1, young leaf 2, mature leaf and senescent leaf in *Miconia albicans* (Sw.) Triana from a Cerrado *sensu stricto* remnant in Itirapina, São Paulo state, southeastern Brazil. The box extends from the 25th to 75th percentiles, the continuous line within the box shows the median, and error bars represent 5th and 95th percentiles (n = 5 plants). Distinct letters indicate significant differences between leaf phases by Tukey test ($P < 0.05$).

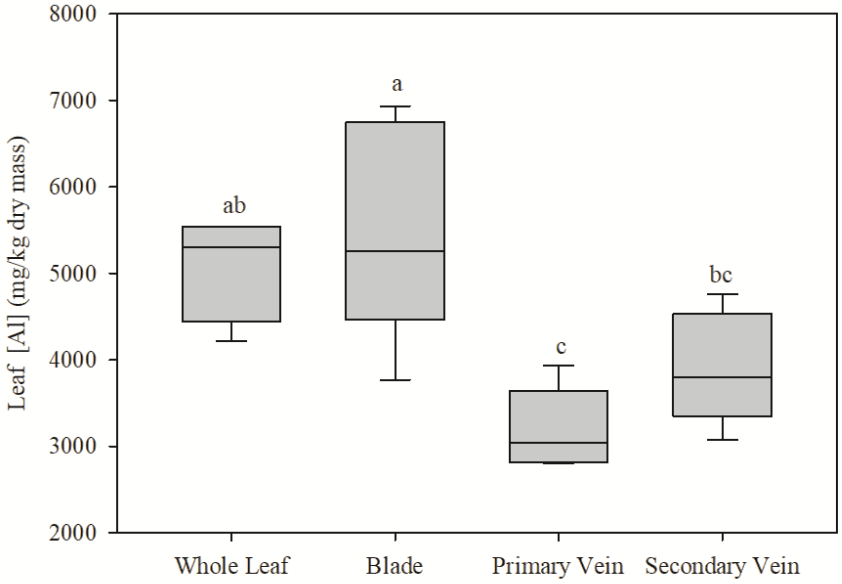

Fig. 3 Aluminum (Al) concentration in whole leaf, leaf blade, primary and secondary leaf veins of *Miconia albicans* (Sw.) Triana from a Cerrado *sensu stricto* remnant in Itirapina, São Paulo state, southeastern Brazil. The box extends from the 25th to 75th percentiles, the continuous line within the box shows the median, and error bars represent 5th and 95th percentiles (n = 5 plants). Distinct letters indicate significant differences between leaf phases by Tukey test ($P < 0.05$).

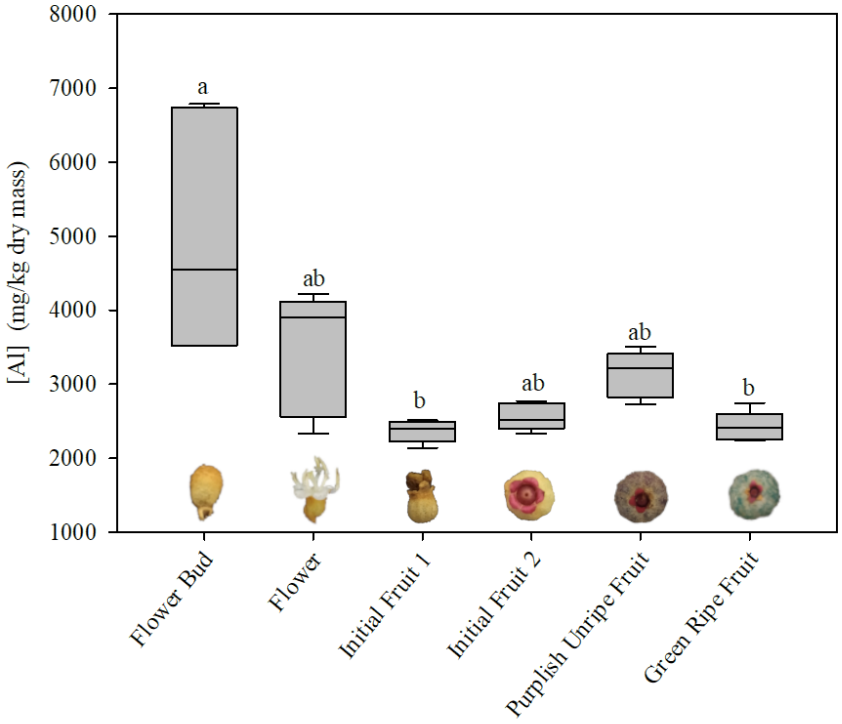

Fig. 4 Aluminum (Al) concentration in flower bud, flower, initial fruit 1 and 2, purplish unripe fruit and green ripe fruit of *Miconia albicans* (Sw.) Triana from a Cerrado *sensu stricto* remnant in Itirapina, São Paulo state, southeastern Brazil. The box extends from the 25th to 75th percentiles, the continuous line within the box shows the median, and error bars represent 5th and 95th percentiles (n = 5 plants). Distinct letters indicate significant differences between leaf phases by Tukey test ($P < 0.05$).

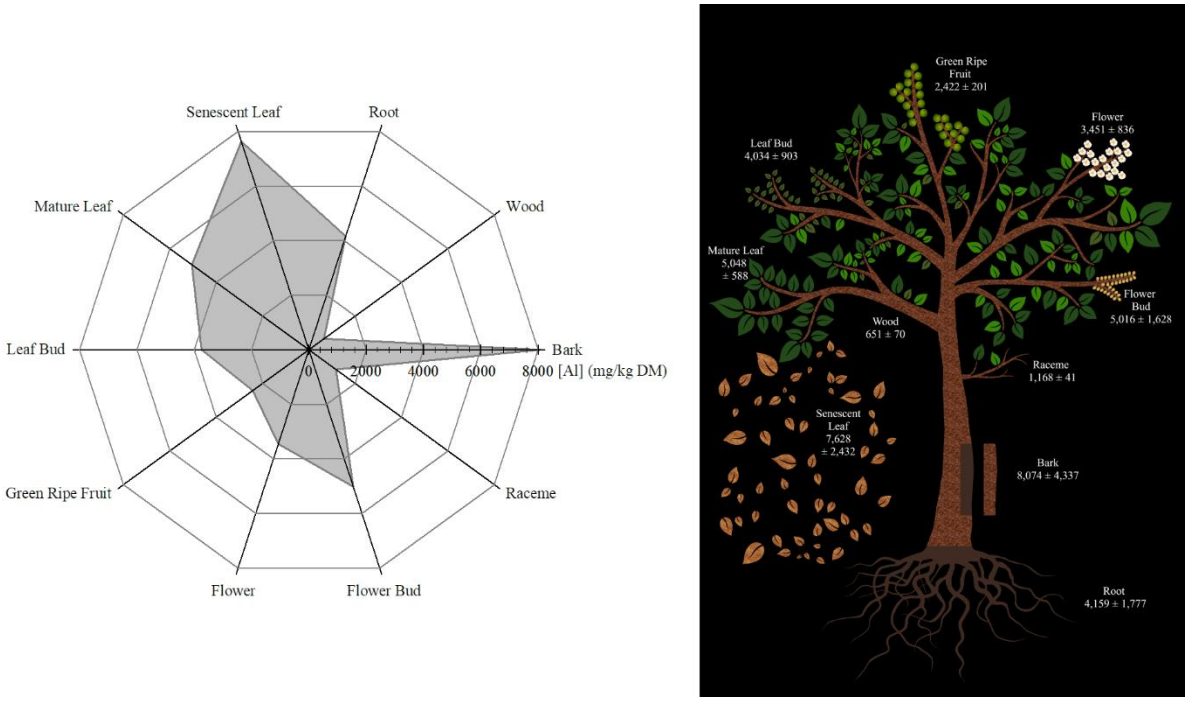

Fig. 5 Range (A) and mean values (± SD) (B) of Al concentration in vegetative organs (leaf bud, mature leaf, senescent leaf, and root), wood, bark, and reproductive organs (raceme, flower bud, flower, and green ripe fruit) of *Miconia albicans* (Sw.) Triana from a Cerrado *sensu stricto* remnant in Itirapina, São Paulo state, southeastern Brazil.

Supplementary Material:

Figure S1. Details of the inflorescence and flower of *Miconia albicans* (Sw.) Triana from a Cerrado *sensu stricto* remnant in Itirapina, São Paulo state, southeastern Brazil.

CAPÍTULO 3

Aluminum-accumulating *Miconia* species across an elevation gradient in the Tropical Atlantic Rain Forest in Brazil

Luá Taibo Timpone[1], Gustavo Habermann[2]*

[1]Programa de Pós-Graduação em Biologia Vegetal, Departamento de Biodiversidade, Instituto de Biociências, Universidade Estadual Paulista, UNESP, Av. 24-A, 1515; 13506-900, Rio Claro, SP, Brazil;
[2]Departamento de Biodiversidade, Instituto de Biociências, Universidade Estadual Paulista, UNESP, Av. 24-A, 1515; 13506-900, Rio Claro, SP, Brazil. E-mail: gustavo.habermann@unesp.br; Tel: +0055 19 3526-4210 (*Corresponding author)

Abstract

Aluminum (Al) occurs in acidic soils (pH < 5.0) as Al^{3+}, being toxic to most plant species, but plants can exudate organic acids avoiding Al uptake or accumulate it in leaves. Al accumulation seems to be a trait mainly of tropical woody species from Melastomataceae, Rubiaceae, Symplocaceae, and Vochysiaceae. The neotropical *Miconia* genus (Melastomataceae) is predominantly found in moist forests, although Al-accumulating *Miconia* species were already recorded in the Brazilian savanna (Cerrado vegetation) in South America. We looked for Al-accumulating *Miconia* species across an elevation gradient in the Atlantic Rain Forest, southeastern Brazil. We also expected to find non-accumulators of this genus as these were already reported at 20% within the genus. Soil Al saturation (m%) was measured along with leaf Al concentration. We found 27 Al-accumulation (and none non-accumulating) *Miconia* species, with some surpassing 25,000 mg Al Kg^{-1} dry leaf. These species grow on distrophic soils with m% above 70%. When a species occurred in at least three altitudes, m% did not drive Al accumulation. Therefore, other factors influencing leaf Al accumulation in *Miconia* species may be exerting action in *Miconia* species from the Atlantic Forest, and non-accumulators within this genus should be sought to form congeneric pairs helping future research on Al accumulation within this genus.

Key words: *Miconia spp.*, Altantic forest, Al-accumulating, acidic soil.

1 Introduction

Aluminum (Al) is the third most abundant element in the Earth's crust. It is present in soils as Al silicates and oxides (von Uexküll and Mutert 1995). In acidic soils (pH < 5.0), however, it is solubilized into monomeric metallic forms (Al^{3+}), which is toxic to many plant species, including crop plants (Kochian et al. 1995; Singh et al. 2017; Hajiboland et al. 2022).

Native plant communities, however, show plant species growing on acidic soils with high Al saturation (m%) without phytotoxic symptoms. Forests from temperate humid climates in the northern hemisphere as well as tropical humid forests in Asia, Africa, Oceania, and Americas, all contain plants that cope with distrophic soils (Brunner and Sperisen, 2013; Schmitt et al. 2016). Thus, plants from these environments have developed either resistance mechanisms against Al in the rhizosphere (external tolerance) (Brunner and Sperisen, 2013; Bittencourt et al. 2020) or internal mechanisms, including Al accumulation in leaves (Metali et al. 2012).

To be considered an Al accumulator it must accumulate at least 1000 mg Al kg^{-1} dry leaf (Chenery et al. 1948). The most studied Al-accumulating species belong to few families: Melastomataceae, Rubiaceae, Symplocaceae, Theaceae, and Vochysiaceae (Chenery et al. 1948; Haridasan 1982; Jansen et al. 2002; Bressan et al. 2016; Timpone and Habermann 2022). In South America, the Cerrado vegetation, known as 'Brazilian savanna,' shows Al-accumulators and non-accumulators in the woody plant community, being the former comprised of few species from Melastomataceae (*Miconia* genus), Rubiaceae (*Rudgea* genus), and Vochysiaceae (*Qualea and Vochysia* genera). *Miconia albicans*, *M. pohliana*, and *M. rubiginosa* typical plant species from the Cerrado, accumulate between 5,500 and 8,600 mg Al kg^{-1} dry leaf (Haridasan 1982; Bressan et al. 2016; Souza et al. 2018; Timpone and Habermann 2022). This genus also occurs in tropical humid forests in South America, as no geographical barriers exist between the Cerrado and Atlantic rain forest (Ratter et al. 2003), where it is significantly important. *Miconia* is the largest genus in this vegetation, including three lineages, *Leandra* s.s. clade (ca. 215 spp.), *Miconia* section Chaenanthera (24 spp.), and *Miconia* discolor clade (estimated to 77 spp.) (Caddah et al. 2022). Therefore, it is feasible that the same *Miconia* genus occurring as Cerrado Al-accumulating species could be found as accumulators in the Atlantic rain forest, where the soil is also acidic (pH < 4.7) and show m% above 70% (Mariano et al. 2020). Although Al accumulation is frequent in Melastomataceae (and *Miconia*) (Jansen et al. 2002), these authors point out that Al accumulation have poor phylogenetic signals within

Melastomataceae, and approximately 20% of non-accumulators can be found within *Miconia*. For these authors, environmental factors (soil) should also play a role in this context.

Significant regions of the Atlantic rain forest are mountainous vegetation. In the state of São Paulo and Minas Gerais, Southeastern Brazil, it occurs from 0–50 m altitude up to 1900–2000 m a.s.l. (Pompeu et al. 2014; Cardoso et al. 2019; Mariano et al. 2020). Therefore, soil fertility parameters can vary in response to the altitude, and m% from these soils could also influence the accumulation capacity of possible Al-accumulating *Miconia* species to be identified in the Atlantic rain forest.

In the present study, we looked for *Miconia* species in the Atlantic rain forest, expecting to find Al-accumulating and non-accumulating species. Additionally, we evaluated whether soil Al saturation varies in response to an altitudinal gradient and, if so, could *Miconia* spp. occurring in repeated altitudinal elevations respond to m%? For this, we collected leaves of *Miconia* species and soil samples in four fragments of the Atlantic rain forest on the Brazilian coast.

2 Material and methods

2.1 Experimental areas

Plants of *Miconia* genus were sought in four experimental areas in southeastern Brazil, in the states of Minas Gerais (MG) and São Paulo (SP) (Fig. 1). Area 1 (Itamonte, MG) was comprised of ombrophilous high montaine dense forest located in a private reserve of natural heritage (RPPN) of Instituto Alto Montana (22° 21’ 09.5’’ S, 44° 47’ 45.9’’ W), and collections were performed at 1500, 1700, 1900, and 2100 m of altitude. The climate of this area, according to Köppen (1948), is *Cwb*, humid temperate with dry winter. The mean air temperature in January (hot and wet season) and July (dry and cold season) were 21.7 °C and 15.5 °C, respectively, and annual rainfall was approximately 1,140 mm (Fig. 2A).

Area 2 (Cunha, SP) was comprised of ombrophilous Atlantic dense forest located at the ‘Serra do Mar’ state Park (núcleo Cunha) (23º 14' 09" S, 45º 01' 17" W), and collections were performed at 1,000 and 1,200 m of altitude. The climate of this area, according to Köppen (1948), is *Cfb*, humid temperate. The mean air temperature in January (hot and wet season) and July (dry and cold season) were 21.9 °C and 15.5 °C, respectively, and annual rainfall was approximately 1,145 mm (Fig. 2B).

Area 3 (Legado, SP) was comprised of ombrophilous dense forest located at Instituto Legado das Águas, Miracatu, SP (24°03’12” S, 47°12’55” W), and collections were performed at 400, 600, and 800 m of altitude. The climate of this area, according to Köppen (1948), is *Cfa*,

hot and subtropical. The mean air temperature in January (hot and wet season) and July (dry and cold season) were 23 °C and 17.5 °C, respectively, and annual rainfall was approximately 1,425 mm (Fig. 2C).

Area 4 (Picinguaba, SP) was comprised of low land ombrophilous dense forest and transition to high restinga (coastal areas with plant formations occurring in sandy coastal plains) and submontaine forest, located at 'Serra do Mar' state Park, Ubatuba, SP (núcleo Picinguaba) (21' 30" S, 44º 51' 00" W), and collections were performed at 50 and 100 m of altitude. The climate of this area, according to Köppen (1948), is humid tropical or rainy tropical, with rain occurring practically the year round. The mean air temperature in January (hot season) and July (cold season) were 24.6 °C and 19.5 °C, respectively, and annual rainfall was approximately 2,300 mm (Fig. 2D).

2.2 Plant material

In the four experimental areas, collections were performed throughout a 12-month period (2021/2022). Adult tree plants were randomly selected within the vegetation limits at each experimental site, and five individuals per species were collected at each altitudinal elevation. Mature, healthy, fully expanded leaves with petioles were collected from four quadrants of each tree canopy (N, S, E, and W). The leaves were washed in deionized water, dried in forced-air oven at 60 °C and sent to a plant nutrition laboratory to measure leaf Al concentration.

To confirm the species, exsicates of each plant containing vegetative (and reproductive structures when available) were mounted and sent to Dr. Renato Goldenberg (Federal University of Paraná — UFPR, Curitiba, Brazil), who is specialist on the taxonomy of *Miconia* genus. After identification, the exsiccates were deposited in herbaria at São Paulo State University (Unesp), Campus of Rio Claro (HRCB) and Federal University of Paraná (UFPR), Campus of Curitiba (UPCB).

2.3 Experimental design

Plants of *Miconia spp*. (Melastomataceae) were sought in the four experimental sites of the Atlantic Rain Forest. Each identified species found at each altitude (50, 100, 400, 600, 800, 1000, 1200, 1500, 1700, 1900, and 2100 m) had its leaf Al concentration measured to classify them as Al-accumulating (above 1,000 mg Al kg^{-1} dry mass) or non-accumulating species. Soil samples were also collected at each altitude of each experimental area to measure physical and chemical properties, including soil pH, Al saturation (m%), among other parameters. Then,

correlation between altitudes and m% was tested, and m% influencing leaf Al concentrations was tested in species that were observed in at least three altitudinal elevations.

2.4 Aluminum concentration in plant material

The leaf samples were sent to the plant nutrition laboratory at Instituto Agronômico de Campinas (IAC, Campinas, SP). They were ground and digested in a solution of nitric:perchloric acids (3:1, v/v). The concentration of Al was assessed by the inductively coupled plasma optical emission spectroscopy (ICP-OES) method and expressed as mg Al per kg dry mass.

2.5 Soil sampling

Five holes for soil sampling (30–40 cm in depth) were randomly arranged at each elevation in each experimental area, also trying to be as close as possible to sampled plants. The soil was collected with an auger and samples were bagged, identified, and sent to the soil science laboratory at University of São Paulo (USP, Esalq, Piracicaba, SP). Chemical (fertility) analysis, including soil pH (in $CaCl_2$), Al saturation (m%) among other parameters was performed according to van Raij et al. (2001). Physical analysis (percentage of sand, silt, and clay) was done according to protocols described by Embrapa (1997).

2.6 Data analysis

Shapiro-Wilk test was used to verify the data normality. A linear correlation between altitude and m% was used to test the influence of the former on the latter. In addition, leaf Al concentration data were submitted to one-way analysis of variance (ANOVA), and mean values of each species were compared between altitudinal elevations by Tukey test ($P < 0.05$).

3 Results

The four experimental areas showed acidic soils, with pH varying from 3.88 to 4.51, low P values and m% all above 70% (Table 1). Soils from 2,100 and 1,900 m (Itamonte) showed organic matter (OM) between 55 and 60 g dm^{-3}, while those from the remainder altitudes showed 16–36 g dm^{-3} of OM (Table 1). Cation exchange capacity (CEC) was 80–180 mmol charges dm^{-3} for soils from most areas, while those collected at 50 m showed 49 mmol charges dm^{-3} (Table 1). Soils from almost all areas showed a considerable clay concentration, being classified as ‘clay soils’, while the soil collected at 50 m was sandy (Table 2). Therefore, the soils from all areas were dystrophic. In addition, a linear regression showed low correlation (R^2

= 0.37) between altitudes and m%, indicating the high m% range (above 70%) was observed regardless of the altitude (Fig. 3).

We collected 27 different *Miconia spp.* in the four experimental areas, and all of them accumulated more than 1000 mg Al kg^{-1} dry leaf, except for *M. brunnea* collected in the Cunha experimental area, at 1,200 m altitude (Table 3). Of these 27 species, 11 (40.7%) showed at least one species per altitude with more than 10,000 mg Al kg^{-1} dry leaf, 6 (22%) with more than 15,000 mg Al kg^{-1} dry leaf, and 3 (11.1%) above 20,000 mg Al kg^{-1} dry leaf (Table 3).

The leaf Al concentration of species that occurred in at least three altitudes did not respond to m% (Fig. 4). *Miconia cinnamomifolia* (Fig. 4A), *M. dasytricha* (Fig. 4B), *M. latecranata* (Fig. 4D), *M. sellowiana* (Fig. 4F), *M. shepherdii* (Fig. 4G), and *M. tristis* (Fig. 4H) decreased their leaf Al concentration with the increase of soil Al saturation. *Miconia formosa* did not alter leaf Al concentration in response to m% (Fig. 4C), while *M. pusilliflora* showed a puzzled response pattern (Fig. 4E). Therefore, m% does not seem to influence leaf Al accumulation capacity of these *Miconia* species.

4 Discussion

In the present study, we sought *Miconia spp.* in the Atlantic rain forest and found 27 species that could be considered Al-accumulating species. We also asked whether the altitudes of the montane relief of the Atlantic rain forest could determine m% and whether this parameter could influence leaf Al concentration. We found distrophic soils with m% above 70% regardless of the altitude and *Miconia* species not responding to m%, suggesting other factors controlling Al accumulation in the leaves of these plants.

To be considered an Al-accumulating species it must accumulate more than 1,000 mg Al kg^{-1} dry leaf (Chenery, 1948; Jansen et al. 2002). A remarkable characteristic of plants from *Miconia* genus is leaf Al accumulation (Jansen et al. 2002), especially in the neotropics, including the Cerrado vegetation, where *M. ferruginata*, *M. pohliana* (Haridasan 1982) and *M. albicans* (Souza et al. 2018; Timpone and Habermann 2022) accumulate between 4,000 and 7,500 mg kg^{-1} dry leaf. A survey of species of this genus from the literature, showed that of 384 species, 314 (80%) are potentially accumulators, evidenced by qualitative histochemical tests (Jansen et al. 2002). The 27 species we found in the Atlantic rain forest showed leaf Al concentration varying from 1,200 (*M. sellowiana*) up to 26,400 (*M. prasina*) mg Al kg^{-1} dry leaf (Table 3). Therefore, the Al accumulation range of *Miconia spp.* found in the Atlantic rain forest is approximately 20-fold higher than the range found in the Cerrado, where Al-accumulation species from Melastomataceae, Rubiaceae, and Vochysiaceae, which

characteristically occur in the Cerrado, were first described (Haridasan 1982). In addition, as far as we are concerned, the present study is the first report of Al-accumulating plants in the Atlantic rain forest.

We expected to find Al-accumulators and non-accumulators, as *Miconia* species screened from the literature showed approximately 20% potentially non-accumulating species (Jansen et al. 2002). Indeed, *M. brunnea* collected at 1,200 m of altitude showed 595.6 ± 327.6 mg Al kg^{-1} dry leaf (Table 3). At 800 m of altitude, however, it accumulated 1859.1 ± 305.1 mg Al kg^{-1} dry leaf (Table 3). This points out that some *Miconia* species '*may not*' be considered an Al-accumulator or that its leaf Al concentration depends on soil properties. The soil Al saturation from the site where *M. brunnea* did not accumulate above 1000 mg Al kg^{-1} dry leaf, Cunha (area 2), was 92.4%, while m% from the site where it accumulated 1859 mg Al kg^{-1} dry leaf, Legado (area 3), was 91.2%, indicating m% was not the main factor influencing Al accumulation of these species, and other factors could be involved.

Soil Al saturation of all experimental areas was above 70% (Table 1) regardless of the altitude (Fig. 3), and the increase in m% was not associated with *Miconia* species accumulating more Al in their leaves (Fig. 4). *Miconia cinnamomifolia* (Fig. 4A), *M. dasytricha* (Fig. 4B), *M. latecranata* (Fig. 4D), *M. sellowiana* (Fig. 4F), *M. shepherdii* (Fig. 4G), and *M. tristis* (Fig. 4H) showed lower leaf Al concentration when growing on soils with higher m%. Similarly, no direct relationship between m% and Al accumulation were observed in Vochysiaceae species from the Cerrado (Andrade et al. 2011). Even when *Qualea grandiflora* and *Q. parviflora* (Vochysiaceae) grow naturally on Cerrado calcareous soils, with m% not higher than 3%, they accumulate approximately 6,000 and 17,000 mg Al kg^{-1} dry leaf, respectively (Nogueira et al. 2019). Therefore, the Al accumulation found in *Miconia* species in the Atlantic rain forest follows the same pattern of Al-accumulation observed in species from the Cerrado, apparently '*not depending on m%*' for this phenomenon.

Thus, it could be a binary phenomenon: it accumulates or does not accumulate! As discussed above, a striking characteristic of *Miconia* species is Al accumulation in their structures, especially in the leaves, and Al accumulation '*appears*' to be a primitive trait within the Miconieae tribe (Jansen et al. 2002). Nevertheless, these authors reveal that 20% *Miconia* species are non-accumulators, leading us to questions like, why congeneric *Miconia* species present such antagonistic response? We expected to find non-accumulating *Miconia* species, which we believe exist, but we were not able to find in the Atlantic rain forest. In addition, *Miconia albicans* saplings grow better and produce more and greener leaves when cultivated in distrophic soils, in relation to to calcareous soils (Haridasan, 2008). At the same time, *Vochysia*

tucanorum seedlings start dying within seven days in nutrient solution without Al (Bressan et al. 2021). These two species from the Cerrado may then, show, benefic effects of Al (*M. albicans*) while some sort of "essentiality" could be inferred to *V. tucanorum*. Therefore, in the present study, we show for the first time Al-accumulating *Miconia* species occurring in the Atlantic rain forest, accumulating Al at a 20-fold higher range than Al accumulation observed in the Cerrado, and non-accumulating *Miconia* species remain to be found, not only in the Atlantic rain forest, but in other vegetations.

Acknowledgements

This study was financial in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) _ Finance Code 001. We thank Instituto Alto Montana da Serra Fina, Legado das Águas (Grupo Votorantim), Parque Estadual da Serra do Mar – Núcleos Cunha e Picinguaba – Alexander D. da Silva, Nangel, Gabriel Mesquita, Miguel M. F. de Jesus for field assistance and Renato Goldenberg (Federal University of Paraná — UFPR, Curitiba, Brazil), for identification of *Miconia spp.*

5 References

Andrade LRM, Barros LMG, Echevarria GF, do Amaral LIV, Cotta MG, Rossatto DR, Haridasan M, Franco AC. 2011. **Al-Hyperaccumulator Vochysiaceae from the Brazilian Cerrado store aluminum in their chloroplasts without apparent damage**. Env Exp Bot 70:37–42

Bittencourt, BMOC, Silva, CMS, Filho, SZ, Habermann G. 2020. **Aluminum (Al)-induced organic acid exudation in an Al-accumulating species from the Brazilian savanna**. Trees 34, 155–162

Bressan ACG, Coan AI, Habermann G. 2016. **X-ray spectra in SEM and staining with chrome azurol S show Al deposits in leaf tissues of Al-accumulating and non-accumulating plants from the cerrado**. Plant Soil 404: 293–306

Bressan ACG, Bittencourt BMOC, Silva GS, Habermann G. 2021. **Could the absence of aluminum (Al) impair the development of an Al-accumulating woody species from Brazilian savanna?** Theor Exp Plant Physiol 33:281–292

Brunner I, Sperisen C. 2013. **Aluminum exclusion and aluminum tolerance in woody plants**. Front Plant Sci 4: 1–12

Caddah MK, Meirelles J, Nery EK, Lima DF, Nicolas AN, Michelangeli FA, Goldenberg R. 2022. **Beneath a hairy problem: Phylogeny, biogeography, and morphology circumscribe the new *Miconia* supersection Discolores (Melastomataceae: Miconieae)**. Mol Phylogen Evol 171: 107461

Cardoso FCG, Zwiener VP, Marques MCM. 2019. **Tree phenology along a successional gradient of tropical Atlantic Forest**. Journal of Plant Ecology, 12, 272–280

Chenery EM. 1948. **Aluminium in the plant world. part I: general survey in dicotyledons**. Kew Bull 1948:173–183

Embrapa (Empresa Brasileira de Pesquisa Agropecuária). 1997. **Manual for Methods of Soil Analyses**. Second ed. Embrapa, Rio de Janeiro.

Hajiboland R, Panda CK, Lastochkina O, Gavassi MA, Habermann G, Pereira JF. 2022. **Aluminum toxicity in plants: Present and future**. Journal of Plant Growth Regulation

Haridasan M. 1982. **Aluminium accumulation by some Cerrado native species of central Brazil**. Plant Soil 65:265–273

Haridasan M. 2008. **Nutritional adaptations of native plants of the cerrado biome in acid soils**. Braz J Plant Physiol 20: 183–195

Jansen S, Watanabe T, Smets E. 2002. **Aluminium accumulation in leaves of 127 species in Melastomataceae, with comments on the order Myrtales**. Ann Bot 90:53–64

Kochian LV. 1995. **Cellular mechanisms of aluminum toxicity and resistance in plants**. Annu Rev Plant Physiol Plant Mol Biol 46: 237–260

Köppen W. 1948. **Climatología con un estudio de los climas de la Tierra**. Fondo de Cultura Económica-Pánuco, Mexico. Ed.

Mariano RF, Rezende VL, Mendes CN, Santos RMD, Souza CRD, Moura ASD, Machado FS, Pompeu PV, Carvalho WAC, Fontes MAL. 2020. **Phylogenetic beta diversity in an upper montane Atlantic Forest along an altitudinal gradient**. Plant Ecology 221(1) 671– 682

Metali F, Salim KA, Burslem DFRP. 2012. **Evidence of foliar aluminium accumulation in local, regional and global datasets of wild plants**. New Phytol 193:637–649

Nogueira MA, Bressan ACG, Pinheiro MHO, Habermann G. 2019. **Aluminum-accumulating Vochysiaceae species growing on a calcareous soil in Brazil**. Plant Soil 437:313–326

Pompeu PV, Fontes MAL, Santos RM, Garcia PO, Batista TA, Carvalho WAC, Filho ATO. 2014. **Floristic composition and structure of an upper montane cloud forest in the Serra da Mantiqueira Mountain Range of Brazil**. Acta Botanica Brasilica 28: 456-464

Ratter J, Bridgewater S, Ribeiro JF. 2003. **Analysis of the floristic composition of the Brazilian Cerrado vegetation**. III: comparison of the woody vegetation of 376 areas. Edinb J Botany 60: 57-109

Schmitt M, Boras S, Tjoa A, Watanabe T, Jansen S. 2016. **Aluminum accumulation and intra-tree distribution patterns in three Arbor aluminosa (Symplocos) species from Central Sulawesi**. PLoS ONE 11:e0149078

Singh S, Tripathi DK, Singh S, Sharmac S, Dubeyb NK, Chauhana DK, Vaculíke M. 2017. **Toxicity of aluminium on various levels of plant cells and organism: A review**. Env Exp Bot 137: 177–193

Souza, MC, Rosa, AL, Poschenrieder, C, Tolrà, R, Da Costa, FB. 2018. **Fingerprinting metabolomics in tropical mistletoes: A case study with facultative aluminum-accumulating species**. Photochem. Lett. 25, 90–94

Timpone LT, Habermann G. 2022. **Is aluminum (Al) eliminated by senescent structures of *Miconia albicans*, an Al-accumulating species from Brazilian savanna?** Flora 289:152036

van Raij B, Andrade JC, Cantarella H, Quaggio JA. 2001. **Análise química para avaliação da fertilidade de solos tropicais**. Instituto Agronômico de Campinas (IAC), Campinas

von Uexküll HR, Mutert E .1995. **Global extent, development and economic impact of acid soils**. Plant Soil 171:1–5

Tables:

Table 1. Macro- and micronutrients and fertility parameters of soils from experimental sites in the Atlantic Forest. southeastern Brazil.

Altitude	pH	OM	P resin	Al^{3+}	$H^{+}+Al^{3+}$	K^{+}	Ca^{2+}	Mg^{2+}	BS	CEC	V%	m%	B	Cu	Fe	Mn	Zn
m	$CaCl_2$	g dm^{-3}	mg dm^{-3}	---------- mmol charges dm^{-3} ----------							(%)	(%)	---------- mg dm^{-3} ----------				
2100	3.95	60.28	5.12	37.36	180.80	1.09	0.50	0.54	2.24	183.04	1.20	93.80	0.09	0.05	38.60	0.86	0.12
	±0.08	±30.27	±1.66	±11.83	±27.24	±0.49	±0.00	±0.18	±0.78	±27.41	±0.45	±2.86	±0.08	±0.00	±12.99	±0.72	±0.10
1900	4.09	54.02	4.22	31.12	149.60	1.16	0.50	0.48	1.86	151.46	1.20	94.40	0.17	0.05	29.62	2.78	0.16
	±0.13	±14.64	±1.80	±5.05	±12.77	±0.47	±0.00	±0.15	±0.57	±13.17	±0.45	±1.14	±0.08	±0.00	±6.10	± 1.49	±0.09
1700	3.89	38.48	3.98	31.48	151.28	1.12	0.76	0.60	2.56	153.78	1.60	92.40	0.13	0.05	47.98	2.28	0.36
	±0.06	±6.44	±0.61	±5.21	±21.78	±0.54	±0.58	±0.10	±1.06	±21.92	±0.89	±2.60	±0.06	±0.00	±8.57	±0.89	±0.31
1500	3.99	36.82	6.14	19.96	114.32	1.96	3.86	2.46	8.28	126.20	6.80	72.00	0.13	0.20	61.10	10.32	0.60
	±0.08	±8.51	±1.54	±6.03	±18.79	±0.94	±4.41	±1.85	±7.11	±21.06	±5.36	±19.34	±0.09	±0.10	±13.23	±3.54	±0.29
1200	3.92	30.34	5.28	22.26	120.38	0.61	0.40	0.82	1.84	122.22	1.60	92.40	0.23	0.20	213.9	1.68	0.34
	±0.14	±4.84	±1.34	±5.03	±22.99	±0.14	±0.30	±0.22	±0.49	±23.10	±0.55	±2.07	±0.06	±0.17	±131.91	±0.24	±0.27
1000	4.24	27.36	4.60	18.38	98.14	0.95	1.32	1.52	3.80	101.94	3.80	82.80	0.32	0.48	130.90	2.78	0.38
	±0.23	±6.04	±1.10	±3.57	±19.94	±0.26	±0.75	±0.47	±1.03	±20.45	±0.84	±4.44	±0.07	±0.41	±50.29	±1.18	±0.19
800	3.91	28.76	3.50	32.86	133.86	0.63	0.82	1.58	3.02	136.88	2.40	91.20	0.26	0.05	90.82	1.94	0.12
	±0.08	±7.55	±0.00	±7.91	±32.01	±0.06	±0.50	±1.05	±1.46	±31.30	±1.67	±5.12	±0.08	±0.00	±37.85	±1.99	±0.04
600	3.95	27.72	4.64	25.38	108.04	1.11	1.62	1.68	4.40	112.44	4.60	83.80	0.31	0.13	103.50	2.82	0.38
	±0.11	±7.81	±2.55	±8.47	±34.52	±0.49	±1.24	±1.20	±1.97	±33.84	±2.61	±8.70	±0.11	±0.11	±71.09	±2.62	±0.24
400	3.88	17.40	3.50	16.94	78.42	0.85	1.78	2.06	4.70	83.12	6.00	78.00	0.28	0.11	57.14	6.00	0.22
	±0.10	±3.82	±0.00	±2.22	±12.41	±0.31	±0.80	±0.48	±1.02	±11.97	±1.87	±5.38	±0.06	±0.05	±12.12	±8.43	±0.19
100	4.17	16.42	3.86	14.72	78.66	0.71	1.66	2.34	4.70	83.36	7.00	73.80	0.08	0.11	36.58	5.18	0.52
	±0.31	±4.90	±6.29	±5.36	±29.22	±0.35	±1.04	±1.47	±2.86	±28.34	±7.31	±20.87	±0.05	±0.11	±13.80	±8.36	±0.28
50	4.51	23.10	2.96	10.14	45.12	1.38	1.62	1.10	4.12	49.24	9.00	69.60	0.05	0.24	25.88	1.52	0.40
	±0.15	±14.77	±1.71	± 3.74	±11.10	±0.77	±0.41	±0.31	±1.02	±10.51	±4.64	±12.97	±0.00	±0.05	±4.56	±0.33	±0.00

OM = Organic matter; BS = Base saturation ($K^{+} + Ca^{2+} + Mg^{2+}$); CEC = Cation exchange capacity ($K^{+} + Ca^{2+} + Mg^{2+} + H^{+} + Al^{3+}$); V% = Fertility rate [$V = 100 (K^{+} + Ca^{2+} + Mg^{2+}) CEC^{-1}$];
Soil Al saturation (m%) = $(100 \times Al^{3+})/(BS + Al^{3+})$
Mean values ($n = 5$) ± standard deviation

Table 2. Texture (sand, silt, and clay) of soils from experimental sites in the Atlantic Forest, southeastern Brazil.

Altitude (m)	Total sand ($g\ Kg^{-1}$)	Total sand (%)	Clay (in NaOH) ($g\ Kg^{-1}$)	Clay (%)	Silt ($g\ Kg^{-1}$)	Silt (%)	Texture
2100	191.00 ± 58.52	19.10	545.80 ± 67.44	54.57	263.40 ± 53.86	26.33	Clay
1900	153.80 ± 45.92	15.21	627.60 ± 40.80	62.07	229.80 ± 25.40	22.72	Very clayey
1700	163.80 ± 69.80	16.38	596.60 ± 80.34	59.66	239.60 ± 35.75	23.96	Very clayey
1500	210.00 ± 79.10	21.00	546.20 ± 78.08	54.61	244.00 ± 50.04	24.39	Clayey
1200	440.20 ± 97.68	44.03	343.20 ± 38.51	34.33	216.40 ± 68.39	21.64	Clayey
1000	554.00 ± 119.09	55.40	314.00 ± 105.68	31.40	132.00 ± 44.06	13.20	Clay mean
800	289.60 ± 60.63	28.95	495.40 ± 77.22	49.52	215.40 ± 66.96	21.53	Clayey
600	491.40 ± 114.74	49.13	405.80 ± 140.60	40.57	103.00 ± 50.82	10.30	Clayey
400	448.80 ± 40.82	44.90	459.60 ± 51.23	45.98	91.20 ± 22.54	9.12	Clayey
100	502.00 ± 123.20	50.20	380.00 ± 135.55	38.00	118.00 ± 35.89	11.80	Clayey
50	724.00 ± 112.38	72.40	177.40 ± 60.91	17.74	98.60 ± 58.67	9.86	Sandy mean

Mean values ($n = 5$) ± standard deviation.

Table 3. Mean values of leaf aluminum concentration of 27 *Miconia* species from experimental sites in the Atlantic rain forest, southeastern Brazil.

Species	Altitude (m)	Al [mg kg^{-1}]
M. atlântica Caddah & R.Goldenb.	100	16,068.05 ± 10,225.32
M. brackenridgei (A.Gray) R.Goldenberg.	800	8,594.12 ± 660.66
M. brunnea DC.	1200	595.56 ± 327.56 **b**
	800	1,859.08 ± 305.08 **a**
M. budlejoides Triana	800	11,536.76 ± 1,082.41 **a**
	400	12,425.36 ± 1,159.40 **a**
M. calvences DC.	50	11,135.58 ± 2,958.23
M. cinerascens Miq.	2100	18,028.00 ± 2,752.78 **a**
	1900	12,689.20 ± 2,245.98 **b**
M. cinnamomifolia (DC.) Naudin	800	7,663.29 ± 1,903.99 **b**
	600	11,018.54 ± 1,935.01 **a**
	400	9,505.37 ± 1,225.61 **ab**
M. cubatanensis Hoehne	1200	7,513.88 ± 1,497.27
M. dasytricha (A.Gray) R.Goldenberg.	800	$8,756.60^{a}$ ± 1,543.22 **b**
	600	$10,904.16^{b}$ ± 689.83 **a**
	400	10,771.82 ± 930.01 **ab**
	100	$8,822.58^{a}$ ± 1,184.85 **a**
M. fasciculata Gardner	50	9,463.24 ± 1,205.82
M. flammea Casar.	1200	1,587.16 ± 262.10
M. formosa Cogn.	800	4,568.78 ± 1,456.09 **a**
	600	5,778.23 ± 1,353.45 **a**
	400	5,949.83 ± 1,536.90 **a**
M. goldenbergiana Caddah	1000	7,828.50 ± 1,333.36
M. latecrenata (DC.) Naudin	1200	7,737.90 ± 706.56 **b**
	1000	8,365.33 ± 1,041.99 **b**
	600	8,051.16 ± 1,966.96 **b**
	400	11,206.16 ± 2,051.15 **a**
M. melastomoides (Raddi) R.Goldenb.	1700	25,022.00 ± 5,558.63 **a**
	1500	25,264.00 ± 2,168.94 **a**
M. paniculata (DC.) Naudin	1000	3,274.18 ± 738.24

M. prasina (Sw.) DC.	50	26,401.06 ± 1,935.68
M. pusilliflora (DC.) Naudin	2100	8,928.40 ± 2,189.44 **a**
	1900	8,171.20 ± 1,658.23 **ab**
	1700	5,781.20 ± 1,384.52 **bc**
	1500	4,249.20 ± 1,597.52 **c**
	1200	3,535.09 ± 1,211.61 **c**
	800	6,500.86 ± 1,403.93 **abc**
M. racemifera (DC.) Triana	1000	7,231.61 ± 1,736.74
M. sellowiana Naudin	2100	2,802.00 ± 996.70 **a**
	1900	2,418.80 ± 481.46 **ab**
	1700	2,109.20 ± 187.90 **ab**
	1500	2,714.60 ± 1,252.16 **a**
	1000	1,942.98 ± 341.31 **ab**
	800	1,289.08 ± 150.15 **b**
	600	1,968.42 ± 743.05 **ab**
M. serrulata (DC.) Naudin	50	2,3294.72 ± 4479.25
M. shepherdii R.Goldenb. & Reginato	1900	14,900.00[a] ± 1,781.98 **b**
	1700	19,888.00[b] ± 1,989.72 **a**
	1500	16,460.00 ± 2,428.32 **ab**
M. sublanata (Cogn.) R.Goldenb.	2100	3,394.80 ± 1,371.97 **b**
	1900	4,955.60 ± 1,352.91 **a**
M. theaezans (Bonpl.) Cogn.	1500	24,534.00 ± 1,975.15
M. tristes Spring	1000	4,148.88 ± 407.59 **b**
	600	5,404.53 ± 1,343.84 **ab**
	400	7,185.18 ± 136.32 **a**
M. valtheri Naudin	800	9,494.99 ± 1,348.59 **a**
	600	11,006.64 ± 1,106.46 **a**
M. willdenowii Klotzsch ex Naudin	1200	1,441.05 ± 342.33

Mean values ($n = 5$) ± standard deviation. For each species, distinct letters indicate significant differences ($P < 0.05$) by Tukey test between altitudes.

Figure captions:

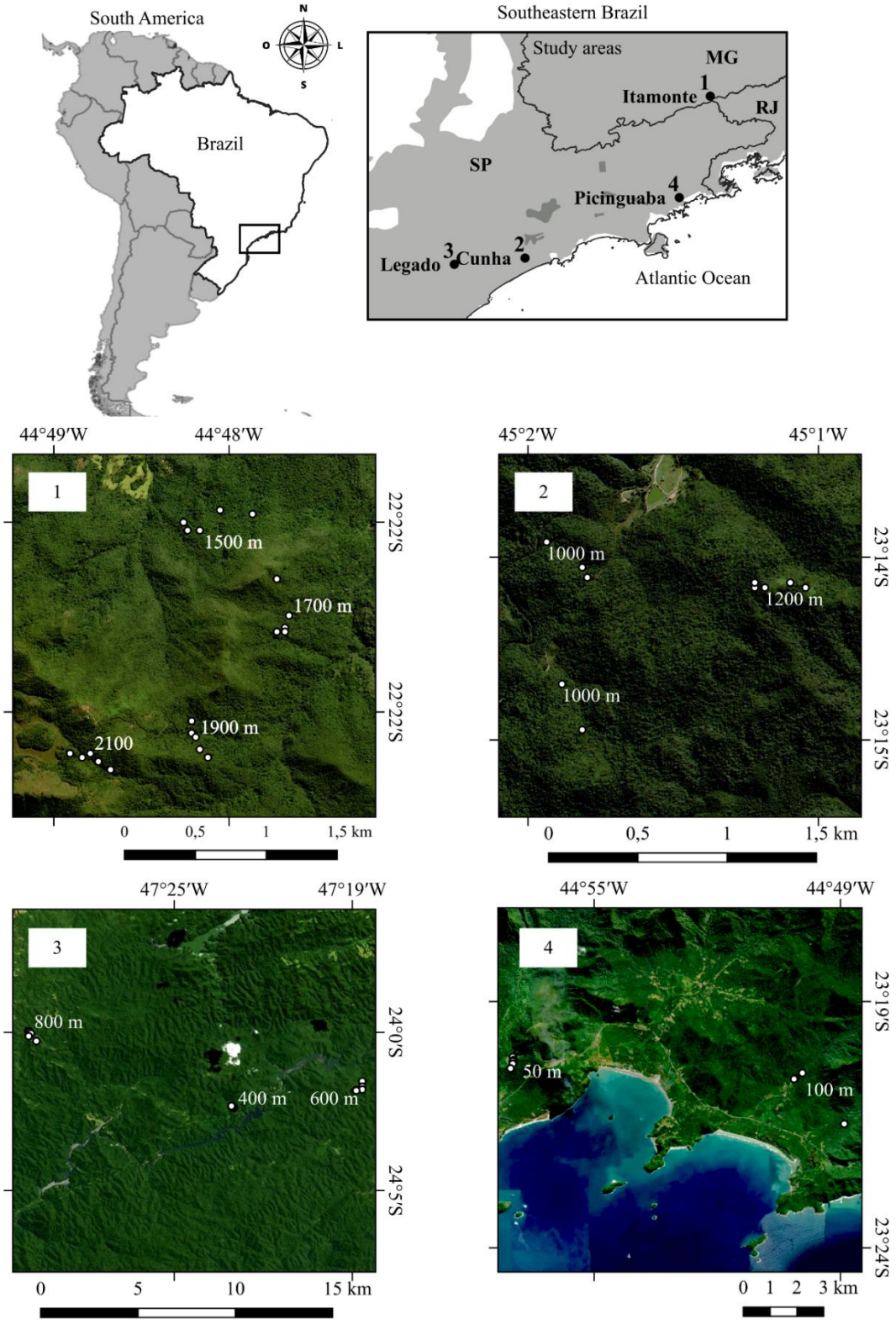

Fig. 1 Location map of study area and arrangement of the location soil sampled over altitudes in Atlantic rain forest in the municipality of Itamonte, Minas Gerais state (**1**) and in the mucipality of Cunha (2), Juquitiba (3) and Picinguaba (4), district of Ubatuba, São Paulo state, southeastern Brazil, in South America.

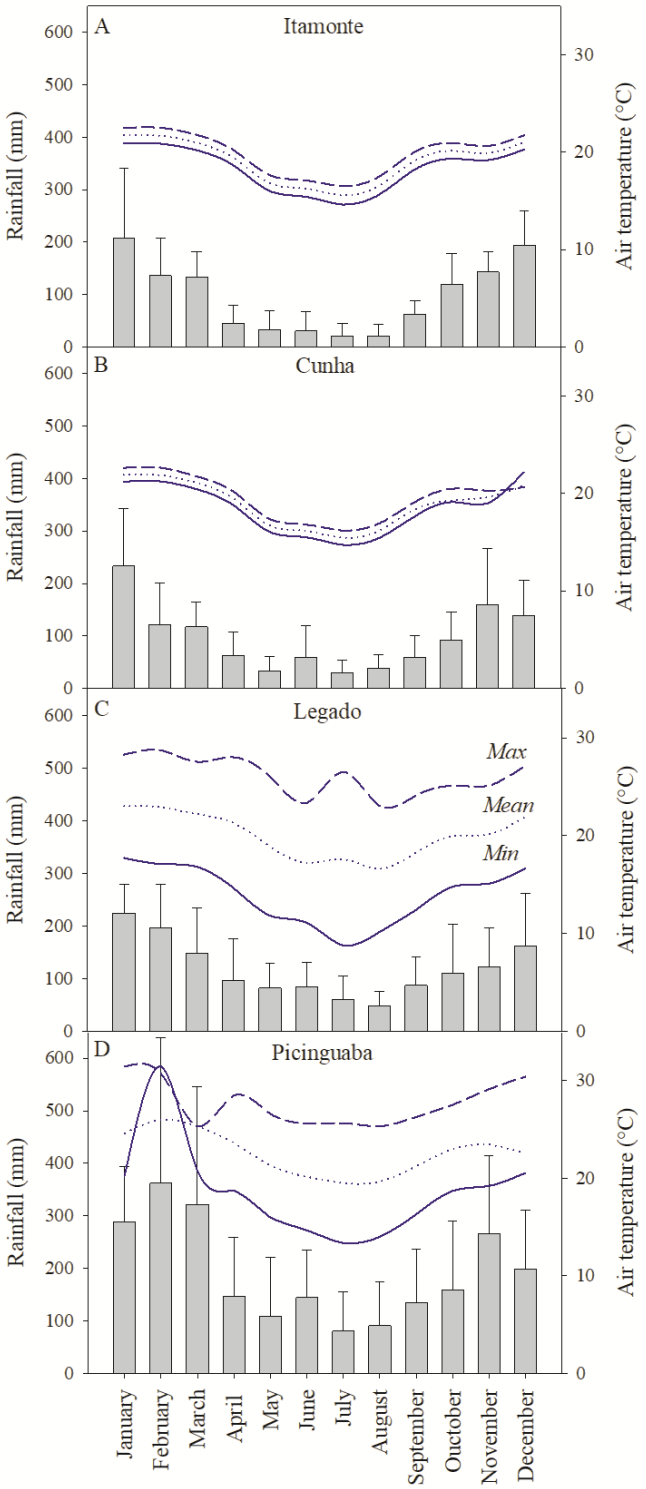

Fig. 2 Climatic diagram displaying the annual rainfall (mm) and monthly averages of maximum, mean, and minimum air temperature (ºC) for a period of 10 years (2012–2022) in Itamonte (MG) (A), Cunha (SP) (B), Juquitiba (SP) (C), and Ubatuba (SP) (D) of the Atlantic rain forest.

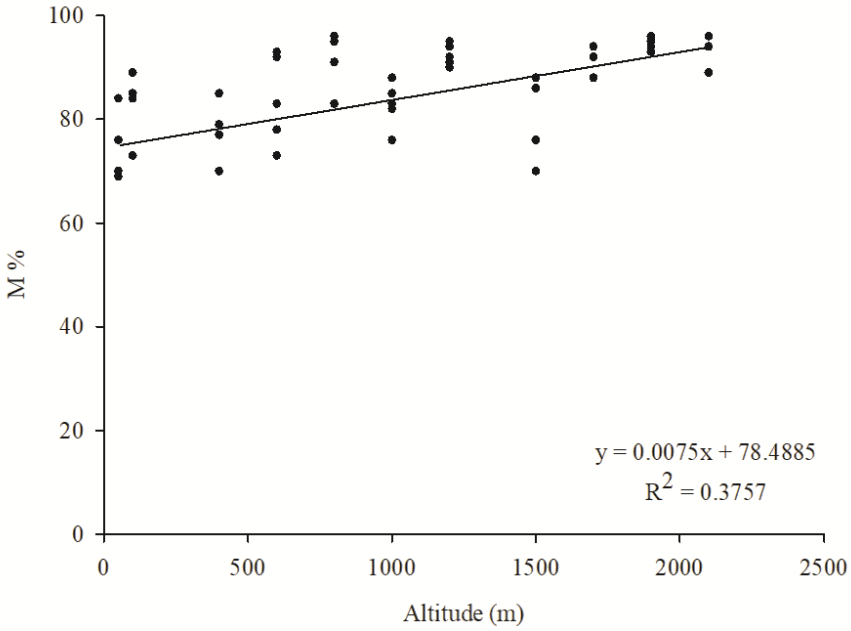

Fig 3. Linear regression between soil aluminum saturation (M%) and altitudes in four sites of the Atlantic rain forest (Itamonte, MG; Cunha, Juquitiba, and Ubatuba, SP) in southern Brazil.

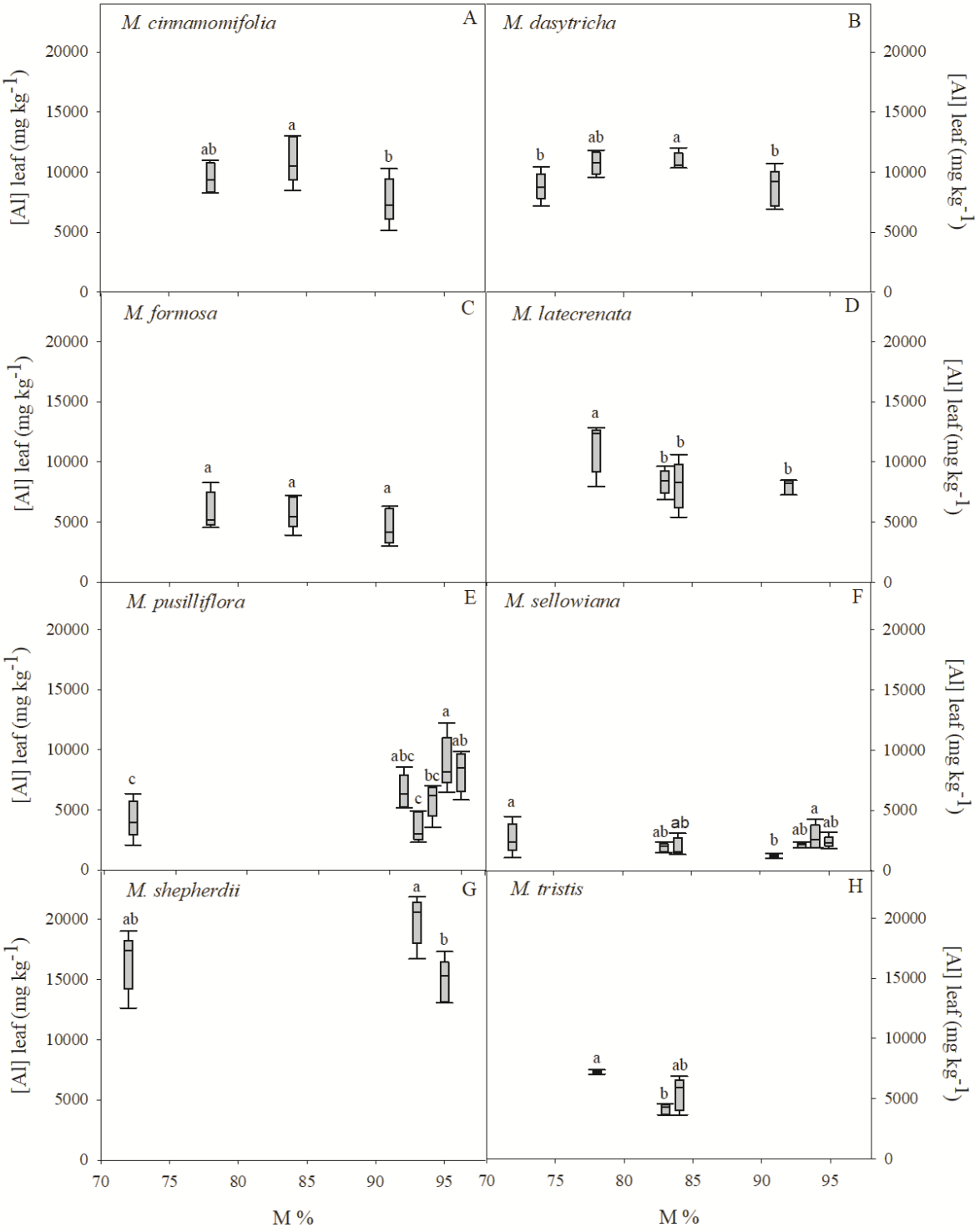

Fig. 4 Mean values (n = 5 plants) of leaf aluminum (Al) concentration of species that occurred in at least three altitudes (*Miconia cinnamomifolia* (A), *M. dasytricha* (B), *M. formosa* (C), *M. latecranata* (D), *M. pusilliflora* (E), *M. sellowiana* (F), *M. shepherdii* (G), and *M. tristis* (H)) in response to soil Al saturation (M%) in in four sites of the Atlantic rain forest (Itamonte, MG; Cunha, Juquitiba, and Ubatuba, SP), southeastern Brazil. The boxplots extend from the 25th to 75th percentiles, the continuous line within the box shows the median, and error bars represent 5th and 95th percentiles (n = 5 plants). Distinct letters indicate significant differences by Tukey test ($P < 0.05$).

Supplementary Material:

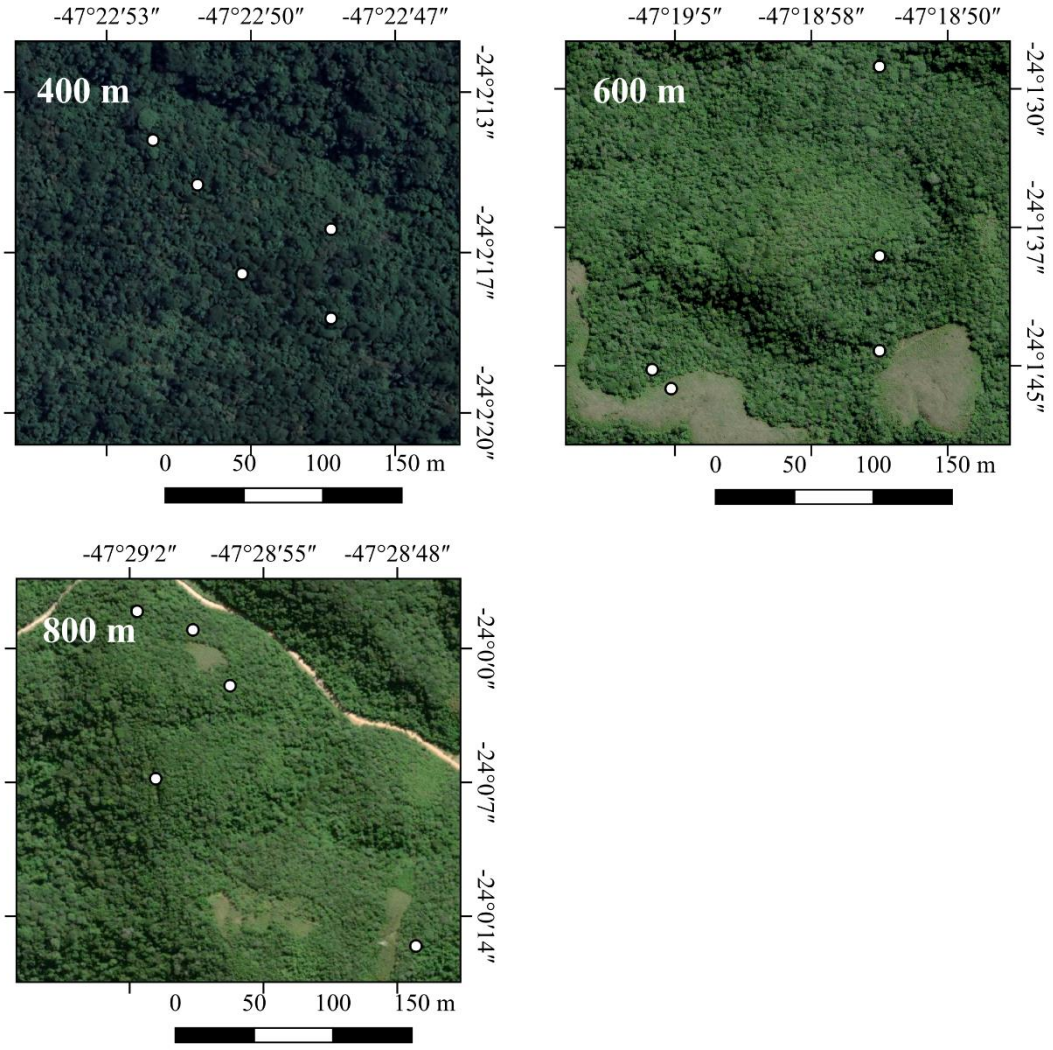

Figure S1. Location map of study area and arrangement of the location sampled soil over three different altitudes in Atlantic Rain Forest in the municipality of Juquitiba (3), São Paulo state, southeastern Brazil, in South America.

Considerações finais

Os solos ácidos (pH < 5,0) estão distribuídos em diversas regiões da crosta terrestre e nessa condição os compostos edáficos de Al são solubilizados à Al^{3+}, tornando-se tóxico para a maioria das espécies vegetais. Plantas que são adaptadas a alta saturação do solo por Al (m%) geralmente não mostram sintomas de toxicidade. Dentre estas espécies, há as não acumuladoras e as acumuladoras. As não acumuladoras geralmente restringem a absorção de Al pela raiz da planta. Por outro lado, as acumuladoras são capazes de absorver, transportar e distribuir por meio de ácidos orgânicos e acumular o Al em diferentes partes da planta, além de suas folhas. Assim, verificamos a capacidade das espécies nativas em direcionar alta concentração de Al para outros órgãos/tecidos.

Vochysia tucanorum acumulou nas folhas 7,8 vezes mais Al do que *Q. grandiflora e M. albicans*. Por outro lado, as não-acumuladoras mostraram baixa concentração de Al em suas raízes assim como em suas folhas. Logo, concluímos que as espécies acumuladoras de Al acumulam mais de 1000 mg Al kg^{-1} de folhas secas e concentrações variáveis de Al nas raízes. Porém, as espécies não-acumuladoras são "evitadoras", retendo baixas concentrações de Al nas raízes e folhas.

Em *M. albicans*, o acúmulo de Al não se limita às folhas, mas também ocorre em suas raízes, casca e caule. Com destaque para os órgãos senescentes – folhas senescentes e casca – que apresentaram concentrações de Al maiores quando comparado aos tecidos foliares maduros, o que pode ser uma via de eliminação de Al da planta. Além disso, durante o período reprodutivo encontramos que os botões e as flores acumulam mais Al do que os frutos imaturos e maduros.

Quando investigamos se o acúmulo de Al no tecido foliar de espécies de *Miconia* acumuladoras de Al na Mata Atlântica poderia estar associado à disponibilidade de Al no solo, encontramos que não existe uma relação direta entre esses dois fatores. E que o acúmulo de Al “parece” ser uma característica primitiva dentro da Tribo Miconieae. Portanto, o acúmulo de Al é uma característica binária: a planta acumula ou não, mas os mecanismos de controle desta característica dentro do gênero *Miconia* ainda não foram identificados. Para isso seria importante encontrar as espécies de *Miconia* não acumuladoras de Al.