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Accumulation capacity of nickel and zinc in yerba mate cultivated in soils with contrasting parent materials

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Abstract

Yerba mate (Ilex paraguariensis St. Hill.) has shown a relatively high capacity for metal absorption and accumulation under field conditions. To further evaluate the accumulation capacity of Ni and Zn, yerba mate clonal seedlings were grown in containers under five rates of Ni or Zn (0, 0.5, 2, 10, and 40 mg kg-1) with three soils originating from different parent material (basalt, rhyodacite, and sandstone). After 10 months, plants were harvested, divided into component parts (leaves, branches, and roots), and evaluated for 12 elements. Use of Zn and Ni enhanced seedling growth under rhyodacite- and sandstone-derived soils at the first application rate. Application of Zn and Ni resulted in linear increases based on Mehlich I extractions; recovery of Ni was smaller than Zn. Root Ni concentration increased from approximately 20 to 1000 mg kg-1 in rhyodacite-derived soil and from 20 to 400 mg kg-1 in basalt- and sandstone-derived soils; respective increases in leaf tissue were ~3 to 15 mg kg-1 and 3 to 10 mg kg-1. For Zn, maximum obtained values were close to 2000, 1000, and 800 mg kg-1 for roots, leaves, and branches for rhyodacite-derived soils, respectively. Corresponding values for basalt- and sandstone-derived soils were 500, 400, and 300 mg kg-1, respectively. Although yerba mate is not a hyperaccumulator, this species has a relatively high capacity to accumulate Ni and Zn in young tissue with the highest accumulation occurring in roots.

Introduction

Yerba mate (*Ilex paraguariensis* St. Hill.) is a species native to the austral region of South America that naturally occurs in Brazil, Argentina, and Paraguay. In 2020, Brazil produced 953,000 tonnes of yerba mate, followed by Argentina (812,000 tonnes), and Paraguay (150,000 tonnes) [1,2]. In South America, the annual average consumption per capita (kg) for major consumers are: Uruguay = 8.6; Argentina = 6.5; Paraguay = 2.5; and Brazil = 0.8 [3]. Yerba mate is exported for consumption to other countries such as Germany, the United States, Chile, France, Spain, Syria, and Bolivia [4]. The main use of yerba mate is for preparing hot infusion products ("*chimarrão*") using a composition of ~70% leaves and ~30% thin branches. In addition, yerba mate is utilized in other infusion products, energy drinks, cosmetics, and drugs [5, 6].

Regions where yerba mate occurs have a diversified geology, with portions occupied by eruptive rocks ranging from basic (basalt) to acidic (rhyodacite), and some areas with sedimentary rocks [7]. Studying yerba mate grown without chemical fertilization in the three states of southern Brazil, Motta et al. [8] found total concentrations of 40, 5, and 14 mg kg^{-1} for Ni and 128, 112, and 18 mg kg^{-1} for Zn on soils originating from basalt, rhyolite/rhyodacite, and sedimentary (shale, claystone, and sandstone) parent materials, respectively. Magri et al. [9] found pseudo-total concentrations in soils originating from basalt of 25 and 24 mg kg^{-1} for Ni, and 66 and 103 mg kg^{-1} for Zn in Argentina and Paraguay, respectively. These findings suggest that yerba mate can grow within a large spectrum of Zn and Ni variation.

High soil acidity favors availability of toxic Al and cationic micronutrients [10]. This fact is relevant to yerba mate since most soils in regions of occurrence in Brazil, Argentina, and Paraguay are acidic and have high Al [9]. Aluminum toxicity has not been a limiting factor for growth of yerba mate since most plantations do not receive lime to correct soil acidity [11]. Furthermore, there are indications of greater growth of root systems when Al is used in nutrient solutions [12]. However, the combination of abiotic factors (e.g., high acidity and high Mn concentrations in some soils) with genetic factors of this species, suggests that yerba mate is a species with a high capacity for accumulating metals. In this regard, foliar Mn levels above 10,000 mg kg^{-1} were recently

recorded in yerba mate plants under field conditions [8] and in mineral-enriched soil without decreased growth [13].

These combinations of factors may also be responsible for high concentrations of Ni and Zn micronutrients reported in yerba mate leaves from both fertilized and unfertilized areas [14, 15]. However, wide variations in foliar micronutrient concentrations have been recorded for yerba mate. Assessments of yerba mate samples from commercial products sold in South American countries found 2.7 mg kg^{-1} for Ni and 79.4 mg kg^{-1} for Zn (Argentina), 2.4 mg kg^{-1} for Ni and 44.2 mg kg^{-1} for Zn (Brazil), 3.2 mg kg^{-1} for Ni and 57.1 mg kg^{-1} for Zn (Uruguay), and 2.8 mg kg^{-1} for Ni and 77.3 mg kg^{-1} for Zn (Paraguay) [16]. Leaf levels of Ni and Zn in yerba mate can also exhibit high variation within the same country. For example, an assessment of southern states in Brazil (*Rio Grande do Sul*, *Santa Catarina,* and *Paraná* states) noted that levels ranged between 0.40 and 6.7 mg kg^{-1} for Ni and 14 to 179 mg kg^{-1} for Zn in leaves [8]; this variation pattern was also observed in commercial products with levels ranging between 2.2 to 3 mg kg^{-1} for Ni and 54.5 to 67.6 mg kg^{-1} for Zn [17]. However, high levels and variations in Ni and Zn are not restricted to yerba mate leaves for this can occur in other plant tissues. Frigo et al. [18] reported mean Ni concentrations of 0.9, 4.2, and 2.5 mg kg^{-1} and Zn concentrations of 81.2, 44.3, and 101.1 mg kg^{-1} for branches, new leaves, and old leaves, respectively.

Variations observed at both macro- and microscales may be associated with differences in soils (weathering and parent material), genetic material, and management. The influence of soil parent material in southern Brazil was reported by Motta et al. [8]; lower foliar Ni was found for yerba mate grown on soils originating from rhyodacite/rhyolite compared to basalt and sedimentary derived soils, but this was not true for Zn. Magri et al. [9] also reported large variations in foliar concentrations of Zn (17 – 191 mg kg^{-1}) and Ni (1.4 – 5.1 mg kg^{-1}), with the highest concentrations being associated with plants grown on soils originating from basalt parent material. However, the influence of genetic variation on Ni and Zn foliar concentrations is difficult to quantify since the vast majority of plants used today were planted from seeds of native and unselected matrices. Additionally, most yerba mate plants investigated in Brazil were selected from spontaneously regenerated plants found under canopies of native Araucarian forests [19].

Differences in mobility and redistribution of elements within plants can also influence variations in element concentrations among plant tissue types. In general, elements such as Cu, Ni, Zn, Fe, and others have higher concentrations in root tissue compared to those found in shoot tissue [10]. Reis et al. [20] suggested that about 50 to 80% of absorbed Ni stays in the root system. Concentrations of 1.26 (mature leaves), 2.59 (stems), and 54.75 mg kg^{-1} (feeder roots) found in *Camellia sinensis* also illustrates that more accumulation occurs in root tissue [21].

The wide variation in Ni and Zn concentrations noted in yerba mate, as well as the high values observed in processed yerba mate or in leaf and branch tissues collected in the field, may be associated with geological/pedological differences, genetic variation, management, and the wide capacity for nutrient acquisition that is difficult to individualize at the field level. Thus, the objective of this study was to evaluate the ability of uniform yerba mate (clones) to accumulate Ni and Zn in leaves, branches, and roots when grown in containers using soils originating from the most common parent materials of southern Brazil.

Materials and methods

Experimental conditions

Experiments were conducted at a nursery of the Federal University of Paraná – UFPR, in the city of *Curitiba* (*Paraná* state). At this nursery, plants were grown under Araucaria tree canopy cover (*Araucaria angustifolia*) to mimic natural native growth conditions. Two experiments were simultaneously conducted (respectively for Ni and Zn evaluation). These consisted of five application rates of Ni or Zn (0, 0.5, 2, 10, and 40 mg kg^{-1}) and three soils from different parent materials (basalt, rhyodacite, and sandstone). Soils originating from basalt (27°33'50.57" S and 52°24'4.01" W) and rhyodacite (28°54'50.89" S and 52° 7'52.38" W) were collected from the municipalities of *Barão de Cotegipe* and *Ilópolis*, *Rio Grande do Sul* state of Brazil, respectively. Soil originating from sandstone was collected from the municipality of *São João de Triunfo* (25°40'45.05" S, 50°18'36.04" W), *Paraná* state, Brazil. The collection sites had no history of herbicide, fertilizer, or lime usage. After removal of the surface litter layer, soils were collected to a depth of 20 cm.

Samples of each soil were air-dried and passed through a 2 mm sieve for chemical and granulometric analysis. Soil analysis results are shown in Table 1. Determined soil chemical attributes were: pH (0.01 mol L^{-1} $CaCl_2$), potential acidity (H+Al), exchangeable acidity (Al^{3+}, extracted by 1 mol L^{-1} KCl; quantification by titration), exchangeable Ca^{2+} and Mg^{2+} (extracted by 1 mol L^{-1} KCl; determined by atomic absorption spectrophotometry), K^+ (extracted with Mehlich-I; determined by flame photometry), available P, Ni, and Zn (extracted by Mehlich-I; P determined by the molybdenum blue colorimetry method; Ni and Zn determined by ICP-OES), and organic C (extracted by sodium dichromate; determined by colorimetry). Chemical determinations followed methodologies described by Marques and Motta [22], while soil granulometry was determined by the Bouyoucos hydrometer method [23].

Table 1 – Chemical and textural properties of basalt, sandstone, and rhyodacite derived soils.

Soil	pH $CaCl_2$	Al	Ca	Mg	K	P	Ni	Zn	C	Clay	Silt	Coarse sand	Fine sand
	---- $cmol_c$ dm^{-3} ----					mg dm^{-3}	mg kg^{-1}		g dm^{-3}	------- g kg^{-1} -------			
Basalt	3.9	0.7	3.0	1.0	0.1	8.8	0.3	2.7	26.0	713	225	48	14
Sandstone	3.9	1.5	2.2	0.3	0.3	14.7	0.3	1.1	33.0	200	263	287	250
Rhyodacite	3.5	3.7	1.1	0.2	0.1	22.6	0.2	1.0	38.3	213	588	52	147

Experiment implementation

Three-month-old clonal yerba mate seedlings (cultivar BRS BDL Yari – [24]) grown in a commercial substrate (100 cm^3 tubes filled with a mixture of rice husks, vermiculite, and peat) were treated with nickel sulfate ($NiSO_4.6H_2O$ P.A.) and zinc sulfate ($ZnSO_4.H_2O$ P.A.) as sources of Ni and Zn, respectively. These sources were diluted in 200 mL of water to achieve the desired application concentrations. Immediately after Zn and Ni applications, seedlings (~25 cm in size) were transplanted into 5 L pots. Each experimental unit consisted of a pot (5 L), containing either basalt, rhyodacite, or sandstone derived soil, and a clonal yerba mate seedling that had been treated with the proper rate of either Ni or Zn. There were five treatments applied to the three soils of

distinct materials of origin for Ni and for Zn, and for each treatment four (4) repetitions were performed, totalling 120 experimental units.

Throughout the study, plants were irrigated as needed. Two applications of nitrogen were carried out at 152 and 209 days after transplanting. These applications consisted of 50 mg kg^{-1} of urea (diluted in 100 mL of water), which corresponded to a total of 45 mg kg^{-1} of nitrogen. Total experiment duration was 306 days (15 May 2020 to 17 March 2021).

Plant tissue collection and analysis

At study termination, plants were separated into old leaves, branches (up to 8 cm in diameter; commercial standard), and roots. Since there were only four experimental units that had plants with approximately two new leaves, we only used old leaves for analysis. Samples were rinsed in running water and washed with reverse osmosis water. Old leaves, branches, and roots samples were placed in separate kraft paper bags and dried in a forced-air oven (65º C) until constant mass prior to dry matter measurements. Dry samples were ground to pass through a 1 mm sieve.

For digestion of plant tissue, 1g of sample was weighed into porcelain crucibles and ashed (500° C for 3 hours). Ash was solubilized in 3 mol L^{-1} hydrochloric acid while heated on a hot plate (70ºC) for 20 minutes. Extracts were filtered (blue stripe filter paper; 5-8 micrometers) and brought to 100 mL with reverse osmosis water. Quantification of Al, Ba, Ca, Cu, Cr, Fe, K, Mg, Mn, Ni, P, and Zn was performed via Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES, Varian 720-ES). Chemical determinations followed modified methodologies of Martins and Reissmann [25]. Operational conditions of the ICP-OES with an axial configuration were: radio frequency power = 1200 W; replicate = 2; plasma gas flow rate = 15 L min^{-1}; auxiliary gas flow rate = 1.5 L min^{-1}; sample uptake rate = 1.0 mL min^{-1}; nebulizer gas flow rate = 0.5 L min^{-1}; nebulizer type = seaspray; spray chamber = time cyclonic; quartz torch, signal integration = 15 s. Wavelength settings were: Al 396.152 nm, Ba 233.527 nm, Ca 317.933 nm, Cu 327.395 nm, Cr 267.716 nm, Fe 238.204 nm, K 766.491 nm, Mg 280.270 nm, Mn 257.610 nm, Ni 231.604 nm, P 81.604 nm, and Zn 213.857 nm.

Statistical analysis

Statistical analysis was performed using R software [26]. Analysis of variance (ANOVA) for normality and homogeneous data (checked by Shapiro-Wilk and Bartlett testes, respectively) were performed to validate the difference induced by treatments on Ni and Zn concentrations in soil and plant tissue. After validation, regression analyses were applied to determine the response of variables to supply of Ni or Zn. Leaf, branch, and root dry matter were converted to relative yield based on the control (zero dose rate) corresponding to 100% yield. In addition, Pearson correlations of leaf elemental composition were performed for each evaluated soil, and Zn and Ni translocation indices (TI) were determined [27] based on elemental concentrations in shoots (leaves, branches, and leaves + branches) and roots (mg kg^{-1}).

Results

Soil availability of Ni and Zn

Results indicated a linear increase in Ni and Zn availability in the three tested soils. Comparatively, increases were greater for Zn in relation to Ni, indicating a lower degree of Zn adsorption (Figure 1). Regarding soil parent material, higher Ni availability was observed in soil originating from sandstone with increases of 0.56 mg kg^{-1}, compared to 0.37 (basalt) and 0.39 mg kg^{-1} (rhyodacite) for each 1 mg kg^{-1} of Ni applied (Figure 1). Zinc recovery was also higher in soil originating from sandstone (linear coefficient of 0.64); this was close to soils originating from basalt (0.60) and rhyodacite (0.49).

Growth with Ni application

Nickel application increased leaf and branch dry matter of plants cultivated in soils originating from rhyodacite and sandstone (Figure 2A and B). Increases in rhyodacite derived soil was more prominent at rates of 0.5 and 2 mg kg^{-1} for leaves and branches, respectively. The relative production of leaves and branches was also higher than the control at rates of 2 and 10 mg kg^{-1}. In sandstone derived soil, leaf increases were restricted to lower rates (0.5 and 2 mg kg^{-1}), while branch increases were observed at intermediate rates (2 and 10 mg kg^{-1}). For plants grown in basalt derived soil, relative production of leaves and branches was lower than the control at all Ni rates.

Unlike observations for yerba mate leaves and branches, Ni use compromised root growth even at the lowest application rate (Figure 2C). Although the supply of Ni impaired relative plant growth at some rates, even the highest rate caused no observable foliar symptoms indicative of plant toxicity.

Elemental composition with Ni application

Leaf Ni concentration in the control treatment was ~2 mg kg^{-1} and reached 17.9, 11.7, and 8.8 mg kg^{-1} in plants grown in soils originating from rhyodacite, sandstone, and basalt, respectively (Figure 3A). Despite the high rate (40 mg kg^{-1}), this was not sufficient to attain maximum potential Ni accumulation in plants grown in rhyodacite and sandstone derived soils. For plants grown in basalt derived soil, the quadratic equation indicated that the highest foliar Ni concentration occurred near the 25 mg kg^{-1} rate. For branches, the highest Ni concentrations of 7.6, 5.6, and 5.2 mg kg^{-1} were for plants grown in soils originating from rhyodacite, basalt, and sandstone, respectively (Figure 3B).

Roots showed the greatest response to Ni supply (Figure 3C), with the largest change in Ni concentration (from 15 mg kg^{-1} to 1036 mg kg^{-1}) occurring in the rhyodacite derived soil. Lower variations in Ni root concentrations were observed with soil originating from basalt and sandstone, which changed from 26 and 22 mg kg^{-1} to 564 and 568 mg kg^{-1}, respectively (Figure 3C). Nickel TI was low for all soils, reaching a maximum value of 15% (0.5 mg kg^{-1} rate in basalt derived soil). In addition, TI decreased with Ni application, and there was more significant translocation to leaves compared to branches for all rates (Figure 4).

Nickel concentration in leaves and branches were lower in plants grown in soil originating from sandstone, despite the higher Ni availability of this soil (Figure 1). The maximum increases in Ni concentration for roots, leaves, and branches were observed in rhyodacite derived soil; values were 41, 10, and 7-fold higher than the control. On the other hand, corresponding values were 21, 5, and 4 times higher and 22, 5, and 5 times higher for plants grown in basalt and sandstone derived soils, respectively.

Aluminum, Fe, Cu, and Cr generally accumulated in roots, while Mn concentration was higher in leaves (Table S1). The use of Ni resulted in an antagonistic interaction with Mn, Zn, and Cu in plants grown in soil originating from basalt (Figure 5A). For plants grown in rhyodacite derived soil, antagonistic interaction occurred for practically all elements (Figure 5C), while antagonism was not observed plants grown in soil originating from sandstone (Figure 5B).

Growth with Zn application

Application of Zn increased leaf, branch and root dry matter of plants grown in all evaluated soils (Figure 6). Figure 6A shows that leaf increases occurred at the 2 mg kg^{-1} rate (rhyodacite), the 0.5 and 10 mg kg^{-1} rates (sandstone), and the 0.5 and 2 mg kg^{-1} rates (basalt). For branches, increased dry matter occurred at all rates for plants cultivated in rhyodacite derived soil and at the 2 and 40 mg kg^{-1} rates for basalt derived soil (Figure 6 B). Root dry matter increased at the 2, 10, and 40 mg kg^{-1} rates for rhyodacite derived soil, at the 0.5, 10, and 40 mg kg^{-1}rates for sandstone derived soils, and at all rates for basalt derived soil (Figure 6C). Similar to Ni, plant leaves did not display any visual symptoms of Zn toxicity.

Elemental composition with Zn application

Similar to Ni observations, the highest concentrations of Zn were observed in roots, followed by leaves and branches. However, concentration differences among tissues were much smaller for Zn, indicating wider distribution in plants (Figure 7). On the other hand, there was a great influence of soil parent materials. For plants not receiving Zn (i.e., control), the concentration of this element in the leaves was 24.9, 108.3, and 91.6 mg kg^{-1} for plants grown in soils originating from sandstone, basalt, and rhyodacite, respectively. Leaf Zn concentrations attained maximum valuea of 1127, 674, and 539 mg kg^{-1} for plants grown in rhyodacite, basalt, and sandstone derived soils, respectively (Figure 7A). Corresponding values were 801, 371, and 366 mg kg^{-1} for branches (Figure 7B) and 2230, 809, and 858 mg kg^{-1} for roots (Figure 7C).

Zinc concentration in roots and leaves of plants grown in rhyodacite and basalt derived soils displayed stabilization above the 10 mg kg^{-1} rate. On the other hand, plants grown in sandstone derived soil displayed a linear response, indicating that maximum rate of 40 mg kg^{-1} was not sufficient to achieve maximum Zn accumulation in roots and leaves. Zinc TI was higher than for Ni, reaching 65% (1.0 mg kg^{-1} rate in basalt derived soil); unlike Ni observations, application of Zn resulted in small or no reduction in TI (Figure 8). In rhyodacite and sandstone derived soils, there was more significant Zn translocation to branches compared to leaves at lower rates.

Similar to Ni observations, there was an accumulation of Al, Cu, Cr, and Fe in roots and Mn in leaves (Table S3). The use of Zn showed an antagonistic effect with Mg and Mn in plants grown in basalt derived soil (Figure 9A), while antagonism occurred with Al, Cr, Cu, Ni, and Mg for plants grown in sandstone derived soil (Figure 9B) and with Mg, Mn, and P for plants grown in rhyodacite derived soil (Figure 9C).

Discussion

Effect of Ni application

The positive effects of Ni on yerba mate leaf and branch production has been observed in other crops such as pecan and soybean [28, 29]. Nickel participates in the structural composition of the urease enzyme [30], which contributes to better N use and consequent production of more shoot dry matter, and plays a physiological role in herbivory defense [31] and stress tolerance modulation [32]. General recommendations for Ni fertilization are still in formative stages since there are few studies investigating this requirement. Some studies have reported positive responses to Ni application in crops such as soybean, bean, pecan, and coffee [28, 29, 33, 34]. Similarly, our study indicated positive initial growth responses to Ni supply for yerba mate grown in rhyodacite and sandstone derived soils, primarily at rates between 0.5 and 10 mg kg^{-1} (Figure 2).

Root Ni concentration increased on the order of 20 to 40 times at the maximum application rate, while this increase was 4 to 8 times in shoot tissues as reflected in low TI (Figure 3C; Figure 4). According to Reis et al. [20], ~50 to 80% of Ni absorbed by plants can be retained in root systems. Accumulation of heavy metals in roots and low translocation to aerial parts are considered mechanisms by which root systems can contribute to heavy metal tolerance in tree species [35, 36]. Although high, Ni values in roots (564 to 1036 mg kg^{-1}; Figure 3C) were below the variation range of 1500 to 2700 mg kg^{-1} reported for white clover, ryegrass, cabbage, and corn [37]. Higher Ni accumulation in roots (compared to shoots) has also been observed in *Camellia sinensis* [21]. Unlike yerba mate, branches of *C. sinensis* showed greater increases than leaves.

Foliar Ni concentrations under natural soil conditions (control) were close to 2 mg kg^{-1} (Figure 3A), which can be considered relatively high since Kabata-Pendias [10] suggested that Ni concentration in plant tissues is generally less than 1 mg kg^{-1}. However, this value is within the 0.05 - 5 mg kg^{-1} range indicated for plants grown in uncontaminated soils [38, 39]. Values for treatments without Ni addition were within the range of 0.4 to 6.7 mg kg^{-1} observed by Motta et al. [8], and close to ranges (1.4 - 5.1 mg kg^{-1} and 1.01 - 2.15 mg kg^{-1}) reported by Magri et al. [9] and Barbosa et al. [40], respectively. Since Ni was not applied in these cited studies, values observed were directly associated with yerba mate grown under natural soil conditions. Although soil originating from sandstone had the highest Ni content, our hypothesis that this soil would have more plant available Ni was declined. Soil originating from rhyodacite provided higher Ni availability based on higher levels in plants. High Ni values have been reported in several surveys for *C. sinensis* tea leaves in China: concentration ranges of 4 - 12 mg kg^{-1} (old leaves) and 8 - 12 mg kg^{-1} (new leaves) [41]; 3.00 to 7.57 mg kg^{-1} (old leaves) and 4.84 - 14.4 mg kg^{-1} (new leaves) [42]; and average value of 14.03 mg kg^{-1} with minimum of 4.26 mg kg^{-1} and maximum of 24.08 mg kg^{-1} [43]. The high levels in both yerba mate and tea can be related to acidic soil conditions in these systems.

In the present study, foliar Ni concentrations much lower (8.8, 11.7, and 17.9 mg kg^{-1}; Figure 3A) than root values confirms low Ni translocation to shoots. Such values can cause a decrease in plant development [44, 45] since foliar levels of 10 - 50 mg kg^{-1} are considered toxic in non-hyperaccumulating species [39]. Decreased aboveground productivity in plants grown in sandstone and rhyodacite derived soils at the highest Ni rate supports this possibility (Figure 2). Reported values were very far from the 1000 mg kg^{-1} level that can be attained by Ni hyperaccumulating species; hyperaccumulators found in Niquelândia, Goiás state, Brazil had foliar concentrations upwards to 10,610 mg kg^{-1} [46]. Yang et al. [37] suggested that resistance to Ni toxicity is related to low influx into roots and low transport from roots to shoots. Therefore, high concentrations observed in yerba mate roots (Figure 3C) indicate probable resistance to transport only. Nickel (Ni) concentration in commercial yerba mate products (including leaves and branches) from South American countries varies between 2.2 and 3 mg kg^{-1} [17], which was close to control observations in this study (Figure 3). Commercial *C. sinensis* products

have higher values (6.27 to 14.5 mg kg^{-1}) as reported by Ma et al. [47]. Other observations by Zhong [48], Marcos [49], Nookabkaew [50], and Brzezich-Cirocka [51] included means and ranges (in mg kg^{-1}) of 7.55 (2.7 to 13.41), 7.27 (2.99 to 22.56), 5.63 (2.28 to 9.19) and 5.2 to 8.2, respectively. Szymczycha-Madeja et al. [52] indicated that *C. sinensis* tea products are considered Ni-rich with values ranging from 3.79 to 6.91 mg kg^{-1}. Again, both yerba mate and *C. sinensis* plants are preferentially grown in acidic soils and likely share similar soil exudation capacities. For plants grown in soils derived from rhyodacite and sandstone (in some cases), there is no explanation why shoots continue to grow at low Ni rates when root growth has been compromised. Furthermore, it is also difficult to understand why plants grown in basalt derived soil displayed significant growth decreases but contained lower Ni levels.

The lowest Ni concentrations were found in branches with and without Ni application (Figure 3B); this supports observation of Frigo et al. [18] for yerba mate plantations on soils derivied from rhyodacite and basalt. However, similar increases observed between branches and leaves at the maximum rate (on the order of 5 times) suggest uniform enrichment (Figure 3). That is, levels in leaf and branch tissues will increase proportionally when Ni is applied. Since branches are part of commercial yerba mate products, their inclusion can benefit the product by reducing Ni concentration; this would be dependent on the leaf to branch ratio of the resulting mixture. According to Reis et al. [20], Ni is located in the vascular cylinder of roots and is associated with chelators in vacuoles, cell walls, trichomes, and epidermis in stems and leaves.

Nickel use resulted in antagonistic interactions with Mn, Zn, and Cu in plants grown in soil originating from basalt, Mn in soil originating from sandstone, and several other elements in soil originating from rhyodacite (Figure 5). Antagonistic interactions between Ni and divalent cations (Fe^{2+}, Zn^{2+}, Cu^{2+}, Mn^{2+}) were expected since they compete for the same ion channel for entry into root systems [20]. However, site competition from Fe, Zn, and Cu may be greater since they have greater chemical similarities (valence and ionic radius) with Ni [53]. Antagonistic interactions confirmed by Palacios et al.[54] indicated a greater interaction intensity between Ni and Mn. Roveda et al. [55] reported that an antagonistic interaction did not produce visual leaf symptoms attributable to Fe deficiencies in strawberry.

Effect of Zn application

At most Zn rates, yerba mate did not present visual leaf toxicity, but rather displayed healthy leaves and positive growth aspects (Figure 6). Although roots attained greater accumulations of Zn than shoots, this accumulation is very relative to the plant species, but in a much lower proportion than Ni. According to Kabata-Pendias [10], higher Zn levels are often found in roots, especially if plants are grown in Zn-rich soils. Silva et al. [50] reported values between 589 and 2866 mg kg^{-1} in root tissue of four *Eucalyptus* species; these values were ~2 to 15 times higher than that observed in shoots. In *Crambe abyssinica*, Tito et al. [57] reported that Zn concentrations were higher in shoots (from 36.3 to 419.8 mg kg^{-1}) compared to roots (45.4 to 330.6 mg kg^{-1}). In 45 *C. sinensis* plantations in China, Li et al. [42] found higher mean Zn values in roots (44.0 mg kg^{-1}) compared to mature leaves (13.6 mg kg^{-1}).

Foliar Zn concentrations in the control were close to 100 mg kg^{-1} for plants grown in soils derived from basalt and rhyodacite and 25 mg kg^{-1} for those grown in sandstone derived soil. This great difference could be explained by the natural presence of Zn in the soil parent material since Zn is more abundant in igneous versus sandstone materials [58]. Furthermore, values obtained for plants grown in soils derived from basalt and

rhyodacite without Zn addition were at the limit of what is generally considered toxic to plants, which is between 100 and 500 mg kg^{-1} [10].

Maximum foliar Zn concentrations were 1127, 674, and 539 mg kg^{-1} for plants grown in soils originating from basalt, rhyodicite, and sandstone, respectively. These values were several times higher than the maximum levels found in the literature: 192, 88, 108, 191, and 314.8 mg kg^{-1} reported by Motta et al. [8], Magri [13], Toppel et al. [15], Frigo et al. [18] and Reissmann et al. [59], respectively. This finding is very significant since Zn values in yerba mate are already naturally high and no Zn toxicity symptoms were observed in our or the above-mentioned studies. Therefore, the 100 - 500 mg kg^{-1} toxicity range suggested by Kabata-Pendias [10] may not be true for yerba mate. Our values were above the 79.4 and 80 mg kg^{-1} found in commercial yerba mate products (including leaves and branches) reported by Pozebon et al. [16] and Giulian et al. [60], respectively.

Similarities between yerba-mate and *C. sinensis* observed for Ni were not noted for Zn. Values of Zn were higher in yerba mate compared to *C. sinensis*. Ma et al. [47] reported average values of 33.8 and 52.7 mg kg^{-1} for Puerch and Black teas, respectively. In China, mature leaves of *C. sinensis* had average, minimum, and maximum Zn values of 52.4, 31.68, and 7515 mg kg^{-1}, respectively [43]. Also, Li et al. [42] found mean values of 13.6 and 45.0 mg kg^{-1} for young and old *C. sinensis* leaves, respectively.

Unlike Ni, branches had a greater response to Zn application with values close to those observed in leaves; this is important since thin branches are also used in commercial yerba mate products. Zinc can have high solubility (45 to 80 %) in commercial yerba mate products with consumption of *chimarrão* contributing up to 10 % of the recommended dietary allowance [17]. Since Zn plays an important role in human nutrition, the high concentration in branches increases amounts of Zn in commercial yerba mate products and consequent ingestion of this element by consumers via infusion drinks. Corroborating our results, Frigo et al. [18] found higher mean Zn values in branches than in mature leaves of plants grown in soil derived from basalt, while the opposite was true for plants grown in soil derived from rhyodacite.

Zinc showed an antagonistic relationship with Mn in plants grown in soil derived from basalt (Figure 9). For plants grown in soil derived from sandstone, Zn showed an antagonistic relationship with Cr, Ni, Mg, and Cu. For soil derived from rhyodacite, this relationship was observed with Mg and Mn. Corroborating our results, Kabata-Pendias [10] indicated that Zn addition decreased absorption of most nutrients and potentially toxic elements such as Cd, Cu, Fe, As, P, Ca, and Mg. This antagonistic relationship among elements probably occurred due to competition for the same absorption sites.

Conclusion

Sandstone and rhyodacite derived soils were not able to supply sufficient Zn and Ni to seedlings, which confirms a general lack of Zn in many Brazilian soils. However, the response to Ni addition was unusual and requires more research to confirm actual responses under field conditions. Yerba mate displayed a high potential for accumulating Zn and Ni in plant tissues, which supports previous field observations. In leaf tissue, Zn and Ni reached values as high as 1000 and 15 mg kg^{-1}, respectively. Unlike Ni, Zn showed small differences between root, branch, and leaf concentrations, which suggests better transport to leaves and redistribution to shoot tissues. This is important since fine branches are used in commercial beverage products. High Ni values in the roots compromised their growth. Clearly, yerba mate has a relatively high capacity to accumulate Ni and Zn in

young tissue with the highest accumulation occurring in roots. Although yerba mate is not a hyperaccumulator, additional research is needed to determine how Ni and Zn applications affect the elemental composition of commercial yerba mate products and consequent impacts to human nutrition and health.

Declarations

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Competing Interests

The authors have no relevant financial.

Author contributions

Nayara Caroline Majewski Ulbrich: Writing - Original Draft, Conceptualization, Methodology, Investigation, Software, Formal analysis. Antônio Carlos Vargas Motta: Writing - Review & Editing, Conceptualization, Methodology, Investigation. Ederlan Magri: Writing - Review & Editing, Conceptualization, Investigation, Software, Formal analysis. Stephen A. Prior: Writing - Review & Editing, Investigation. Carla Gomes de Albuquerque - Review & Editing, Methodology, Investigation. Fabiana Gavelaki - Review & Editing, Methodology, Investigation. Julierme Zimmer Barbosa - Review & Editing, Investigation. Ivar Wendling - Review & Editing, Investigation. Giovana Clarice Poggere - Review & Editing, Conceptualization, Methodology, Investigation.

Data Availability

All data generated or analysed during this study are included in this published article [and its supplementary information files].

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent to publish

Not applicable.

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Figures

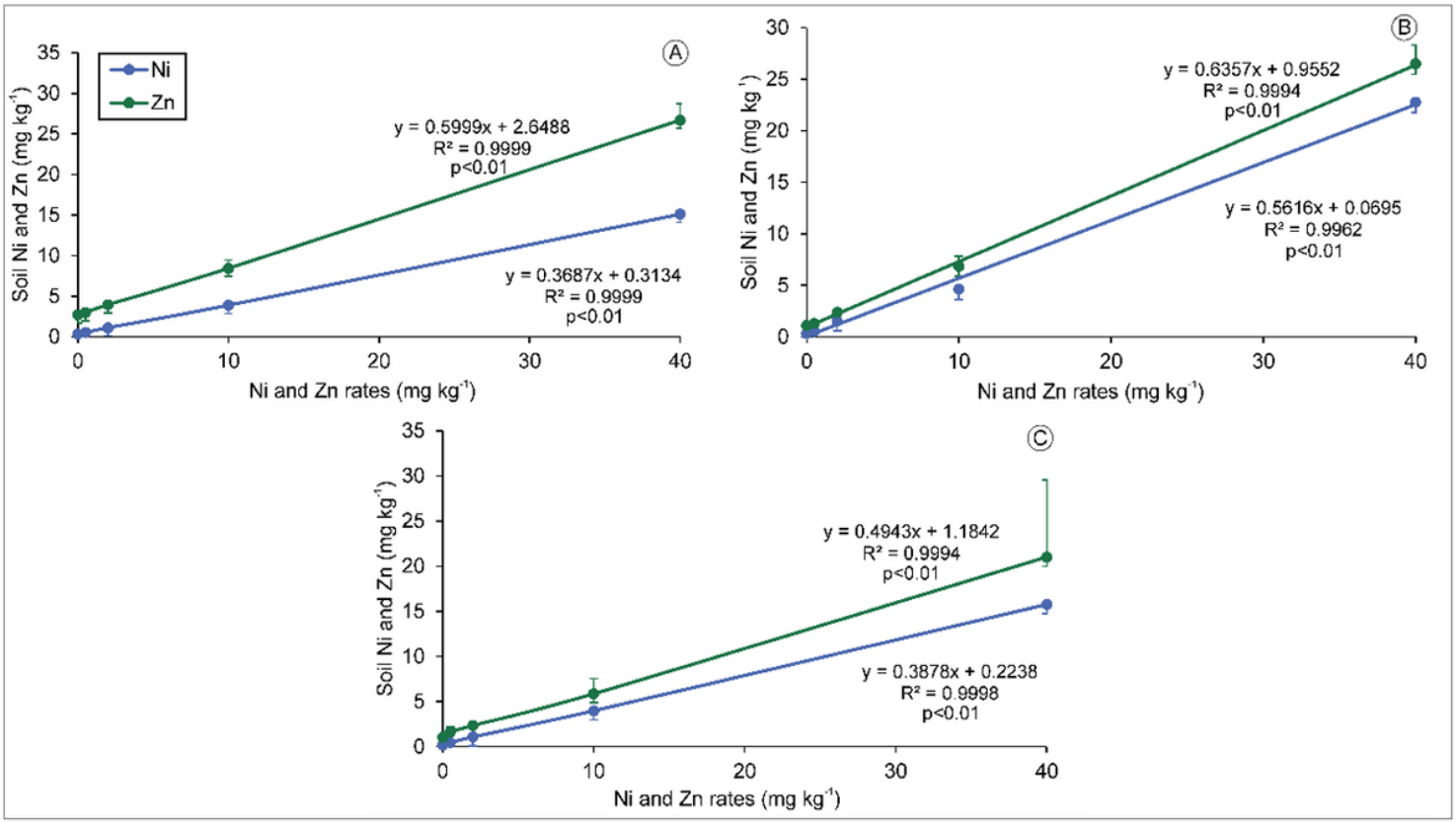


Figure 1

Nickel (Ni) and Zn concentrations (Mehlich-I extraction) in soils originating from basalt (A), sandstone (B), and rhyodacite (C) in response to Ni and Zn application (0, 0.5, 2, 10, 40 mg kg^{-1}). Regression equations, means, and standard deviations are shown.

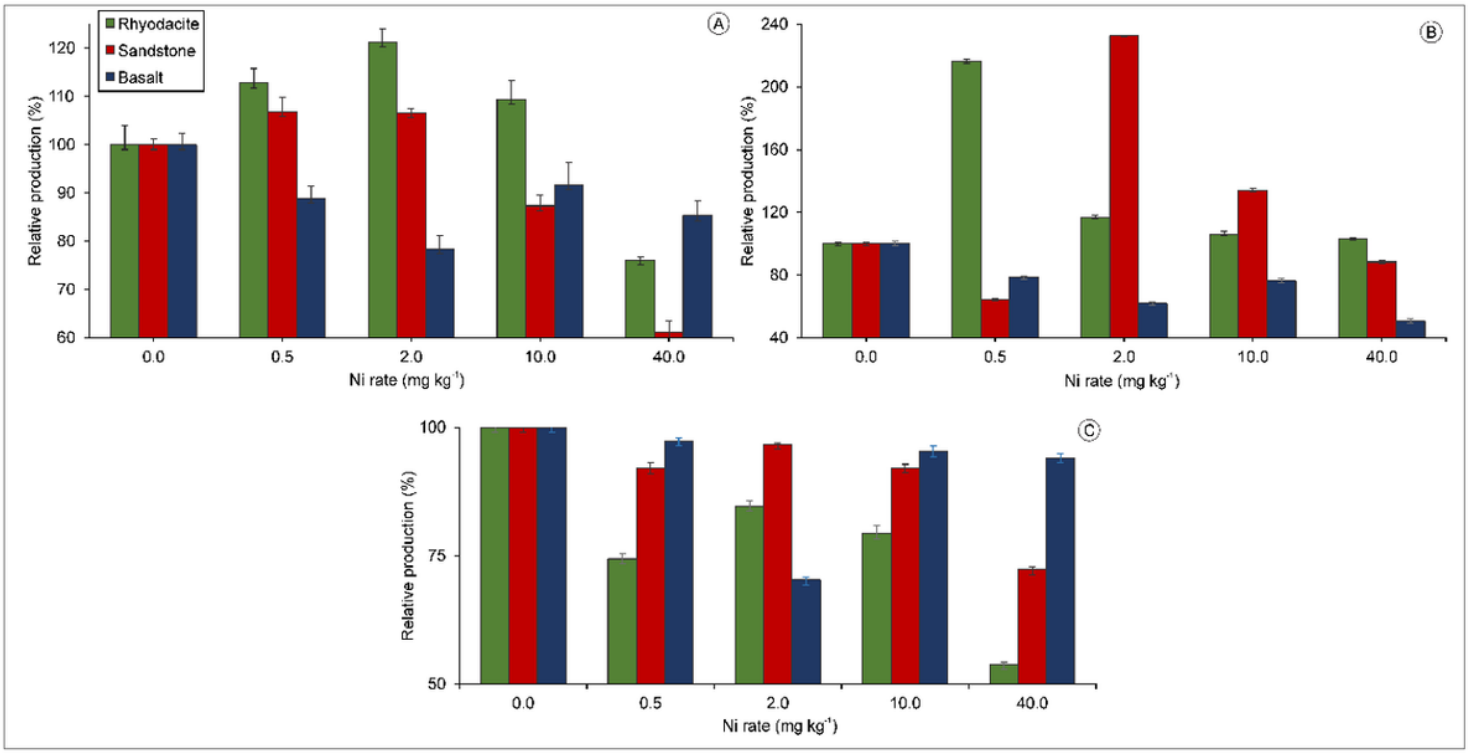


Figure 2

Relative dry matter production of leaves (A), branches (B), and roots (C) of yerba mate (*Ilex paraguariensis*) clones grown in soil originating from basalt, sandstone, and rhyodacite in response to Ni application (0, 0.5, 2, 10, 40 mg kg^{-1}). Means and standard deviations are shown.

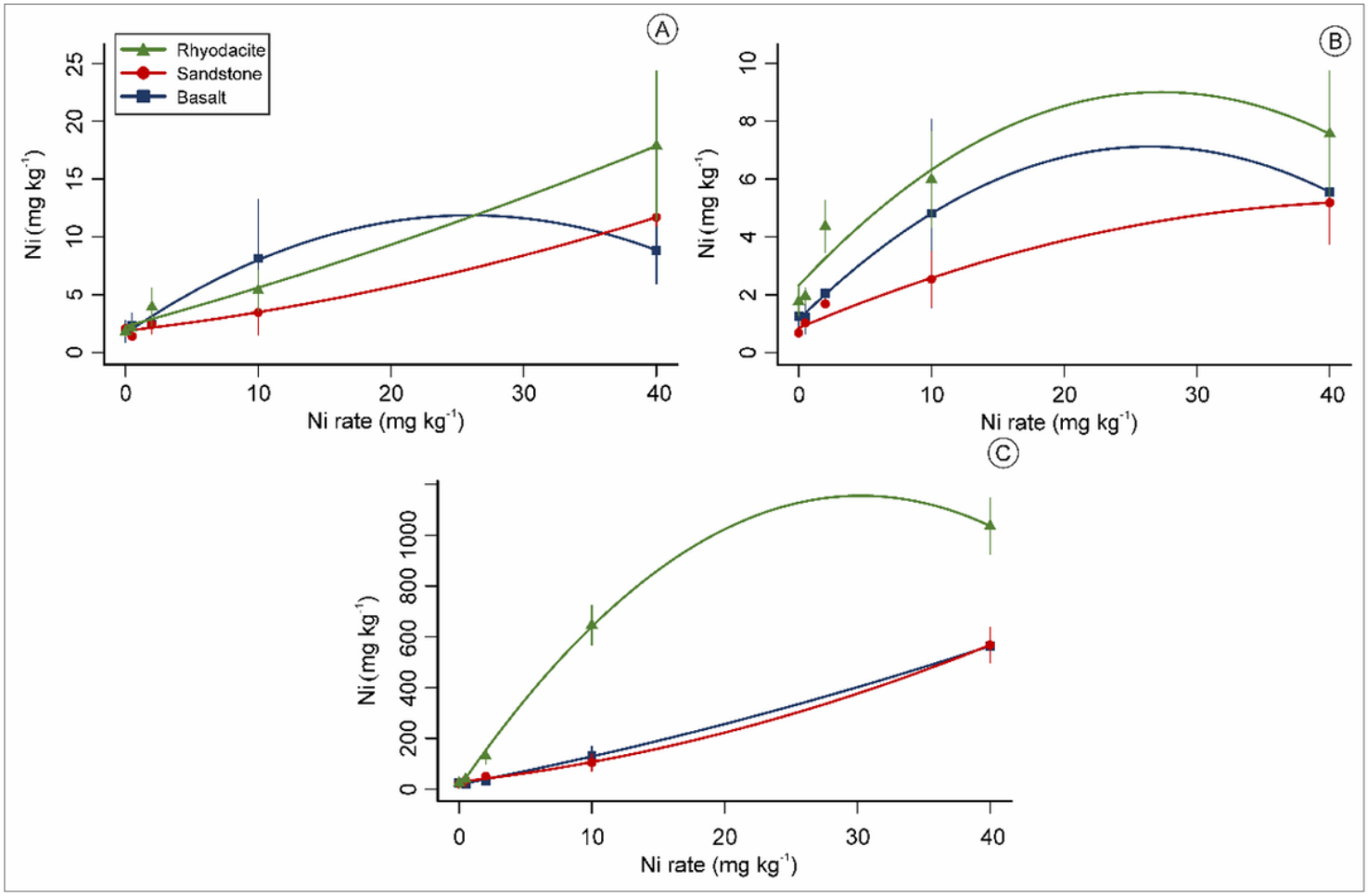


Figure 3

Nickel (Ni) concentration (means and standard errors) in leaves (A), branches (B), and roots (C) of yerba mate (*Ilex paraguariensis*) clones grown in soils originating from basalt, sandstone, and rhyodacite in response to Ni supply (0, 0.5, 2, 10, 40 mg kg^{-1}). Model regression equations are presented in Table S2.

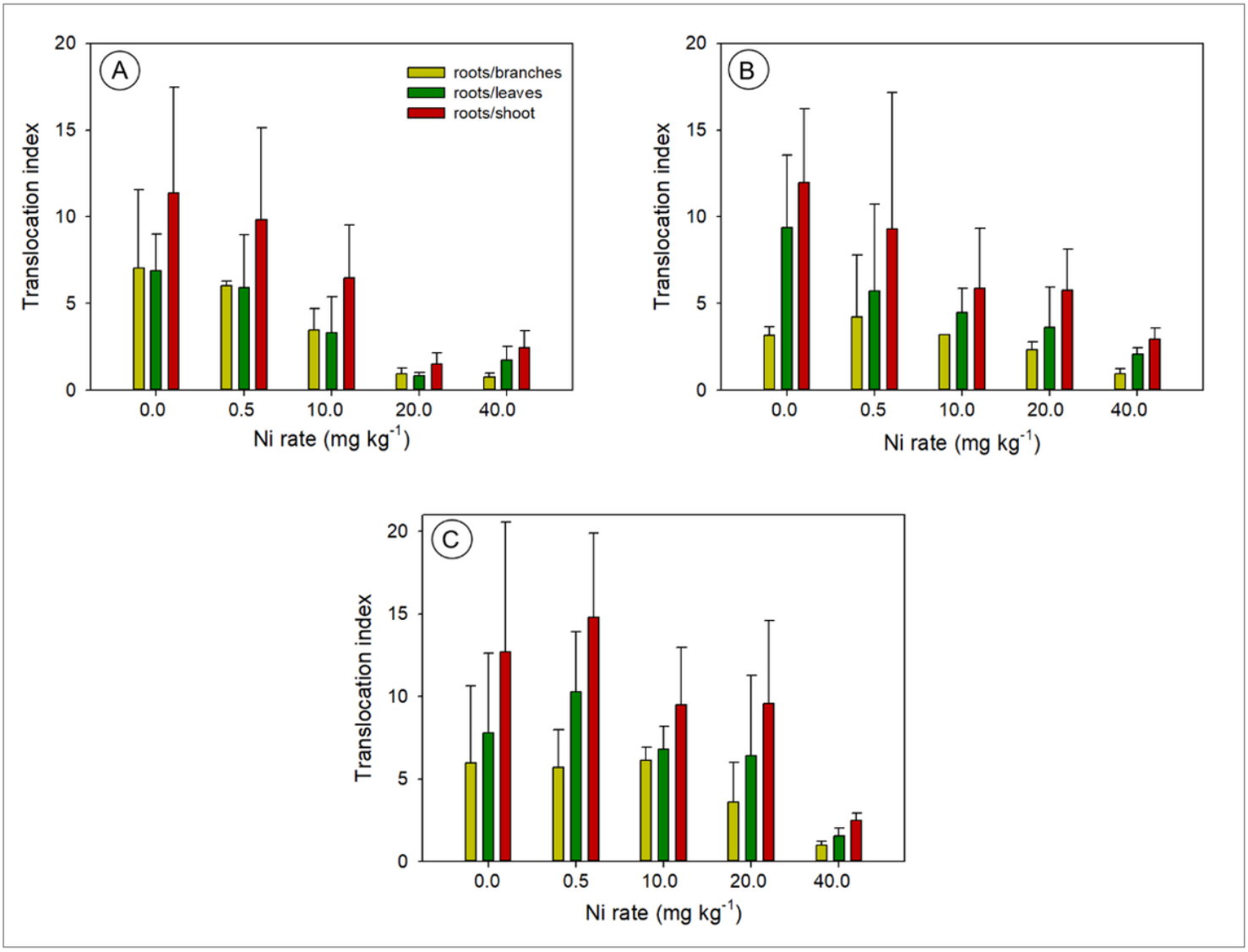


Figure 4

Translocation index of yerba mate (*Ilex paraguariensis*) cultivated in soils originating from rhyodacite (A), sandstone (B), and basalt (C) in response to Ni supply (0, 0.5, 2, 10, 40 mg kg^{-1}). Means and standard deviations are shown.

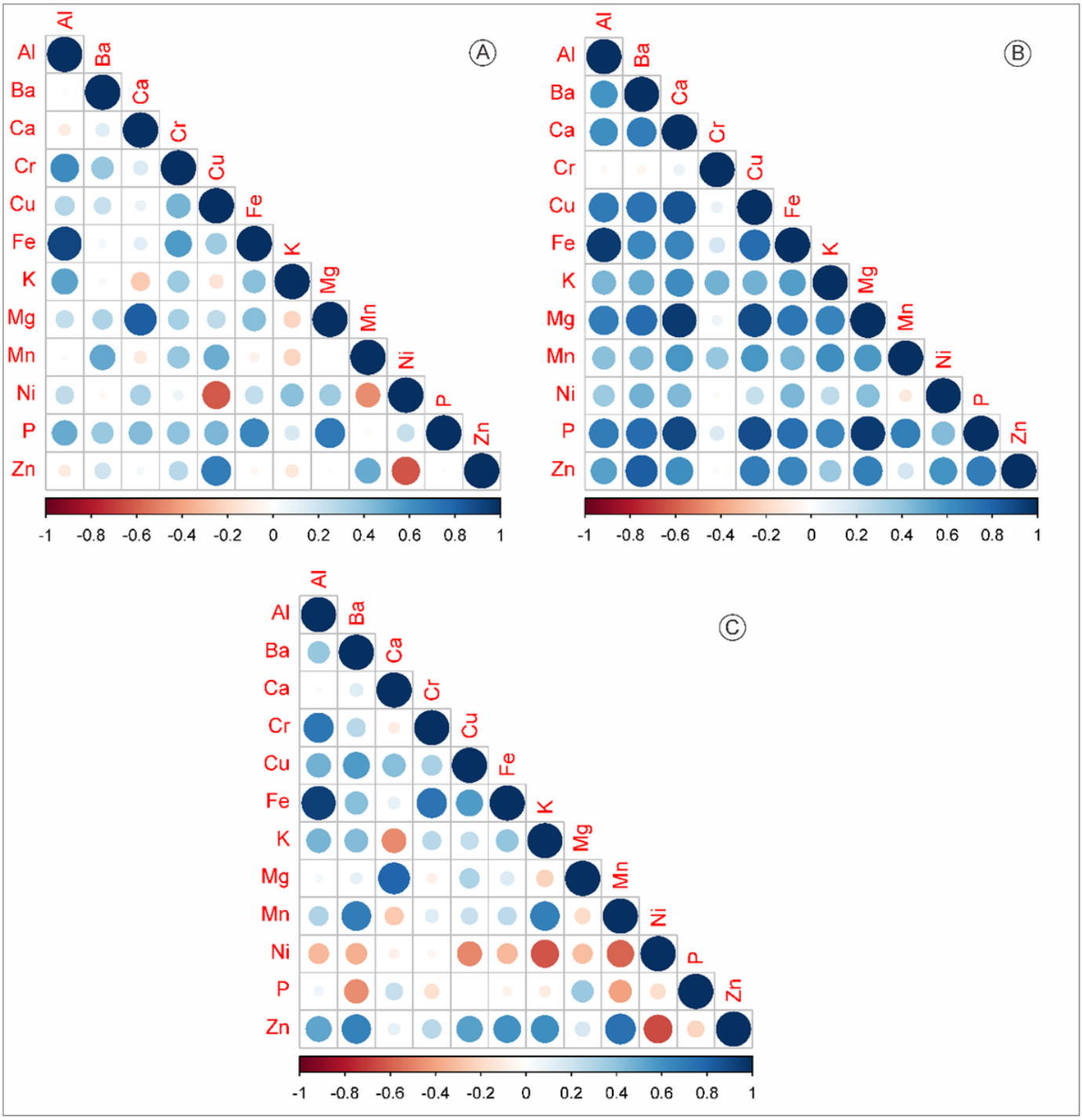


Figure 5

Correlation of elemental composition of yerba mate (*Ilex paraguariensis*) leaves cultivated in soils originating from basalt (A), sandstone (B), and rhyodacite (C) in response to Ni supply (0, 0.5, 2, 10, 40 mg kg^{-1}).

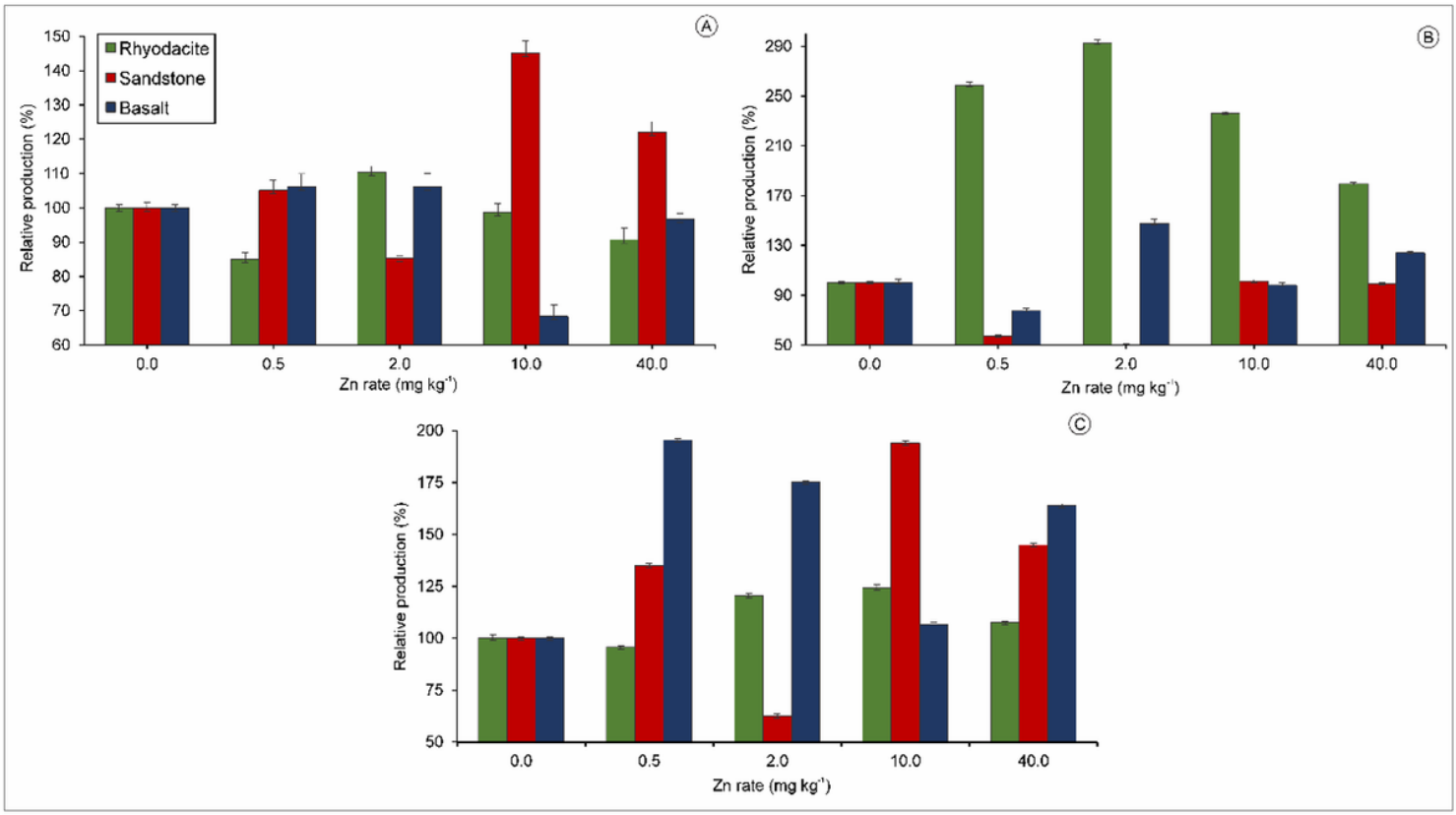


Figure 6

Relative dry matter production of leaves (A), branches (B), and roots (C) of yerba mate (*Ilex paraguariensis*) clones cultivated in soils originating from basalt, sandstone, and rhyodacite in response to Zn supply (0, 0.5, 2, 10, 40 mg kg^{-1}). Means and standard deviations are shown.

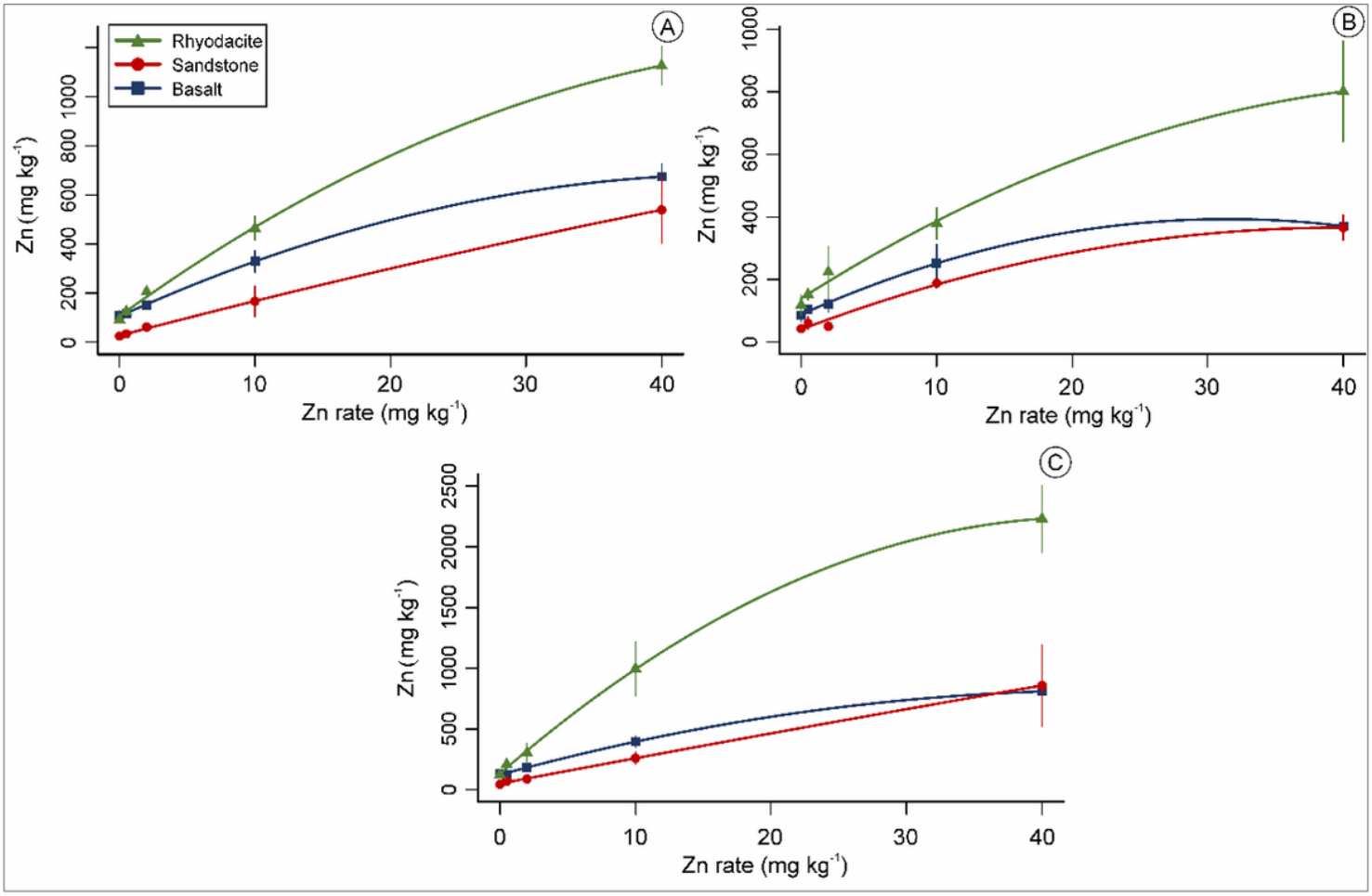


Figure 7

Zinc (Zn) concentration in leaves (A), branches (B), and roots (B) of yerba mate (*Ilex paraguariensis*) cultivated in soils originating from basalt, sandstone, and rhyodacite in response to Zn supply (0, 0.5, 2, 10, 40 mg kg^{-1}). Model equations are presented in Table S4. Means and standard deviations are shown.

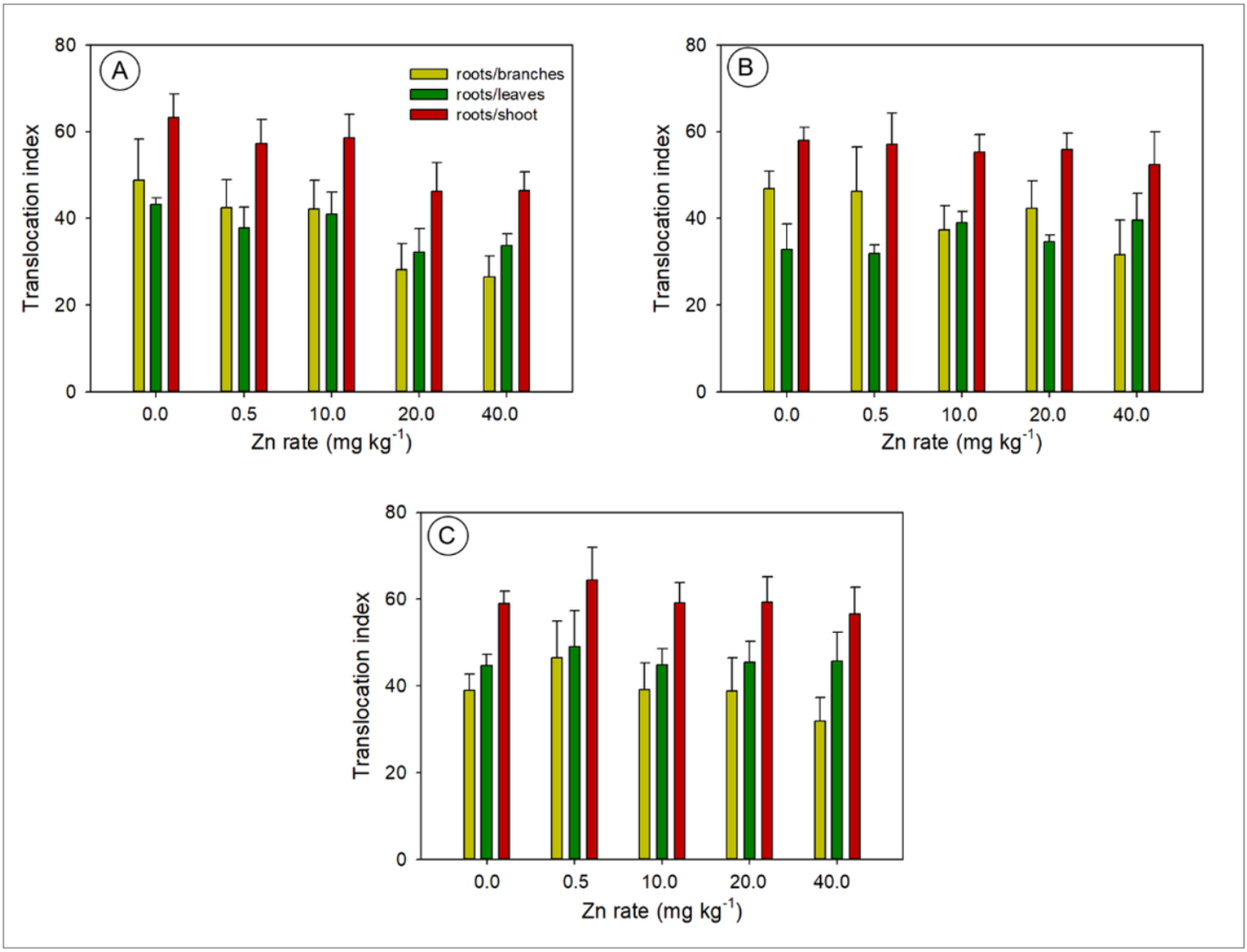


Figure 8

Translocation index of yerba mate (*Ilex paraguariensis*) cultivated in soils originating from rhyodacite (A), sandstone (B), and basalt (C) in response to Zn supply (0, 0.5, 2, 10, 40 mg kg^{-1}). Means and standard deviations are shown.

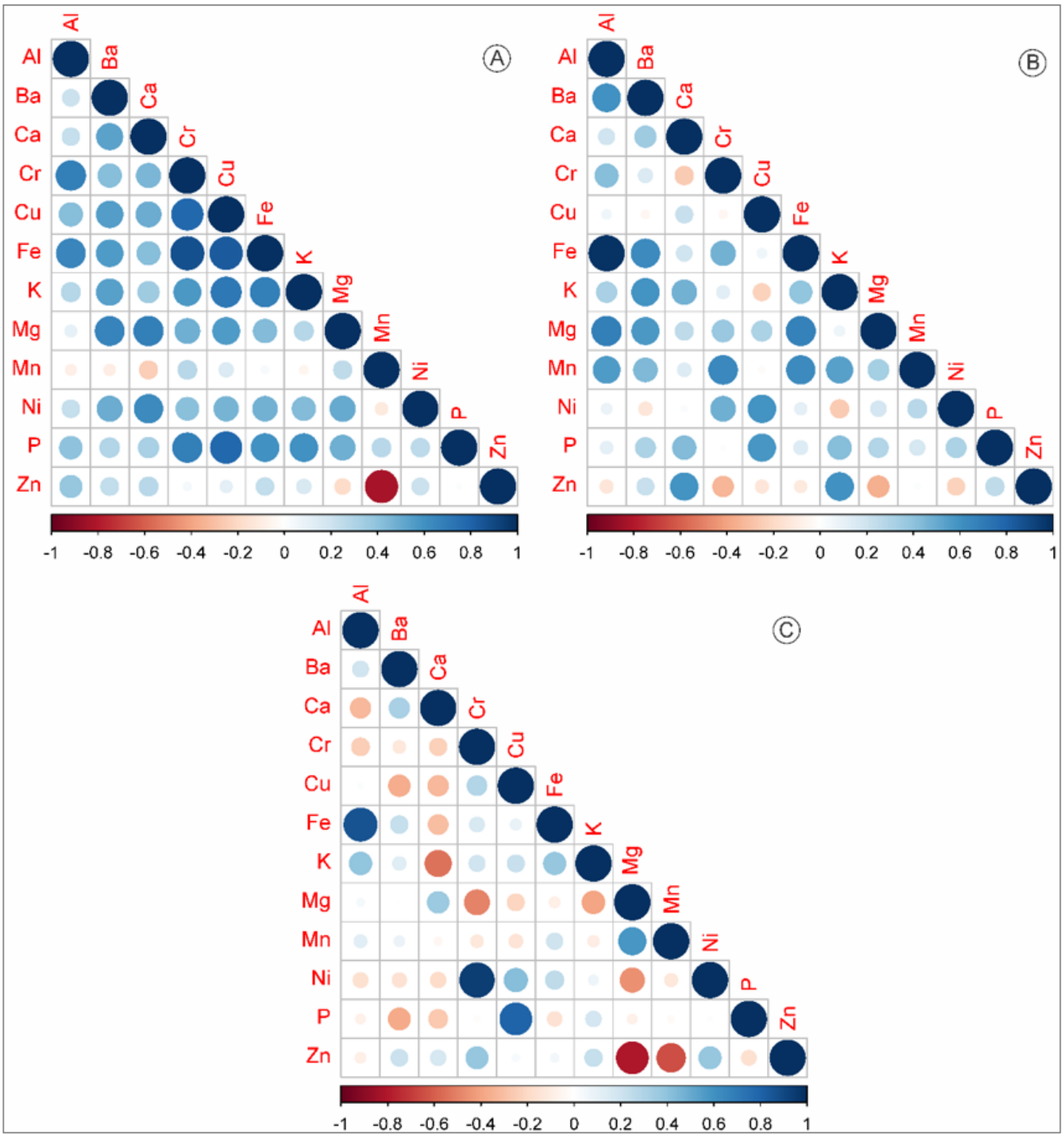


Figure 9

Correlation of elemental composition of yerba mate (*Ilex paraguariensis*) leaves cultivated in soils originating from basalt (A), sandstone (B), and rhyodacite (C) in response to Zn supply (0, 0.5, 2, 10, 40 mg kg^{-1}).

Supplementary Files

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