Ecotoxicology and Environmental Safety 203 (2020) 111010

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Manganese hyperaccumulation capacity of *Ilex paraguariensis* A. St. Hil. and occurrence of interveinal chlorosis induced by transient toxicity

Ederlan Magri[a,*], Eduardo Kieras Gugelmin[b], Felipe Augusto Piacentini Grabarski[a], Julierme Zimmer Barbosa[c], André Carlos Auler[d], Ivar Wendling[e], Stephen Arthur Prior[f], Alice Teresa Valduga[g], Antônio Carlos Vargas Motta[d]

[a] Postgraduate Program in Soil Science, Department of Soils and Agricultural Engineering, Federal University of Paraná (UFPR), Curitiba, Paraná, Brazil
[b] Agronomy, Federal University of Paraná, Brazil
[c] Federal Institute of Southeast Minas Gerais, Barbacena, Minas Gerais, Brazil
[d] Department of Soils and Agricultural Engineering, Federal University of Paraná (UFPR), Curitiba, Paraná, Brazil
[e] Brazilian Agricultural Research Corporation - Embrapa Forestry, Colombo, Paraná, Brazil
[f] USDA-ARS National Soil Dynamics Laboratory, 411 South Donahue Drive, Auburn, AL, USA
[g] Postgraduate Program in Ecology, Regional Integrated University of Alto Uruguai and Missões (URI), Erechim, RS, Brazil

A R T I C L E I N F O

Keywords:
Yerba mate
Nutritional imbalance
Elemental composition
X-ray microanalysis

A B S T R A C T

Manganese (Mn) toxicity is common in plants grown on very acid soils. However, some plants species that grow in this condition can take up high amounts of Mn and are referred to as hyperaccumulating species. In this study, we evaluated the capacity of *Ilex paraguariensis* to accumulate Mn and the effect of excessive concentrations on plant growth and nutrition. For this, a container experiment was conducted using soils from different parent materials (basalt and sandstone), with and without liming, and at six doses of applied Mn (0, 30, 90, 270, 540 and 1,080 mg kg^{-1}). Clonal plants grown for 203 days were harvested to evaluate yield, and leaf tissue samples were evaluated for Mn and other elements. Without liming and with high Mn doses, leaf Mn concentrations reached 13,452 and 12,127 mg kg^{-1} in sandstone and basalt soils, respectively; concentrations in excess of 10,000 mg kg^{-1} are characteristic of hyperaccumulating plants. Liming reduced these values to 7203 and 8030 mg kg^{-1}. More plant growth accompanied increased Mn leaf concentrations, with a growth reduction noted at the highest dose in unlimed soils. Elemental distribution showed Mn presence in the mesophyll, primarily in vascular bundles, without high Mn precipitates. Interveinal chlorosis of young leaves associated with high Mn concentration and lower Fe concentrations was observed, especially in sandstone soil without liming. However, the occurrence of this symptom was not associated with decreased plant growth.

1. Introduction

Manganese (Mn) is a plant micronutrient taken up by roots in active or passive forms in the Mn^{2+} oxidation state (Broadley et al., 2012). Functional roles are related to the oxygen evolution complex that aids water photolysis in photosystem-II, chlorophyll synthesis, and activation of numerous enzymes (Schmidt et al., 2016). In vegetative tissues, Mn can accumulate in exchangeable forms, adsorb to negative charges of cell walls, and can exist in available (in cytoplasm) and unavailable (in vacuoles) forms (Junior et al., 2008).

In soil, this micronutrient is the most abundant following iron (Fe) and is mainly found in Mn^{2+} and Mn^{4+} oxidation states, with total amounts varying from 200 to 3,000 mg kg^{-1} (Oliva et al., 2014; Kabata-Pendias, 2010). Manganese plant availability can be altered by pH changes, and is more available in acidic soils, which produces favorable conditions for phytotoxicity in plants (Millaleo et al., 2010; Broadley et al., 2012). In addition, soils derived from igneous sources such as basalt have higher Mn values compared to sedimentary-based soils such as sandstone (Althaus et al., 2018). This difference can affect the amount of Mn availability and uptake by plants (Motta et al., 2020).

* Corresponding author.
E-mail addresses: ederlan.magri@gmail.com (E. Magri), eduardokieras@gmail.com (E.K. Gugelmin), felipegrabarski@gmail.com (F.A.P. Grabarski), juliermezimmer@hotmail.com (J.Z. Barbosa), auler@ufpr.br (A.C. Auler), ivar.wendling@embrapa.br (I. Wendling), steve.prior@usda.gov (S.A. Prior), valice@uricer.edu.br (A.T. Valduga), mottaufpr@gmail.com (A.C.V. Motta).

https://doi.org/10.1016/j.ecoenv.2020.111010
Received 4 April 2020; Received in revised form 30 June 2020; Accepted 3 July 2020
Available online 24 July 2020

Some plants species have the capacity to accumulate high amounts of Mn in tissue without expressing toxicity symptoms or affecting growth. Some species can accumulate leaf concentrations in excess of 1,000 mg kg^{-1}, such as 5,996 mg kg^{-1} in *Phytolacca americana* L. (Phytolaccaceae) (Zhao et al., 2012) and 5,973 to 6,924 mg kg^{-1} in *Alternanthera philoxeroides* (Mart.) Griseb. – Amaranthaceae (Xue et al., 2004). On the other hand, some species are known as hyperaccumulators because they can accumulate Mn in excess of 10,000 mg kg^{-1} (Baker and Brooks, 1989). In a global review of metal hyperaccumulator plants, Reeves et al. (2018) discussed 24 species that presented Mn concentration up to 10,000 mg kg^{-1}. In South America, the tree species *Ilex paraguariensis* A. St. Hil. (Aquifoliaceae) is reported to have a high capacity for accumulating Mn in leaves, with concentrations usually close or up to 1,000 mg kg^{-1} (Reissmann and Carneiro, 2004; Oliva et al., 2014; Barbosa et al., 2018, 2020). Motta et al. (2020) reported Mn concentration of 9,401 ± 1,591 mg kg^{-1} in leaves of *I. paraguariensis* under typical forest conditions, and suggested future research to better explore the Mn accumulation potential of this species.

Another important aspect is that *I. paraguariensis* leaves are used to prepare hot or cold water infusions for human consumption (i.e., "*chimarrão*", "*terrerê*", and various teas) (Valduga et al., 2019). Barbosa et al. (2015) reported that up to 45% of Mn in *I. paraguariensis* leaves can be extracted via hot water infusion. Therefore, attention should be paid to the amount of Mn present in raw material used for yerba mate infusion preparations, since absorption of Mn above recommended maximum amounts can result in neurotoxic effects, higher incidence of acute bronchitis, bronchial asthma, and pneumonia (Leite et al., 2014).

Studies have indicated that Mn is the main micronutrient affected by liming *I. paraguariensis*, where a 57% decrease in Mn leaf concentration has been reported (Reissmann et al., 1999). Santin et al. (2013) reported a similar decrease in leaves (~50%), a high decrease in shoots, and no effect in roots. These evaluations reflect normal field conditions, but behaviors under conditions of high Mn availability are not known. Although the capacity of this species to accumulate significant amounts of Mn in leaves is known (Motta et al., 2020), the maximum accumulation potential, location of Mn within leaf tissues, phytotoxic damage, and consequent effects on plant growth have not been explored.

Therefore, the present study aims to: (1) investigate possible toxicity effects and the consequences of high Mn concentration on growth and leaf elemental composition; (2) determine the maximum Mn accumulation potential of *I. paraguariensis* leaves; (3) investigate the location of Mn within leaf tissue; and (4) assess the effect of soil pH increase on Mn accumulation. Results of the present work will aid in understanding Mn uptake dynamics from soil to *I. paraguariensis* leaves and Mn storage in leaves. Additionally, results may have implications within production and manufacturing systems since *I. paraguariensis* is part of the human diet and excessive Mn consumption may impact human health.

2. Materials and methods

2.1. Experimental design and soil collection

The study was conducted in the understory of an Araucarian forest (*Araucaria angustifolia* L.). This predominantly subtropical region has an altitude of 934 m, with a Köppen classification climate of Cfb (Alvares et al., 2013). The average precipitation during the experimental period was 166.5 mm $month^{-1}$ (67.4–301.2 mm $month^{-1}$) and the average temperature was 21.0 °C (17.3–26.7 °C). The experiment was conducted in a completely randomized design with four replications, in which two soils from different parent materials (basalt and sandstone) were evaluated with and without acidity correction (liming) at Mn application doses of 0, 30, 90, 270, 540, and 1,080 mg Mn kg^{-1} soil.

Soil was collected from the 0–20 cm layer, homogenized, and sieved to pass a 4 mm mesh. Soil formed from basalt was classified as a Ferralsol and was collected in *Barão de Cotegipe*, *Rio Grande do Sul* state, Brazil (27°33′50.71″ S, 52°24′3.83″ W). The soil formed under sandstone was classified as a Cambisol and was collected in *São João do Triunfo*, *Paraná* state, Brazil (25°40′45.05″ S, 50°18′36.04″ W).

2.2. Determination of soil chemical and physical parameters

Prior to experiment installation, texture was determined by the Bouyoucos hydrometer method using as a dispersant mixture of NaOH (4 g L^{-1}) and $(NaPO_3)_6$ (10 g L^{-1}) (Dane et al., 2002). Soil chemical and physical properties are shown in Table 1. After an acidity corrective incubation period, another soil sample was taken and chemical characterization was performed again. In both soil analyses, samples were dried at room temperature and ground to pass a 2 mm mesh sieve to determine the following measures: pH (0.01M $CaCl_2$), potential acidity (H + Al), exchangeable acidity (Al^{3+}, by titration), quantification of exchangeable bases (Ca^{2+}, Mg^{2+} extracted by 1 mol L^{-1} KCl, and K^+ extracted with Mehlich-I), available P (extracted with Mehlich-I), and organic C (wet combustion). Chemical determinations followed the methodologies described by Marques and Motta (2003).

2.3. Correction of acidity and Mn doses

Based on soil analyses, the amount of lime required to achieve a base saturation of 70% was calculated. Thus, 2.80 g kg^{-1} of $CaCO_3$ and 0.28 g kg^{-1} of MgO (both PA reagents) were added to the basalt soil. For the sandstone soil, 4.88 g $CaCO_3$ and 0.50 g MgO per kg of soil were added. Soils were then homogenized, placed in 5 dm^3 containers and incubated for 21 days while keeping soils moist. After incubation, clonal plants of *I. paraguariensis* BRS BLD Yari cultivar propagated by the mini-cuttings technique (Wendling et al., 2017) were transplanted into each container. At 65 days after transplanting, fertilization with N, P, and K equivalent to 150 mg N, 250 mg P_2O_5 and 200 mg K_2O per kg of soil was performed. For this fertilization, we used 117.15 g of K_2HPO_4 + 46.76 g of KH_2PO_4 + 89.08 g of $(NH_4)_2HPO_4$ + 250.59 g of $(NH_4)_2SO_4$ + 26.06 g of KCl diluted in 4.8 L of deionized water and applied 50 mL of solution to each container.

Addition of Mn was split into three application periods to avoid precipitation of Mn and possible plant mortality at high doses. The application of Mn occurred at 41, 65, and 153 days after transplanting. Thus, it was possible to progressively monitor plant stress due to high Mn availability. The source used was manganese sulfate ($MnSO_4$) diluted in deionized water, and three applications of 0, 10, 30, 90, 180, 360 mg kg^{-1} of soil totaled the initially proposed doses of 0, 30, 90, 270, 540 and 1,080 mg Mn kg^{-1} of soil.

2.4. Plant monitoring and elementary quantification

Plants were grown up to 50 days after the last Mn application, which corresponds to 203 days after transplanting. During this period, plants were monitored to diagnose for leaf anomalies indicative of Mn toxicity; symptoms were recorded and characterized in detail. At study termination, plants were cut at the collar region and completely expanded leaves were separated for elemental composition determinations. All plant material was oven dried by forced air circulation at 65 °C until constant mass prior to weight determinations.

Dried fully expanded leaves used to determine elemental composition were ground in a Willey mill to pass a 2 mm mesh sieve. For digestions, 1 g of plant tissue and 10 mL of 3 mol HCl were used and subjected to heat plate digestion at 200 °C for 25 min. After this period, solutions were filtered with 1–2 μm particle retention filter paper, and volume adjusted to 100 mL with deionized water. Elemental quantification of K, Ca, Mg, P, S, Mn, Al, Fe, Zn, B, and Cu was performed using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES, Varian 720-ES). Certified reference material (GBW–10016 *Camellia sinensis*) was utilized to ensure quality control, and the following recovery values were obtained: K – 107%, Ca – 91%, Mg – 113%, P – 95%, S – 91%, Mn – 104%, Fe – 73%, Zn – 100%, and Cu – 79%.

Table 1
Chemical and physical analyses of basalt and sandstone soils with and without lime addition prior to planting.

Soil	pH $CaCl_2$	Al^{3+}	H + Al	Ca^{+2}	Mg^{+2}	K^{+}	P	Mn	OC	Sand	Silt	Clay
		$cmol_c\ dm^{-3}$					$mg\ dm^{-3}$		$g\ dm^{-3}$	$g\ kg^{-1}$		
Without *lime*												
Sandstone	3.5	5.71	20.4	0.7	0.3	0.24	1.5	41	37.3	400	300	300
Basalt	4.4	1.12	12.1	2.8	2.0	0.23	3.8	228	20.1	50	225	725
With *lime*												
Sandstone	5.2	0.00	5.4	10.7	1.7	0.16	9.1	38	37.3	400	300	300
Basalt	5.5	0.00	4.3	9.2	2.9	0.43	7.4	207	20.1	50	225	725

OC = organic carbon.

2.5. Microscopy analysis

Microscopy analysis was performed on plants subjected to Mn doses of 0 and 1,080 mg kg^{-1} (control and maximum dose). After cutting plants, one fully expanded representative leaf from control and maximum dose plants were selected. A section cut from each leaf, resulting in a ~0.5 × 2.0 cm sample that was fixed in the dark (48 h at 4 °C) in 1.5 mL eppendorf microtubes with 1 mL of FAA fixation solution; i.e., 70% ethanol [v/v] + formalin 5% [v/v] + glacial acetic acid 5% [v/v]. After this fixation period, samples were submitted to dehydration by an ethanol series of 80, 90, 95, and 100% (20 min per step). Microanalyses were performed using a SEM (Vega3 LM, Tescan) equipped with an EDX elemental detector (X-Max^N 80 mm^2, Oxford).

2.6. Data analysis

Statistical analyzes were performed using R software, version 3.6.0 (R Core Team, 2019). Data was subjected to Shapiro-Wilk and Bartlett tests to verify the assumptions of residual normality ("shapiro.test" functions) and variance homogeneity ("bartlett.test" function), respectively. In cases where assumptions were not met, data were transformed by the optimal Box-Cox power ("boxcox" function of the MASS package). Analysis of variance for each soil was applied according to the completely randomized design with factorial treatments (2 × 6) with four replications, considering doses (6) as quantitative factors and acidity correction (2) as qualitative, and leaf Mn concentration as the response variable. A 95% confidence level was considered for model validation. After validation, regression analysis was applied to determine the response of Mn concentration as a function of the supply of this element to different soils without and with liming ("lm" function). Plant total dry matter results were converted to relative yield, considering the average repetition result, with the zero dose treatment corresponding to 100% yield, and the other doses proportional to this value.

In addition, a Principal Component Analysis (PCA) of elemental composition and total dry matter ("princomp" function) was performed from the correlation matrix of standardized data for mean zero and variance one (transformed by the "decostand" function of Vegan package). The number of Principal Components (PC) used were selected to explain at least 70% of the variance. Plants with and without leaf anomaly were classified into two distinct groups, and from the observation of PCA, a Discriminant Function Analysis (DFA) was applied to discriminate these two groups (MDA package – "lda" function). In the calculation of DFA, we used the original covariance matrix of the data. Precision of the analysis was determined by the confusion matrix. Additionally, the Student's t-test was applied to compare variables used in DFA according to the created groups.

3. Results

3.1. Leaf Mn concentration and toxicity

Concentration of Mn in leaves of *I. paraguariensis* increased with Mn supply (Fig. 1). The highest Mn concentration in leaves occurred in unlimed soils at the 540 mg kg^{-1} dose, reaching a maximum value of 13,452 mg kg^{-1} for basalt soil and 12,127 mg kg^{-1} for sandstone soil. In limed soils, the respective maximum leaf Mn concentrations of 7,203 mg kg^{-1} and 8,030 mg kg^{-1} for basalt and sandstone soils occurred at the maximum dose.

In some treatments, leaves showed toxicity symptoms (Fig. 2) that was characterized as interveinal chlorosis resulting in leaves without pigmentation in more severe stages (Fig. 2C). This toxicity symptom started from the second Mn application. Symptom intensity was greatest during initial leaf development, becoming less noticeable with leaf aging, and was barely visible or sometimes absent at the end of the experiment.

In unlimed soil, the toxicity symptom was more frequent and

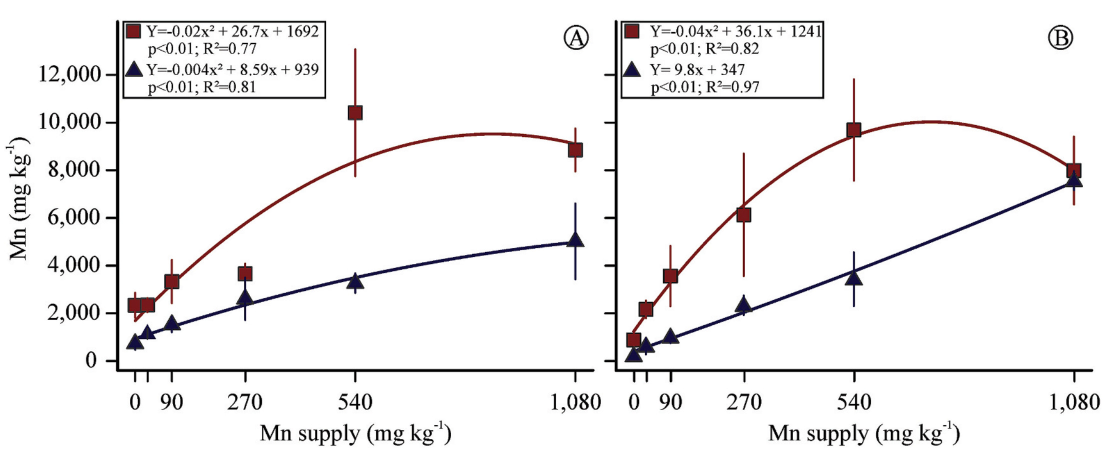

Fig. 1. Mn concentrations (average and error bars) in *Ilex paraguariensis* leaves grown in basalt (A) and sandstone soil (B) without liming (red) and with liming (blue) in response to Mn supply (0, 30, 90, 270, 540, and 1,080 mg kg^{-1}). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

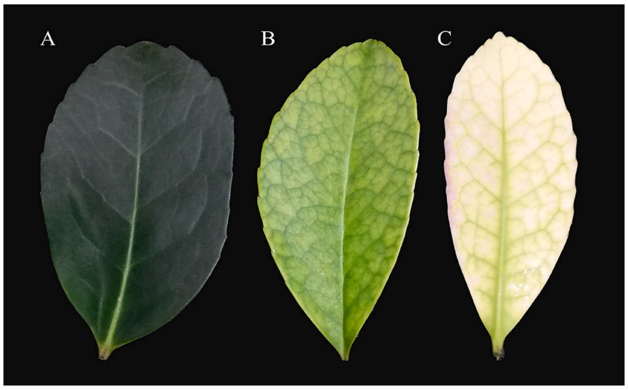

Fig. 2. Normal *Ilex paraguariensis* leaf (A) and leaves with symptoms of interveinal chlorosis (B and C) associated with increased Mn concentration.

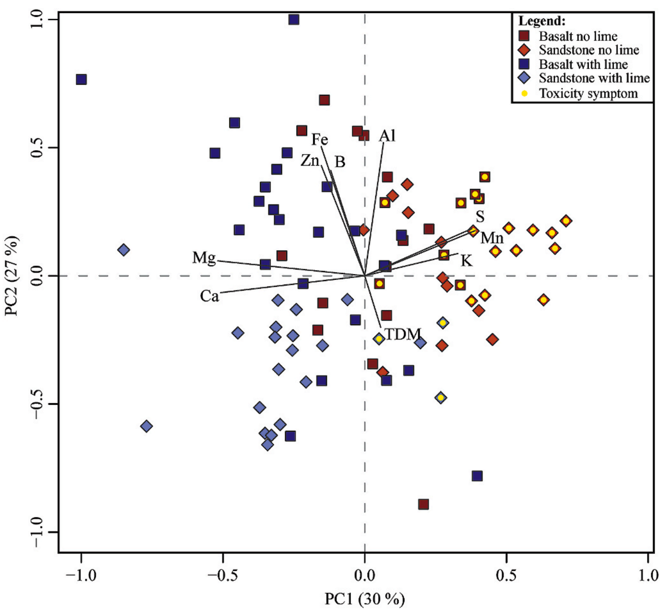

Fig. 3. Principal component analysis of leaf elemental composition of *Ilex paraguariensis* grown in basalt and sandstone soils lime and unlimed, at different supplied Mn doses (0, 30, 90, 270, 540, and 1,080 mg kg^{-1}). TDM: Total dry matter. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

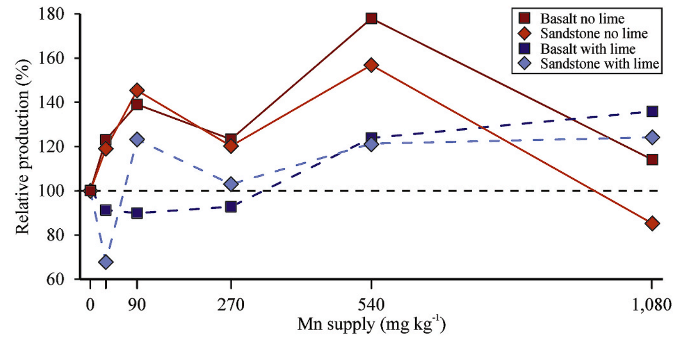

Fig. 4. Relative dry matter production of *Ilex paraguariensis* grown in basalt and sandstone soil without liming and with liming in response to Mn supply (0, 30, 90, 270, 540, and 1,080 mg kg^{-1}). Horizontal dashed line represents the witness (100% relative yield). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

occurred at the Mn dose of 360, 540 and 1,080 mg kg^{-1} in the sandstone soil, and at the Mn dose of 540 and 1,080 mg kg^{-1} in the basalt soils. In limed soils, leaf toxicity occurred only at the maximum dose in the sandstone soil and had less intensive chlorosis (Fig. 2B) than plants grown in unlimed soils. Plants grown in limed basalt soil did not presented toxicity symptoms.

As illustrated by PCA, the predominant occurrence of toxicity symptom in plants indicates that this occurred due to excessive Mn accumulation in leaves of *I. paraguariensis* (Fig. 3). This was confirmed by DFA, which discriminated plants by the presence of toxicity symptom with a 98% accuracy confounded by only one sample from each predetermined group. The main discriminant variables of groups with and without interveinal chlorosis were Mn and Fe leaves concentrations (with negative correlations) based on the discriminant function presented in Eq. (1):

$$D(x) = -0.29Mn[] + 0.23Fe[] - 0.09Al[] + 0.07TDM + 0.04B[] \quad (1)$$

3.2. Plant growth and elemental composition interaction

Increased dose of Mn applied was accompanied by increased plant dry matter yield, with relative dry matter production ~180% and 120% in unlimed and limed basalt soil at the 540 mg kg^{-1} dose, respectively (Fig. 4). In unlimed soils, an increase in total dry matter production was observed at the lowest Mn doses, with a decrease at the maximum dose; a result that follows the behavior of leaf Mn concentration (Figs. 1 and 4) that may be reflective of physiological stress by toxicity. In limed soils, the opposite effect was observed, with a decrease in production at the first Mn dose and an increase at the two highest doses.

Increases in Mn leaf elemental concentration was accompanied by increased in S concentration. This Mn increase did not affect concentrations of any other evaluated element (Table 2).

Three main Principal Components (PC) can explain 70% of data variation for leaf elemental composition and total dry matter (Fig. 3 and Table S1). PC1 showed positive correlation between Mn, S, and K, and these elements were negatively correlated to concentrations of Ca and Mg. Fig. 3 clearly shows the presence of higher Mn concentration in unlimed soils. PC2 represented the positive relationships between Fe, Al, Zn, and B (Table S1). The highest values of these elements were associated with plants grown in soil with Mn doses of 30, 90, 270, and 540 mg kg^{-1} (Table 2). PC3 reflects total dry matter production, which was positively correlated with Mn and S concentrations and negatively correlated with K (Table S1).

3.3. Distribution of elements in yerba mate leaves

Low concentration in the control treatment prevented detection of Mn in yerba mate leaf samples. SEM–EDS evaluations of the maximum dose treatment revealed no presence of Mn precipitates in leaves. This element was concentrated in the mesophyll region, primarily in vascular bundles (Fig. 5).

4. Discussion

4.1. Accumulation of Mn in leaves

The maximum observed Mn concentration (13,452 mg kg^{-1}) (Fig. 1) was approximately a third higher than the previous maximum reported in the literature for *I. paraguariensis* leaves (Motta et al., 2020). Thus, under experimental conditions this species displays characteristics of Mn hyperaccumulating plants by exceeding Mn concentrations of 10,000 mg kg^{-1} (Baker and Brooks, 1989). The maximum concentrations of Mn obtained for both soils without liming were similar. Additionally, for both soils at the highest dose (1,080 mg kg^{-1}), leaf Mn concentration decreased in relation to the previous dose (540 mg kg^{-1}); this suggests

Table 2

Elemental composition (average ± standard derivation) of *Ilex paraguariensis* leaves grown in basalt and sandstone soils with and without lime under Mn doses of 0, 30, 90, 270, 540, and 1,080 mg kg^{-1}.

Doses	K	Ca	Mg	P	S	Al	Fe	Zn	B	Cu
mg kg^{-1}	g kg^{-1}							mg kg^{-1}		
Basalt no liming										
0	11.6 ± 0.5	5.5 ± 0.9	3.9 ± 0.4	0.99 ± 0.16	0.61 ± 0.19	158 ± 48	129 ± 44	155 ± 36	11.8 ± 1.8	2.9 ± 0.7
30	13.5 ± 1.0	5.0 ± 1.4	4.1 ± 0.8	0.70 ± 0.02	0.71 ± 0.41	139 ± 83	105 ± 77	123 ± 69	10.7 ± 5.2	2.3 ± 0.5
90	12.2 ± 1.8	6.0 ± 0.6	4.9 ± 0.3	0.88 ± 0.24	0.55 ± 0.11	190 ± 52	146 ± 53	141 ± 50	15.3 ± 1.2	2.3 ± 0.2
270	12.8 ± 1.5	5.4 ± 0.7	4.3 ± 0.4	0.90 ± 0.27	0.93 ± 0.45	164 ± 60	131 ± 63	116 ± 22	13.4 ± 1.0	2.9 ± 0.3
540	12.5 ± 2.0	5.0 ± 0.4	3.9 ± 0.6	0.79 ± 0.17	0.76 ± 0.26	183 ± 18	126 ± 28	125 ± 21	13.9 ± 2.1	2.7 ± 0.6
1,080	13.1 ± 0.7	4.0 ± 0.4	3.2 ± 0.2	0.84 ± 0.30	1.06 ± 0.32	164 ± 34	102 ± 23	140 ± 10	15.2 ± 2.0	4.1 ± 0.5
Basalt with liming										
0	12.0 ± 0.6	6.9 ± 1.0	4.3 ± 0.4	0.98 ± 0.16	0.35 ± 0.02	102 ± 27	96 ± 34	112 ± 28	13.6 ± 2.5	2.7 ± 0.5
30	12.1 ± 0.4	6.9 ± 0.9	5.1 ± 0.5	1.21 ± 0.28	0.40 ± 0.11	199 ± 60	229 ± 81	146 ± 46	15.5 ± 2.6	2.9 ± 0.8
90	12.8 ± 1.1	7.5 ± 0.4	5.1 ± 0.2	1.15 ± 0.32	0.37 ± 0.10	260 ± 110	185 ± 67	125 ± 24	17.3 ± 2.1	4.1 ± 1.6
270	11.9 ± 2.7	8.2 ± 2.4	5.3 ± 1.0	1.45 ± 0.46	0.46 ± 0.12	219 ± 70	214 ± 94	153 ± 46	21.1 ± 5.4	2.8 ± 0.6
540	12.6 ± 1.7	6.2 ± 1.7	4.2 ± 0.7	1.33 ± 0.25	0.50 ± 0.07	160 ± 73	141 ± 77	100 ± 34	15.1 ± 2.7	2.9 ± 0.1
1,080	12.5 ± 0.8	6.2 ± 0.5	3.7 ± 0.3	1.04 ± 0.19	0.63 ± 0.12	103 ± 28	77 ± 15	114 ± 32	14.5 ± 0.7	3.3 ± 0.5
Sandstone no liming										
0	14.1 ± 0.9	3.4 ± 0.3	3.0 ± 0.2	1.46 ± 0.26	0.31 ± 0.09	182 ± 53	90 ± 23	114 ± 28	12.2 ± 2.0	3.0 ± 0.1
30	14.5 ± 0.2	3.6 ± 0.7	3.6 ± 0.6	1.62 ± 0.43	0.61 ± 0.13	220 ± 35	105 ± 18	101 ± 24	14.2 ± 2.2	1.8 ± 0.3
90	12.9 ± 1.1	3.3 ± 0.7	3.5 ± 0.3	1.37 ± 0.37	0.64 ± 0.20	180 ± 52	92 ± 26	89 ± 42	11.6 ± 1.9	2.4 ± 0.5
270	13.1 ± 1.0	2.6 ± 0.4	2.5 ± 0.3	1.29 ± 0.13	0.94 ± 0.40	176 ± 2	86 ± 22	88 ± 12	10.6 ± 1.3	2.4 ± 0.9
540	12.9 ± 1.5	2.9 ± 0.5	3.1 ± 0.4	1.11 ± 0.30	1.27 ± 0.32	204 ± 18	126 ± 29	90 ± 19	12.4 ± 1.2	3.1 ± 0.8
1,080	13.4 ± 0.8	2.3 ± 0.3	2.7 ± 0.2	1.21 ± 0.43	1.44 ± 0.37	174 ± 18	95 ± 14	115 ± 25	11.5 ± 0.8	4.2 ± 0.6
Sandstone with liming										
0	11.8 ± 1.7	8.2 ± 0.6	4.6 ± 0.7	1.62 ± 0.50	0.40 ± 0.13	108 ± 30	100 ± 29	80 ± 13	9.0 ± 1.7	3.0 ± 0.4
30	13.1 ± 0.3	7.4 ± 1.4	5.2 ± 0.3	1.87 ± 0.53	0.37 ± 0.05	97 ± 21	76 ± 20	78 ± 22	11.8 ± 4.4	3.2 ± 0.6
90	9.1 ± 1.1	9.8 ± 1.4	5.8 ± 0.5	1.01 ± 0.25	0.44 ± 0.17	110 ± 20	82 ± 11	138 ± 101	9.5 ± 2.4	3.0 ± 0.5
270	11.7 ± 0.4	8.7 ± 1.2	5.2 ± 0.2	1.51 ± 0.25	0.42 ± 0.11	90 ± 17	71 ± 17	65 ± 27	12.2 ± 3.1	3.5 ± 1.1
540	11.8 ± 1.2	8.1 ± 1.5	4.5 ± 0.4	1.21 ± 0.26	0.67 ± 0.07	98 ± 23	111 ± 41	81 ± 10	10.9 ± 2.4	2.2 ± 0.8
1,080	11.9 ± 1.1	6.8 ± 1.4	3.4 ± 0.4	1.21 ± 0.21	0.76 ± 0.06	83 ± 14	61 ± 20	66 ± 13	14.7 ± 2.6	3.0 ± 0.6

that these accumulation values are the maximum for this species. Caldeira et al. (2006) found Mn concentrations above 1,000 mg kg^{-1} in *Ilex dumosa* (Reissek) leaves under native conditions, indicating that other species of the genus *Ilex* may accumulate high amounts of Mn in leaves. Given that the critical concentration for the definition of Mn hyperaccumulating species (>10,000 mg kg^{-1}) proposed by Baker and Brooks (1989) has been widely used (Reeves et al., 2018; Van der Ent et al., 2019), *I. paraguariensis* could be considered a Mn hyperaccumulator species under our study conditions. However, other standards has been proposed, such as bioconcentration factor (>1; but often >50), shoot-to-root factor, and the genetic approach (Van der Ent et al., 2013). Motta et al. (2020) reported a bioconcentration factor (plant:soil ratio) between 12 and 14 for *I. paraguariensis* plants with leaf Mn concentrations of 9,401 ± 1,591 mg kg^{-1}; considering the study as a whole (30 sites), the bioconcentration factor ranged from 0.9 to 32.

For healthy growth, plants resistant to high Mn concentration employ mechanisms to maintain this element in non-toxic forms. The most common strategies being associations with the endoplasmic reticulum and Golgi complex, storage in vacuoles and epidermal cells, and Mn chelate formations (Shao et al., 2017). Since toxicity symptoms tend to disappear with leaf aging, *I. paraguariensis* possibly uses some of these mechanisms to maintain Mn in a non-toxic form, which allows mature leaves with Mn concentrations above 10,000 mg kg^{-1} to not manifest visual symptoms in mature leaves. Although present in high concentration, no high Mn precipitates were observed in foliar tissues (Fig. 5), suggesting that this element was homogeneously distributed throughout the mesophyll. As indicated by Junior et al. (2008), Mn may be allocated to negative cell wall charges, cytoplasm, and vacuoles. Thus, distribution in mesophyll indicates Mn was adsorbed to charges of cell walls or existed as free forms in the vascular bundle.

Motta et al. (2020) showed that parent material of soil plays a key role in influencing Mn concentration in *I. paraguariensis* leaves and that concentration in basalt soils is about twice as large as sedimentary and rhyolite/rhyodacite soils. In our study, this effect was clear in unlimed soils without Mn supply, where leaf Mn concentrations were 2,336 ± 457 and 884 ± 295 mg kg^{-1} in basalt and sandstone soil, respectively (Fig. 1). In this context, Barbosa et al. (2018) noted an influence of soil type on leaf Mn concentrations related to crystalline and low crystallinity forms of this nutrient in soil.

Although leaf Mn concentrations followed the amount of soil Mn availability, two other factors can influence this difference: in poorly drained areas (e.g., low slopes or wet slopes), higher Mn availability can be expected (Zengin, 2013); climate variations could increase availability and accumulation of Mn during rainy seasons as has been observed in pastures (Senger et al., 1997; Siman et al., 1974). This implies that a large variation in leaf Mn concentration could occur between plants cultivated within the same location depending on climatic variation during the year and position in regard to landscape relief.

On the other hand, increased pH decreased leaf Mn concentration (Fig. 1), corroborating results of Toppel et al. (2018) who found a relationship between Mn concentrations in native plants of *I. paraguariensis* and soil pH. This indicates that changing soil pH is the most efficient way to reduce Mn concentration in *I. paraguariensis* leaves, and consequently reduce Mn concentration in the raw material used for infusion preparations. As initially reported, this is an important subject since *I. paraguariensis* mate drinks are consumed by a large population (adolescents, adults, and the elderly) and high Mn consumption can be unhealthy (Leite et al., 2014). Almost half of the Mn present in *I. paraguariensis* leaves is known to be soluble in hot water (Barbosa et al., 2015). Therefore, the ecotoxicology of plants can have direct or indirect effects on consumers. However, a better understanding of the amount of Mn that is effectively absorbed by the body is required. On the other hand, there is great potential for use of these Mn-enriched leaves for nutritional supply of both humans (those needing Mn supplements) and breeding animals.

4.2. Plant growth and elemental composition interaction

In comparison to typical cultivated plants, pH increase was accompanied by a reduction in *I. paraguariensis* dry matter accumulation

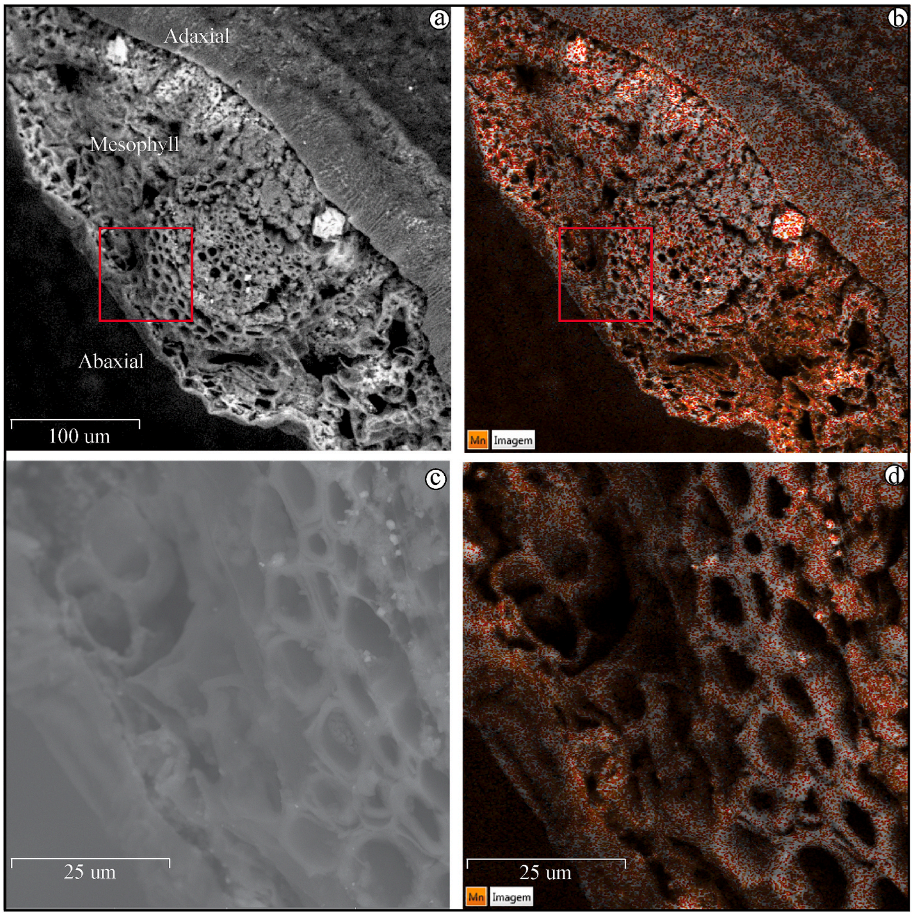

Fig. 5. MEV-EDS photomicrographs showing Mn distribution in *Ilex paraguariensis* leaves from the maximum dose treatment (a and b) and zoomed areas outlined in red (c and d). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

(Fig. 4). This was also observed by Santin et al. (2013) under field conditions. This effect on growth can be linked to the reduction in leaf Mn concentration, since this element was the only one affected in our study. Growth promoted by increased Mn concentration can be associated with distinct physiological effects. First, Mn^{2+} has a similar ionic radius compared to Mg^{2+} and Ca^{2+} (0.075 nm, 0.065 nm and 0.099 nm, respectively) and is able to replace these elements in their functions (Broadley et al., 2012). Another characteristic is that Mn is responsible for cell elongation, and directly affects root growth and subsequent plant growth (Sadana et al., 2002). Finally, high Mn concentrations promote the degradation of Indole-3-Acetic-Acid (IAA) due to increased oxidase and polyphenoloxidase activity (Fecht-Christoffers et al., 2007). Decreased IAA promotes loss of apical dominance and the formation of auxiliary shoots (Bañados et al., 2009), which can contribute to increased leaf emergence and overall increases in plant dry matter. However, it is important to highlight that high pH can harm *I. paraguariensis* due to non-nutritional effects, such as increase in root diseases (Poletto et al., 2011). Plants with toxicity symptom exhibited the highest Mn concentrations (Figs. 1 and 2). However, Mn toxicity is often confused with deficiencies of Fe, Ca, Mg, or Zn (Zanão Júnior et al., 2010). Thus, occurrence of interveinal chlorosis was attributed to excess Mn and associated with lower Fe concentrations. The average concentration of Mn in leaves with interveinal chlorosis was more than three times higher than leaves displaying no toxicity symptoms, while Fe was approximately 20% lower (Table S2). As a consequence, chlorophyll concentration in plants may have decreased due to Fe deficiency induced by excess Mn (Silva et al., 2017). Huang et al. (2016) observed a similar situation in sugarcane with Mn toxicity, where the average amount of total Fe was slightly lower in plants with chlorosis; however, active Fe corresponded to 30% of amounts found in normal plants (i.e., although plants had sufficient Fe, excess Mn inhibited normal functions). This lack of physiologically active Fe is a side effect of Mn toxicity that blocks the synthesis of chlorophyll and results in chlorosis (Subrahmanyam and Rathore, 2000; Huang et al., 2016). In addition, excess Mn can replace Mg in the chlorophyll molecule, causing damage that also results in chlorosis (Clairmont et al., 1986). However, this toxicity symptom (Fig. 2) is probably due to the application of a high dose of Mn to the soil; under these conditions, there are no records of symptoms in leaves with high concentrations of Mn (Motta et al., 2020).

The lower occurrence of interveinal chlorosis in plants cultivated in limed soils is due to increased soil pH along with the addition of Ca and Mg and is very clear in the PCA (Fig. 3). Liming effects are not restricted to soil pH elevation, but are also attributable to increasing Ca amounts near roots, which reduces Mn absorption capacity due to competition for absorption sites (Fernando and Lynch, 2015). In addition, Mg supplied by lime also contributes to reduction of Mn toxicity symptoms in plants (Davis, 1996).

Concentrations of Zn, Fe, and Mg in leaves with interveinal chlorosis were slightly lower than leaves without symptoms (Table 2), while Ca concentration was ~50% lower. However, the literature indicates that Mn toxicity associated with low Ca concentrations causes leaf apex deformation (Broadley et al., 2012), but this was not observed in the present study. Zinc deficiency is associated with reduced growth, while Mg deficiency is symptomatic in old leaves (Broadley et al., 2012). These results reinforce the fact that toxicity is more related to imbalance with Fe than with other nutrients. Regarding nutritional composition (Table 2), an increase in S concentration was expected since the source of Mn used was $MnSO_4$.

There are also reports of mycorrhiza associations with yerba mate roots, but the effect of this association on elemental composition is not known (Bergottini et al., 2017; Silvana et al., 2020). This highlights a knowledge gap that is worthy of future investigation.

5. Conclusion

The maximum concentrations of Mn in *I. paraguariensis* leaves were 13,452 mg kg^{-1} for basalt soil and 12,127 mg kg^{-1} for sandstone soil, indicating that this species may be a Mn hyperaccumulator, which was facultative since this effect was soil-dependent. This is reinforced by the effect of Mn on the physiology of the plant, which showed a positive growth response and had no detrimental effect on nutritional status. Additionally, since leaves show symptoms of phytotoxicity during initial stages of leaf development (likely associated with Fe deficiency) that disappeared with further leaf development, Mn was probably transformed to non-toxic forms with leaf aging. Considering that products derived from *I. paraguariensis* are part of the human diet, increasing pH by lime application can drastically reduce concentrations of Mn in leaves, thereby reducing Mn consumed in infusion products. However, increasing soil pH can decrease *I. paraguariensis* growth. Thus, it is necessary to identify optimal lime doses that decreases Mn in leaves without negatively impacting *I. paraguariensis* yield.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The authors thank the "*Barão de Cotegipe*", "*Rei Verde*" and "*Baldo SA*" industries of yerba mate for their assistance and the Center of Electronic Microscopy of the Federal University of Paraná (CME-UFPR) for SEM-EDS analysis. Antônio Carlos Vargas Motta thanks the National Council for Scientific and Technological Development (CNPq) for research funding (Project No. 306908/2016–6), and Ederlan Magri thanks the Coordination for the Improvement of Personal Higher Education (CAPES) for a scholarship grant.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoenv.2020.111010.

Author statement

Ederlan Magri: Writing - original draft, Conceptualization, Methodology, Software, Formal analysis, Investigation. Eduardo Kieras Gugelmin: Conceptualization, Methodology, Investigation. Felipe Augusto Piacentini Grabarski: Writing - original draft, Formal analysis, Investigation. Julierme Zimmer Barbosa: Writing - review & editing, Supervision. André Carlos Auler: Writing - review & editing, Formal analysis, Investigation. Ivar Wendling: Writing - review & editing, Methodology. Stephen Arthur Prior: Writing - review & editing, Investigation. Alice Teresa Valduga: Writing - review & editing, Supervision. Antônio Carlos Vargas Motta: Writing - review & editing, Conceptualization, Investigation, Methodology, Supervision.

References

Althaus, D., Gianello, C., Tedesco, M.J., Silva, K.J.D., Bissani, C.A., Felisberto, R., 2018. Natural fertility and metals contents in soils of Rio Grande do Sul (Brazil). Rev. Bras. Ciência do Solo 42, 1–15.
Alvares, C.A., Stape, J.L., Sentelhas, P.C., Gonçalves, J.L.M., Sparovek, G., 2013. Köppen's climate classification map for Brazil. Meteorol. Z. 22 (6), 711–728.
Baker, A.J., Brooks, R., 1989. Terrestrial higher plants which hyperaccumulate metallic elements. A review of their distribution, ecology and phytochemistry. Biorecovery 1 (2), 81–126.
Bañados, M.P., Ibáñez, F., Toso, A.M., 2009. Manganese toxicity induces abnormal shoot growth in 'O'Neal' blueberry. Acta Hortic. 810, 509–512.
Barbosa, J.Z., Zambon, L.M., Motta, A.C.V., Wendling, I., 2015. Composition, hot-water solubility of elements and nutritional value of fruits and leaves of yerba mate. Cienc. E Agrotecnol 39 (6), 593–603.
Barbosa, J.Z., Motta, A.C.V., Consalter, R., Poggere, G.C., Santin, D., Wendling, I., 2018. Plant growth, nutrients and potentially toxic elements in leaves of yerba mate clones in response to phosphorus in acid soils. An. Acad. Bras. Ciênc. 90 (1), 557–571.
Barbosa, J.Z., Motta, A.C.V., Reis, A.R., Corrêa, R.S., Prior, S.A., 2020. Spatial distribution of structural elements in leaves of *Ilex paraguariensis*: physiological and ecological implications. Trees (Berl.) 34, 101–110.
Bergottini, V.M., Hervé, V., Sosa, D.A., Otegui, M.B., Zapata, P.D., Junier, P., 2017. Exploring the diversity of the root-associated microbiome of *Ilex paraguariensis* St. Hil. (Yerba mate). Appl. Soil Ecol. 109, 23–31.
Broadley, M., Brown, P., Cakmak, I., Rengel, Z., Zhao, F.-J., 2012. Function of nutrients: micronutrients. In: Marschner, P. (Ed.), Marschner's Mineral Nutrition of Higher Plants, third ed. Academic Press, London, pp. 191–248.
Caldeira, M.V.W., Watzlawick, L.F., Soares, R.V., Valério, A.F., 2006. Teores de Micronutrientes em espécies arbóreas da Floresta Ombrófila Mista Montana-General Carneiro/Pr. Ambiência 2 (1), 29–50.
Clairmont, K.B., Hagar, W.G., Davis, E.A., 1986. Manganese toxicity to chlorophyll synthesis in tobacco callus. Plant. Physl. 80 (1), 291–293.
Dane, J.H., Topp, C.G., Campbell, G.S., 2002. Particle size analysis. In: Methods of Soil Analysis. Part 4: Physical Methods, vol. 5. Soil Science Society of America Book Series, pp. 255–293.
Davis, J.G., 1996. Soil pH and magnesium effects on manganese toxicity in peanuts. J. Plant Nutr. 19 (3–4), 535–550.
Fecht-Christoffers, M.M., Maier, P., Iwasaki, K., Braun, H.P., Horst, W.J., 2007. The role of the leaf apoplast in manganese toxicity and tolerance in cowpea (*Vigna unguiculata* L. Walp). In: Sattelmacher, B., Horst, W.J. (Eds.), The Apoplast of Higher Plants: Compartment of Storage, Transport and Reactions. Springer, Dordrecht, pp. 307–321.
Fernando, D.R., Lynch, J.P., 2015. Manganese phytotoxicity: new light on an old problem. Ann. Bot. 16 (3), 313–319.
Huang, Y.L., Yang, S., Long, G.X., Zhao, Z.K., Li, X.F., Gu, M.H., 2016. Manganese toxicity in sugarcane plantlets grown on acidic soils of southern China. PloS One 11 (3), e0148956.
Junior, J.L., Moraes, M.F., Cabral, C.P., Malavolta, E., 2008. Influência genotípica na absorção e na toxidez de manganês em soja. Rev. Bras. Ciênc. Solo 32 (1), 173–181.
Kabata-Pendias, A., 2010. Trace Elements in Soils and Plants, fourth ed. CRC Press, Pulawy, Poland.
Leite, F.P., Novais, R.F., Silva, I.R., Barros, N.F., Neves, J.C.L., Medeiros, A.G.B., Ventrella, M.C., Villani, E.M.A., 2014. Manganese accumulation and its relation to eucalyptus shoot blight in the Vale do Rio Doce. Rev. Bras. Ciênc. Solo 38 (1), 193–204.
Marques, R., Motta, A.C.V., 2003. Análise química do solo para fins de fertilidade. In: Lima, M.R.de (Ed.), Manual de diagnóstico da fertilidade e manejo dos solos agrícolas, second ed. Departamento de Solos e Engenharia Agrícola, Curitiba, pp. 81–102.
Millaleo, R., Reyes-Diaz, M., Ivanov, A.G., Mora, M.L., Alberdi, M., 2010. Manganese as essential and toxic element for plants: transport, accumulation and resistance mechanisms. J. Soil Sci. Plant Nutr. 10 (4), 470–481.
Motta, A.C.V., Barbosa, J.Z., Magri, E., Pedreira, G.Q., Santin, D., Prior, S.A., Consalter, R., Young, S.D., Broadley, M.R., Benedetti, E.L., 2020. Elemental composition of yerba mate (*Ilex paraguariensis* A. St.-Hil.) under low input systems of southern Brazil. Sci. Total Environ. 736, 139637.
Oliva, E.V., Reissmann, C.B., Gaiad, S., Oliveira, E.B. de, Sturion, J.A., 2014. Composição nutricional de procedências e progênies de erva-mate (*Ilex paraguariensis* St. Hil.) cultivadas em latossolo vermelho distrófico. Ciência Florest. 24 (4), 793–805.
Poletto, I., Muniz, M.F.B., Ceconi, D.E., Blume, E., 2011. Influência da aplicação de NPK e calcário sobre o crescimento da erva-mate, severidade da podridão-de-raízes e população fúngica do solo. Ciência Florest. 21 (3), 429–444.
R Core Team, 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.
Reeves, R.D., Van Der Ent, A., Baker, A.J., 2018. Global distribution and ecology of hyperaccumulator plants. In: Van Der Ent, A., Echevarria, G., Baker, A.J.M., Morel, J. L. (Eds.), Agromining: Farming for Metals. Springer, pp. 75–92.
Reissmann, C.B., Carneiro, C., 2004. Crescimento e composição química de erva-mate (*Ilex paraguariensis* St. Hil.), transcorridos oito anos de calagem. Floresta 34 (3), 381–386.
Reissmann, C.B., Radomski, M.I., Quadros, R.M.B. de, 1999. Chemical composition of *Ilex paraguariensis* St. Hil. under different management conditions in seven localities of Paraná State. Braz. Arch. Biol. Technol. 42 (2), 187–194.
Sadana, U.S., Kusum, L., Claassen, N., 2002. Manganese efficiency of wheat cultivars as related to root growth and internal manganese requirement. J. Plant Nutr. 25, 2677–2688.
Santin, D., Benedetti, E.L., Kaseker, J.F., Bastos, M.C., Reissmann, C.B., Wendling, I., Barros, N.F.de, 2013. Nutrição e crescimento da erva-mate submetida à calagem. Ciência Florest. 23 (1), 55–66.
Schmidt, S.B., Jensen, P.E., Husted, S., 2016. Manganese deficiency in plants: the impact on photosystem II. Trends Plant Sci. 21 (7), 622–632.

Senger, C.C.D., Sanchez, L.M.B., Pires, M.B.G., Kaminski, J., 1997. Teores minerais em pastagens do Rio Grande do Sul. II. Sódio, enxofre, zinco, cobre, ferro e manganês. Pesqui. Agropecu. Bras. 32 (1), 101–108.
Shao, J.F., Yamaji, N., Shen, R.F., Ma, J.F., 2017. The key to Mn homeostasis in plants: regulation of Mn transporters. Trends Plant Sci. 22 (3), 215–224.
Silva, D.M. da, Fonte, N., dos, S., Souza, K.R.D. de, Rodrigues-Brandão, I., Libeck, I.T., Alves, J.D., 2017. Relationship between manganese toxicity and waterlogging tolerance in *Zea mays* L. cv. Saracura. Acta Sci. Agron. 39 (1), 75–82.
Silvana, V.M., Carlos, F.J., Lucía, A.C., Natalia, A., Marta, C., 2020. Colonization dynamics of arbuscular mycorrhizal fungi (AMF) in *Ilex paraguariensis* crops: Seasonality and influence of management practices. J. King Saud Univ. Sci. 32 (1), 183–188.
Siman, A., Cradock, F.W., Hudson, A.W., 1974. The development of manganese toxicity in pasture legumes under extreme climatic conditions. Plant Soil 41 (1), 129–140.
Subrahmanyam, D., Rathore, V.S., 2000. Influence of manganese toxicity on photosynthesis in ricebean (*Vigna umbellata*) seedlings. Photosynthetica 38 (3), 449–453.
Toppel, F.V., Junior, A.M., Motta, A.C.V., Frigo, C., Magri, E., Barbosa, J.Z., 2018. Soil chemical attributes and their influence on elemental composition of yerba mate leaves. Floresta 48 (3), 425–434.
Valduga, A.T., Gonçalves, I.L., Magri, E., Delalibera, F.J., 2019. Chemistry, pharmacology and new trends in traditional functional and medicinal beverages. Food Res. Int. 120, 478–503.
Van der Ent, A., Baker, A.J., Reeves, R.D., Pollard, A.J., Schat, H., 2013. Hyperaccumulators of metal and metalloid trace elements: facts and fiction. Plant Soil 362 (1–2), 319–334.
Van der Ent, A., Ocenar, A., Tisserand, R., Sugau, J.B., Echevarria, G., Erskine, P.D., 2019. Herbarium X-ray fluorescence screening for nickel, cobalt and manganese hyperaccumulator plants in the flora of Sabah (Malaysia, Borneo Island). J. Geochem. Explor. 202, 49–58.
Wendling, I., Santin, D., Nagaoka, R., Sturion, J.A., 2017. BRS BLD Aupaba e BRS BLD Yari: cultivares clonais de erva-mate para produção de massa foliar de sabor suave. Embrapa Florestas-Comunicado Técnico (INFOTECA-E).
Xue, S.G., Chen, Y.X., Reeves, R.D., Baker, A.J., Lin, Q., Fernando, D.R., 2004. Manganese uptake and accumulation by the hyperaccumulator plant *Phytolacca acinosa* Roxb.(Phytolaccaceae). Environ. Pollut. 131 (3), 393–399.
Zanão Júnior, L.A., Fontes, R.L.F., Neves, J.C.L., Korndörfer, G.H., Ávila, V.T.de, 2010. Rice grown in nutrient solution with doses of manganese and silicon. Rev. Bras. Ciênc. Solo 34 (5), 1629–1639.
Zengin, F., 2013. Biochemical and physiological effect of excess manganese (Mn) in bean (*Phaseolus vulgaris* L. cv. Strike). Proc. Natl. Acad. Sci. India B Biol. Sci. 83 (4), 651–657.
Zhao, H., Wu, L., Chai, T., Zhang, Y., Tan, J., Ma, S., 2012. The effects of copper, manganese and zinc on plant growth and elemental accumulation in the manganese-hyperaccumulator *Phytolacca americana*. J. Plant Physiol. 169 (13), 1243–1252.