Bioavailable Nickel and Zinc in Polluted Soil Alter the Growth of Non-pioneer Trees in the Brazilian Atlantic Forest

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Abstract

**Background and aims**

The Brazilian Atlantic forest has been affected by the deposition of Ni and Zn, among other heavy metals adsorbed from atmospheric particles, which can be incorporated into the soil. If available in the soil, they can be absorbed by plant roots. The study aimed at testing experimentally the hypotheses: 1) Ni and Zn depositions increase their bioavailable fractions in the soil; 2) pioneer tree species demonstrate a greater potential to absorb Ni and Zn from the soil and less growth changes than non-pioneer species.

**Methods**

The experiment was carried out with six pioneer and non-pioneer species native to the Atlantic Forest, grown for 90 days in soil from an urban fragment of the Atlantic Forest, according to the treatments: soil with balanced fertilization (control) and soil enriched with Ni, Zn and Ni+Zn. At the end, the concentrations of Ni and Zn were determined in four soil fractions (F1: soluble; F2: linked oxides/hydroxides; F3: organic matter: F4: residual metals) and in leaves, stems/branches and roots. Mobility factors in soil, concentration ratios between treatments and respective controls, translocation index and relative growth rate in height, leaf number and total biomass were also calculated.

**Results**

The results showed that Ni and Zn concentrations increased significantly in the bioavailable soil fractions (F1, F2). The absolute content of Ni and Zn in the plants directly reflected the soil level in the available forms.

**Conclusions**

The metal accumulation in the species occurred regardless of the successional group to which they belonged. Non-pioneer species showed greater susceptibility to the metals.

**Introduction**

Fragmented forests in urban areas have been affected by a diversity of pollutants emitted by a large flow of both light and heavy vehicles (Ferreira et al., 2017, Kalaiarasan et al., 2018). Atmospheric particulate matter from urbanization represents a continuous source of heavy metal soil pollution, such as nickel (Ni) and zinc (Zn) (Kabata-Pendas, 2011; Keshavarzi and Kumar, 2019). This same particulate matter represents a significant potential risk to urban forest fragments, due to long-term persistence in the environment, bioaccumulation and acute and chronic toxicity (Chen et al., 2019; Motuzova et al., 2014; Wu et al., 201). Heavy metals like Ni and Zn, in high concentrations in plants, cause damage to the cellular and physiological processes of plants, such as photosynthesis interruption, which leads to alterations of growth and decreases in yield and biomass production (Patra et al., 2020).
In general, the cycling of heavy metals in the ecosystem is conditioned by the chemical and physical attributes of soil, which interfere with the adsorption / desorption, precipitation / dissolution, complexation, chelation and oxy-reduction reactions (Shah & Daverey, 2020). Among the soil attributes that interfere with metal bioavailability are acidic pH, organic matter content, redox potential, texture and the presence of exudates released by roots (Rieuwerts et al., 2006).

Metals in soluble and exchangeable forms are considered readily mobile and available to plants and those precipitated with Fe, Mn and Al oxides or complexed by organic matter are considered less available (Violante et al., 2010). In general, the bioavailable fraction of these elements in the soil is higher in acidic soils, such as those that occur in tropical regions. However, Rieuwerts (2007), in a review paper, added that the predictive models of soil mobility and bioavailability of heavy metals, which were proposed for temperate regions, may not be applicable in the tropics, due to differences in chemical characteristics. This alert reinforces the need for further studies on the topic in the tropics, including in Brazil, where the degradation of natural ecosystems has been increasing.

Trees play a major role in the circulation of heavy metals in forest ecosystems (Rai 2016; Sánchez-López et al., 2015; Song et al., 2015), due to their ability to absorb them naturally from the soil (Naidu et al., 2001; Sarma et al., 2011). However, plant species differ markedly in their ability to absorb and accumulate heavy metals (Karmakar & Padhy, 2019; Zhao et al., 2020). The Atlantic Forest is among the most biodiverse biomes in the world and the most threatened by anthropic expansion (Lira et al. 2012; Marques et al. 2021). It has potentially been affected by the deposition of heavy metals, since a substantial part of this biome has been reduced to small remnants found in degraded areas, agricultural fields and urban areas (Joly et al., 2014; Nakazato et al. 2021). However, its high biodiversity restricts a comprehensive analysis of the level of risk posed by these heavy metals to the urban remnants of this important Brazilian biome. This difficulty may be overcome by considering functional traits of the plant community, such as the response patterns of pioneer and non-pioneer trees (Uriarte et al., 2016). This functional approach is based on the premise that different successional groups are characterized by different physiological, molecular and genetic responses in relation to their ability to accumulate metals (sensu Krämer 2010). Pioneer trees in tropical forests are distinguished from non-pioneer trees by their higher solar radiation tolerance, photosynthetic and growth rates, and biomass accumulation, among other functional traits (Favaretto et al. 2011; Macieira et al. 2020; Portes et al., 2010; Rees et al., 2001). Therefore, pioneer species would theoretically have higher absorption rates from soil and lower growth alterations when exposed to metal pollution compared to non-pioneer species.

Bearing in mind that the Atlantic Forest soils in southeastern Brazil are acidic (Lopes et al. 2015) and that urban fragments of this biome have been affected by the deposition of Ni and Zn enriched particulate matter from vehicular emissions (Nakazato et al. 2021), this study aimed to evaluate experimentally whether: 1) the addition of Ni and Zn to soil, from an urban fragment of the Atlantic Forest increases, their bioavailable fractions in the soil; 2) translocation rates and their accumulation potential differ between pioneer and non-pioneer tree species; 3) the growth rates of these species are altered in response to the excess of these metals in the soil. Two hypotheses were tested: 1) Ni and Zn depositions increase
their bioavailable fractions in the soil of urban Atlantic Forest remnants; 2) Pioneer tree species demonstrate a greater potential for absorbing Ni and Zn from the soil and show less changes in growth than non-pioneer tree species.

Materials And Methods

Experimental design

The experiment was carried out in a greenhouse located in the Fontes do Ipiranga State Park (PEFI), located in the city of São Paulo (São Paulo State, SE Brazil). This site contains an important urban remnant of the Atlantic Forest, from which the soil was removed to carry out this experiment. The soil that predominates in the park is Oxisol (Fernandes et al. 2002). The history of anthropic impacts on PEFI was divided into three distinct phases, according to dating carried out on sediments from a lake located next to the forest remnant, in parallel with heavy metal and polycyclic aromatic hydrocarbon (PAHs) analyses (Costa-Böddeker et al. 2012). Phase I (~ 1894-1975) was characterized by low atmospheric contamination, phase II (~ 1975-1990) by an abrupt increase in urbanization and air pollution levels and phase III (~ 1990-2005) by the activities of a neighboring steel company.

The experiment was carried out with six tree species native to the Atlantic Forest in São Paulo: Croton floribundus Spreng., Inga sessilis (Vell.) Mart., Rapanea ferruginea (Ruiz & Pav.) Mez (pioneer species), Eugenia uniflora L., Esenbeckia leiocarpa Engl. and Ocotea odorifera (Vell.) Rohwer (non-pioneer species), belonging to Euphorbiaceae, Fabaceae, Primulaceae, Myrtaceae Rutaceae and Lauraceae, respectively. All species seedlings were donated by the Energy Company of São Paulo (CESP), located in the city of Paraibuna, São Paulo State. The pioneer plants were, on average, 38 cm in height with a 4 mm stem diameter. The non-pioneer plants were 21 cm in height with a 3 mm stem diameter, on average. All seedlings had 5 leaves on average. These plants were transplanted into pots with a 1.7 L capacity, containing topsoil (0-20 cm) collected in the PEFI forest remnant, excluding the litter layer.

The chemical and physical characteristics of the topsoil used in the experiment were determined in five air-dried and sieved (through a 2 mm sieve) samples, as described by Raij et al. (2001). On average, the soil had a medium pH (5.3) and high levels of $\text{P}_{\text{resin}}$ (14 mg/dm$^3$), Ca (66 mmolc/dm$^3$), Mg (11 mmolc/dm$^3$), S (27 mg/dm$^3$), Cu (3.4 mg/dm$^3$), Mn (10.3 mg/dm$^3$), Zn (14.2 mg/dm$^3$), B (58 mg/dm$^3$) and Fe (57 mg/dm$^3$). The soil still had a high content of organic matter (40 g/dm$^3$) and a high cation exchange capacity (109 g/dm$^3$). The soil was also characterized by a clay texture (fine sand: 110 g / kg; coarse sand: 427 g / kg; clay: 380 g / kg and silt: 74 g / kg).

Before plant transplantation, the soil was air-dried, homogenized, ground, passed through a 10 mm mesh sieve and kept moistened (80% of the field capacity) for 15 days. After one week of planting, all plants received 100 ml of nutrient solution containing macronutrients and micronutrients, as synthesized for the control treatment ($T_C$) in supplementary material, Table S2.
After a one month adaptation period, the seedlings of all species were subjected to the following soil treatments: 1) control with balanced fertilization (referred to as T_C); 2) balanced fertilization plus a higher dose of Ni (T_Ni); 3) balanced fertilization plus a higher dose of Zn (T_Zn); 4) balanced fertilization plus higher doses of Ni and Zn (T_{ZnNi}). The doses of Ni and Zn in the solutions used in treatments 2 to 4 (supplementary material, Table S1) were three times higher than the concentrations proposed by the Environmental Company of São Paulo State (CETESB) to prevent risks associated with soil contamination in the State (30 mg Kg^{-1} of soil for Ni and 86 mg.kg^{-1} of soil for Zn; CETESB, 2016). These doses were reached by means of three weekly applications. The experiment lasted 90 days, starting from the first weekly application of the established doses. It was carried out in a 6 x 4 factorial scheme (six species x four soil treatments), with five replicates per treatment and five plants per treatment replicate, totaling 100 plants for each species.

**Measurements and sampling procedures**

Height and number of leaves on the main stem of each plant per treatment replicate was determined every 30 days.

At the end of the experiment, a total of 120 composed soil samples (five composed samples per treatment and species) were submitted to sequential extraction of Ni and Zn.

At the end of the experiment, the roots, stems/branches and leaves of each plant were also separated, and dried in an oven (60°C) to obtain the dry mass. After weighing, the samples of leaves, roots and stem/branches of the plants from each treatment replicate were gathered together in order to obtain five composed samples of each organ, per treatment and species.

**Analytical techniques**

The sequential extraction of Ni and Zn from the soil samples was performed by using the EC Standards, Measurement and Test Program protocol (called the BCR Test) (Janoš et al. 2010; Rauret et al. 1999). The Ni and Zn concentrations were determined in four fractions extracted sequentially from 1 g of each composed soil sample: soluble, exchangeable and bound to carbonates (F1); linked to iron and manganese oxides / hydroxides (F2); linked to organic matter (F3) and residual metals such as silicates (F4). F1 was extracted with acetic acid (0.11M) for 16 h at room temperature; F2 was extracted with hydroxylamine hydrochloride (0.5 M) for 16 h at room temperature; F3 was digested at an elevated temperature with hydrogen peroxide (30%), acidified to pH 2–3 and extracted with ammonium acetate (1 M) and F4 was digested at an elevated temperature with aqua regia (HCL: HNO_3, 3:1). The extraction of F1, F2 and F3 was separated by centrifugation. The supernatant was removed, filtered and nitric acid (2N) was added in order to adjust the pH to 2.0. In the residual fraction (F4) was diluted with deionized water.
The composed samples of leaves, stems and roots were ground in an agate mill and subjected to acid digestion in a closed microwave oven (CEM-Mars-Xpres), using 5 ml HNO$_3$. After digestion, the extracts were diluted with 10 ml of deionized water and stored in polypropylene flasks.

The concentrations of Ni and Zn in soil and plant extracts were determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES), using a piece of Varian equipment, model 720. Analytical curves were prepared with an external standard from the brand Perkin Elmer. The concentration range used to obtain the curves for both elements was between 100 ppb and 1000 ppb. The detection limits were 0.001mg L$^{-1}$ for Zn and 0.002mg L$^{-1}$ for Ni. The quality control of the analyzes was verified by analyzing certified reference material (SRM 1515 – Apple Leaves).

Ni and Zn concentrations in the four soil fractions are expressed as mg g$^{-1}$ of dry soil. The Ni and Zn in leaves, stems/branches and roots per plant are presented as absolute concentrations (concentrations * dry biomass of each organ per plant).

**Ratio/index calculations**

**Soil**

The concentrations and bioavailable levels of Ni and Zn in the contaminated soil were accessed by estimating: a) the sum concentrations found in the soil fractions in the T$_C$, T$_{Ni}$, T$_{Zn}$ or T$_{NiZn}$ treatments, as well as concentration ratios of each fraction, between the values found in T$_{Ni}$, T$_{Zn}$ or T$_{NiZn}$ and those found in T$_C$; b) The soil’s mobility factor (MF), using the formula \[MF = (F1 + F2) / (F1 + F2 + F3 + F4)\] x 100 proposed by Kabala and Singh (2001) with adaptations (F1 to F4 in the formula represent the concentrations of Ni or Zn in the four fractions extracted from the soil).

**Plant material**

The concentration ratios in the different organs were estimated between the values found in each fraction of the soil in the T$_{Ni}$, T$_{Zn}$ or T$_{NiZn}$ treatments and the values found in the control (T$_C$). A heatmap was then proposed using these absolute concentration ratios to visualize the levels of general proportional accumulation of Ni and Zn in the different organs and tree species. All values were transformed into log$_{10}$ (data included in Table S2; supplementary material) and a heatmap was generated using the public software Morpheus (https://software.broadinstitute.org/morpheus). The blue color in the scale (0 to 1) indicates a comparatively low to medium accumulation level and the red color (> 1 to 2) indicates a comparatively high to very high accumulation level.

Absolute concentrations (Ca) obtained from the different plant organs were also used to calculate a translocation index (TI), following a formula proposed by Chanu and Gupta (2016):

\[TI = (Ca \text{ stems-branches} + Ca \text{ leaves}) / Ca \text{ roots}\]

TI > 1 indicates that the element accumulated in a greater proportion in the shoots than in the roots.
Finally, relative growth rates (RGR) for height and leaf number, as well as for the dry biomass of each plant organ, were estimated using the measurements performed at the beginning and end of the experiment, based on the equation proposed by Benincasa (1988):

\[
RGR = \left( \frac{\ln L_2 - \ln L_1}{t_2 - t_1} \right)
\]

Where: \( \ln L_2 \) and \( \ln L_1 \) = natural logarithm of the final and initial value; \( t_2 \) and \( t_1 \) = final time and initial time in days of exposure. The initial values for the dry biomass of each organ were obtained at the beginning of the experiment from an additional lot of plants of each species.

Statistics

Differences in the sum of the concentrations of Ni and Zn in the four soil fractions and the mobility factors of these metals in the \( T_C \), \( T_{Ni} \), \( T_{Zn} \) and \( T_{NiZn} \) treatments were identified using parametric One-way ANOVA (F test) and Tukey test. All the results obtained from the soil used for cultivating the six species were included in these statistics, considering that the different species did not alter significantly the metal concentrations in the soil fractions themselves.

One-way ANOVA, followed by a multiple comparison test (Tukey test), was also applied to compare: the sum of the absolute concentrations (concentration * biomass) of the leaves, stem/branches and roots of each species, to the soil treatments; concentration ratios \( ([Ni]_{treatments}/[Ni]_{control}) \) and \( ([Zn]_{treatments}/[Zn]_{control}) \) for the whole plants (leaves+stems+roots) to the species; translocation indices from roots to shoots to the species and RGR in height, number of leaves and the total biomass of each species between the treatments.

In all cases, the data were transformed into log\(_{10}\), when necessary to meet the requirements of ANOVA (normal distribution and equality of variations).

Results

The sum of the concentrations of Ni (Figure 1 A) and Zn (Figure 1 C) in the four soil fractions were significantly higher in treatments with the addition of these metals than in other treatments. The mobility factor (MF) used by Kabala & Singh (2001) was also applied to the data obtained as an indicator of Ni and Zn bioavailability in the soil. The MF values for Ni (Figure 1 B) and Zn (Figure 1D) were also significantly higher in the treatments with the addition of these metals in relation to their respective controls. In general, higher MF values (between 84.9% and 95.4%) were estimated for Zn compared to MF values for Ni (50.4% to 80.6%).

The estimate of the concentration ratios between treatments, with the addition of metals and control treatment for the different fractions confirmed the greater bioavailability of Zn compared to Ni, when in excess in the soil (supplementary material, Table S2). These estimates indicated that Ni was found in the
highest proportion bound to iron and manganese oxides / hydroxides (F2) in the treatment with an excess of it in the soil (TNi/TC) and bound to organic matter (F3) when Ni and Zn were applied together in the soil (TNiZn/TC). Zn was found proportionally in greater concentration in its soluble, exchangeable forms and bound to carbonates (F1) of the soil, in all cases, in relation to the concentration found in the same fraction control treatment treatments (TZn/TC; TNiZn/TC; TNi/TC) than in the other fractions.

The variations between treatments in the absolute concentrations of Ni and Zn in plants (sum of the values for leaves, stems and roots) of all species followed the overall variations in the mobility factor of both metals in the soil. The total absolute contents of Ni and Zn were at least twice higher in pioneer species than in non-pioneer species grown in all treatments. *R. ferruginea* was the only exception between the pioneer species. Its average absolute Ni and Zn contents were similar to those measured in the non-pioneer trees (Figs. 2 and 3).

The absolute contents of Ni were significantly higher in the plants of all species grown in soil with an excess of Ni (T<sub>Ni</sub>) and of Ni+Zn (T<sub>NiZn</sub>) compared to the control treatment (T<sub>C</sub>). The absolute contents in *I. sessilis*, *R. ferruginea* and *E. uniora* plants exposed to T<sub>Zn</sub> were also significantly higher than those in plants exposed to T<sub>C</sub> (Fig. 2).

The absolute content of Zn was significantly higher in all the tree species samples grown in soil with an excess of Zn (T<sub>Zn</sub>) compared to those in T<sub>C</sub>, T<sub>Ni</sub> and T<sub>NiZn</sub>. Only *C. floribundus* had an absolute content of Zn similar to that of those plants exposed to T<sub>Zn</sub> and T<sub>NiZn</sub> treatments (Fig. 3).

The absolute concentration ratios indicated that Ni and Zn accumulation capacities greatly varied between plant species and different organs independent of their successional stages (Table 1, Fig. 4, Table S3 of supplementary material). Considering the whole plant (leaves + stems/branches + roots), the accumulation of Ni was more accentuated in the plants of *C. floribundus* (Pi) and *I. sessilis* (Pi) grown in soil enriched with Ni (T<sub>Ni</sub>/T<sub>C</sub> = 15.4 ± 0.7 and 14.1 ± 1.2 respectively) and in soil enriched with Ni+Zn (T<sub>NiZn</sub>/T<sub>C</sub> = 12.1 ± 1.3 and 11.2 ± 1.5 respectively). The accumulation of Zn was more accentuated in the plants of *E. leiocarpa* (NPi) and *O. odorifera* (NPi) grown in Zn enriched soil (T<sub>Zn</sub>/T<sub>C</sub> = 15.6 ± 0.9 and 15.1 ± 2.5 respectively). The plants of *C. floribundus* (Pi), *I. sessilis* (Pi) and *E. leiocarpa* (NPi) also showed a more accentuated accumulation capacity for Zn when grown in soil enriched with Ni+Zn (T<sub>NiZn</sub>/T<sub>C</sub> = 6.3 ± 1.1, 6.8 ± 1.1 and 6.4 ± 0.5 respectively) (Table 1).

Proportionally high to very high accumulation capacities were generally estimated for Ni compared to Zn, in plants grown in soil enriched by these metals when compared to the basal values estimated in the control treatment. Additionally, Ni accumulation was proportionally higher in leaves and stems and Zn in the roots (Fig. 4, Table S3 of supplementary material). Therefore, the plants showed a greater ability to transport Ni than Zn to the shoots, although the mobility factors estimated for the soil tended to be higher for Zn than for Ni (Fig. 1).
The leaf accumulation of Ni was proportionally more accentuated in plants of *E. leiocarpa* than in the other species grown with an excess of this metal in the soil, compared to the values obtained in the control treatment \( \frac{T_{Ni}}{T_C} \). *E. leiocarpa* and *E. uniflora* grown in \( T_{Ni} \) were the species that showed proportionally more Ni in their stems compared to the basal contents observed in the control plants. *C. floribundus*, *I. sessilis* and *E. uniflora* showed greater capacity to accumulate Ni in the roots than the other species did, when grown in the treatment with an excess of Ni relative to the root contents of control plants \( \frac{T_{Ni}}{T_C} \) (Fig. 4, Table S3 of supplementary material).

*E. leiocarpa* (NPi) plants grown with an excess of Zn in the soil was the species that accumulated the highest levels of this metal in their leaves and roots relative to the levels obtained in the control plants \( \frac{T_{Zn}}{T_C} \). *R. ferruginea* (Pi), *E. leiocarpa* (NPi) and *O. odorifera* (NPi) cultivated in Zn enriched soil showed the greatest capacity for an increase in the absolute contents of Zn in their stems compared to the control plants \( \frac{T_{Zn}}{T_C} \) (Fig. 4, Table S3 of supplementary material).

The heatmap also revealed that lower Ni and Zn accumulation capacities were generally detected in the plants grown in soil enriched with Ni+Zn compared to basal values \( \frac{T_{Ni, Zn}}{T_C} \), indicating that high levels of one metal in the soil may have inhibited the root uptake of the other (Fig. 4, Table S3 of supplementary material). This inhibition was also evidenced by estimating the translocation index (Table 2).

In this study, the increased metal translocation rates from the roots to the shoots in response to the increase in soil bioavailability was not a characteristic observed in all species. TI \( \leq 1 \) were estimated in the plants of most species treated with the addition of Ni and Zn, indicating that the species tended to immobilize metals in the roots. However, *C. floribundus* (Pi), *R. ferruginea* (Pi), *E. leiocarpa* (NPi) and *O. odorifera* (NPi) stood out again for their high, estimated, TI values for Ni and, in the last two species also for Zn. *E. leiocarpa* (NPi) may be recommended for phytoremediation. It accumulated, on average, 4 and 3.6 times more Ni and Zn, respectively, in the shoots than in the roots when exposed to an excess of those metals in the soil, although it was not the species with the highest absolute content totals of these metals (Table S3 of supplementary material) and its growth in height and leaf number was reduced (Fig. 5, Fig.S1 and S2 of supplementary material).

The RGR in height was not altered in the plants of pioneer species grown in soil with an excess of Ni, Zn and Ni+Zn. However, *C. floribundus* plants showed an increased leaf number and reduced total biomass and *R. ferruginea* a decreased leaf number and total biomass after 90 days of growth in the \( T_{Zn} \) or \( T_{Ni, Zn} \). The TCR for the leaf number of *I. sessilis* was not affected by Ni and Zn treatments (Fig. 5).

The TCR for height, number of leaves and total biomass of non-pioneer species were more clearly affected than those estimated for pioneer species, despite having a lower absolute metal content, partially confirming our second hypothesis. *E. uniflora* (NPi) showed a higher rate in relation to height when grown in soil with Ni and Ni+Zn additions and a lower RGR in leaf number when treated with Zn and Ni+Zn. *E. leiocarpa* (NPi) plants showed the lowest RGR in height when grown in soil with an increase in both metals and a lower RGR in leaf number when treated with Zn and Ni+Zn. Both the height and leaf
production of *O. odorifera* (NPi) were strongly affected. Negative RGR values were estimated in its plants after growing 90 days in soil with the addition of metals, which resulted from the loss of apical bud, and a sharp fall in the number of leaves.

The RGR in total biomass was reduced in non-pioneer species plants treated with an excess of metals (Fig. 5). This reduction in growth seems to indicate that these plants were more susceptible to the stress induced by Ni and Zn than the pioneer species were (Fig. S2 of supplementary material).

**Discussion**

In this study, it was possible to experimentally simulate the deposition of Ni and Zn in the soil of an urban fragment of the Atlantic Forest, to evaluate the interactions between these toxic ions and other soil constituents and to verify whether a high soil contamination would affect the growth of tree species. The results obtained the level of availability of Ni and Zn, when deposited in the soil from the urban forest fragment included in Fontes do Ipiranga State Park (PEFI), in the form of sulfate salts.

The values close to 100% for the mobility factor index, as obtained in the present study, have been interpreted as indicators of high bioavailability of heavy metals in soils (Ma and Rao, 1997). It was added that Zn is more readily available for absorption than Ni, as also observed by other authors (Bogusz and Oleszczuk, 2018; Sanchez-Martin et al., 2007;).

Ni can easily form complexes with Fe oxides (Gonnelli and Renella, 2013), explaining the higher proportion of the metal in F2 verified in TNi in relation to the level observed in the same fraction extracted from TC soil (TNI/TC).

The distinct distribution of both metals among the fractions seemed to be governed by soil physicochemical properties as well as by the different metal properties (Bogusz and Oleszczuk, 2018; Bogusz and Oleszczuk, 2019). In other words, the availability of these metals for plant absorption can be influenced by both their sources and the physical-chemical characteristics of soil (Mao et al., 2017). The soil used in the experiment was acidic (mean pH = 5.3) and was also characterized by high levels of Fe (57 mg / dm3), according to the classification of Raij et al. (2001). According to Brokbartold et al. (2012), lower pH values result in greater cation mobility and, therefore, greater availability for plants. Rieuwerts (2007) concluded that the soils found in the tropics, such as oxisols and argisols (high levels of organic matter, low pH values and high levels of Fe oxides), result in the high mobility and bioavailability of metals. Joris and collaborators (2012) found that the increase in pH caused by liming in a dystrophic red latosol managed by a no-tillage system resulted in a decrease in the availability of Cu, Zn, Cd and Ni, mainly in the topsoil. Based on these studies, we can assume that the chemical characteristics of the urban Atlantic Forest topsoil contribute to an increased bioavailability of Ni and Zn for plants, confirming the first hypothesis of this study.

The absolute contents of Ni and Zn in the species of plants included in this study directly reflected the bioavailability levels found in the soil. These results also showed that the accumulation capacity
appeared to be preponderantly higher in pioneer species than in non-pioneer species, which would confirm our second hypothesis regarding this aspect. Contrasting results were obtained by Nakazato et al. (2021), who sampled the leaves from adult trees of pioneer and non-pioneer species in the urban Atlantic Forest fragment where the soil for this experiment was obtained. The authors measured higher metal concentrations in non-pioneer trees than in pioneer trees. This apparent discrepancy may originate from the fact that we estimated the Ni and Zn accumulation level in each plant organ by multiplying the concentrations per their respective dry masses. It is well known that pioneer tree species have a greater potential for absorbing heavy metals from the soil because they have higher photosynthetic rates and faster growth than non-pioneer species (Boukhris et al., 2016; Favaretto et al., 2011; Porte et al., 2010).

However, our second hypothesis could be evaluated more precisely by estimating absolute concentration ratios between the values obtained in plants grown in the treatments with metal addition and the basal contents determined in plants grown in the control treatment (TNi/TC; TZn/TC; TNiZn /TC).

In addition, a further interpretation of the heatmap included in Fig. 4 led us to reject the second hypothesis regarding the capacity of tree species for absorbing Ni and Zn from the soil. In fact, the Ni and Zn accumulation capacities could not be defined by the successional stage of tree species. Heavy metal stress induces root-level ion flux responses, which can vary among species (Palm et al., 2017). The combined stress of Zn and Ni can induce greater ion flux responses and decrease the entry of metals into plants, as a mechanism to prevent toxic damage.

The translocation index (TI) allowed us to identify a possible plant defense mechanism against an excess of metals in the soil. The immobilization of inorganic contaminants in the roots, restricting their translocation to the shoots and resulting in TI < 1, has been considered a mechanism to prevent damage to leaf metabolic and physiological processes. In contrast, TI > 1 may indicate that plants not only tolerate, but use the contaminant in a beneficial way, a common characteristic of hyper-accumulating plants (Chanu and Gupta, 2016). Therefore, TI > 1 is a decisive factor in categorizing plant species for phytoremediation (Chanu and Gupta, 2016).

The levels of stress caused by toxic levels of heavy metals are dependent on the intensity of exposure, the duration and the sensitivity of the plant to stress, in addition to the combination with other stressors (Shinozaki et al., 2015). The answer initially can lead the plant to enter the alarm phase, which will cause a reversible reaction of growth reduction, entering the resistance phase and, thus, continuing its development. However, an event of very severe stress, whether due to severe deficiency or toxicity, the plant may go into exhaustion, resulting in a decrease in resistance and then death (Shinozaki et al., 2015).

Zn stress can damage the root structure (Ambrosini et al., 2015; Bochicchio et al., 2015), reducing the absorption of water and mineral nutrients from the soil and consequently the plants' growth (Ambrosini et al., 2016). Studies have also shown the negative impacts of Ni on plant growth due to a decrease in chlorophyll content (Sheetal et al., 2016).
Conclusion

The Ni and Zn applied to the soil from an urban Atlantic Forest fragment increased their bioavailable fractions for the plant community, confirming the first hypothesis.

The absolute content of Ni and Zn in plants of the species included in this study directly reflected the level of bioavailability in the soil, being higher in pioneer species than in non-pioneer species. However, the absolute concentration ratios comparing the values obtained from plants grown in the treatments with added metal and the basal contents determined in plants grown in the control treatment indicated that Ni and Zn accumulation capacities varied greatly between plant species and different organs independent of their successional stages. The relative growth rates in height, leaf number and total biomass of non-pioneer species were more evidently affected than those estimated for pioneer species, despite them having a lower absolute content of metals, partially confirming our second hypothesis.

Declarations

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Conflicts of interest/Competing interests

The authors declare that there is no conflict of interest.

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication
Availability of data and material (data transparency)

Data is available by request to the authors.

Code availability

Not applicable.

Author contributions

All authors contributed to the study conception and design. Solange Eulália Brandão, Mirian CS Rinaldi e Marisa Domingos: Conceptualization, methodology, validation, formal analysis, research, writing, visualization and project administration. Geane Martins Barbosa, Matheus Casarini Siqueira, Rafaela de O. A Campos, Ana C.F.Dalsin: Methodology and research. The first draft of the manuscript was written by Solange Eulália Brandão and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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### Tables

**Table 1.** Average concentration ratios of nickel and zinc and respective standard deviations between treatments with the addition of both metals and control treatment ([Ni]_treatments/[Ni]_control; [Zn]_treatments/[Zn]_control) for the whole plants (leaves+stems+roots) of pioneer (Pi) and non-pioneer (NPi) species. Distinct letters indicate significant differences in the concentration ratios between species (p < 0.001).

| Species           | Nickel \( T_{\text{Ni}}/T_{\text{C}} \) | Nickel \( T_{\text{NiZn}}/T_{\text{C}} \) | Zinc \( T_{\text{Zn}}/T_{\text{C}} \) | Zinc \( T_{\text{NiZn}}/T_{\text{C}} \) |
|-------------------|----------------------------------------|----------------------------------------|------------------------------------|----------------------------------------|
| *C. floribundus* (Pi) | 15.4 ± 0.7 a                           | 12.1 ± 1.3 a                           | 6.3 ± 0.7 c                         | 6.3 ± 1.1 a                           |
| *I. sessilis* (Pi)   | 14.1 ± 1.2 a                           | 11.2 ± 1.5 a                           | 8.0 ± 1.0 b                         | 6.8 ± 1.1 a                           |
| *R. ferruginea* (Pi) | 8.7 ± 1.0 c                            | 7.0 ± 1.0 c                            | 7.9 ± 1.3 b                         | 4.2 ± 0.6 b                           |
| *E. uniora* (NPi)    | 12.7 ± 2.2 b                           | 6.9 ± 0.7 c                            | 10.0 ± 2.0 b                        | 5.2 ± 0.7 b                           |
| *E. leiocarpa* (NPi) | 12.6 ± 1.0 b                           | 9.3 ± 1.2 b                            | 15.6 ± 0.9 a                        | 6.4 ± 0.5 a                           |
| *O. odorifera* (NPi) | 11.5 ± 2.0 b                           | 6.9 ± 0.9 c                            | 15.1 ± 2.5 a                        | 5.8 ± 0.6 b                           |

**Table 2.** Mean values ± standard deviations of the translocation index of nickel and zinc from roots to shoots in pioneer (Pi) and non-pioneer (NPi) species grown in metal treatments in soil (\( T_{\text{Ni}}, T_{\text{Zn}} \) and \( T_{\text{NiZn}} \)). Distinct letters indicate significant differences between species for each treatment.
| Species                      | \( T_{Ni} \)   | \( T_{NiZn} \) | \( T_{Zn} \)   | \( T_{NiZn} \) |
|------------------------------|----------------|----------------|----------------|----------------|
| *C. floribundus (Pi)*        | 2.0±0.5 b      | 0.8±0.4 b      | 0.5±0.1 c      | 1.0±0.1 a      |
| *I. sessilis (Pi)*           | 0.5±0.1 c      | 0.6±0.2 b      | 0.5±0.1 c      | 0.6±0.2 b      |
| *R. ferruginea (Pi)*         | 2.0±0.3 b      | 0.7±0.2 b      | 1.2±0.4 b      | 0.2±0.03 c     |
| *E. uniflora (NPi)*          | 1.1±0.5 c      | 0.8±0.4 b      | 0.2±0.04 c     | 0.7±0.2 b      |
| *E. leiocarpa (NPi)*         | 4.0±1.2 a      | 0.7±0.2 b      | 3.6±0.4 a      | 1.1±0.3 a      |
| *O. odorifera (NPi)*         | 2.2±0.4 b      | 1.8±0.1 a      | 2.4±0.5 a      | 0.5±0.2 b      |

**Figures**
Sum of the concentrations of Ni (A) and Zn (C) in soluble, exchangeable and bound to carbonates (F1), linked to iron and manganese oxides / hydroxides (F2), linked to organic matter (F3) and residual metals such as silicates (F4) of the soil from the control (TC), Ni (TNi), Zn (TZn) and Ni+Zn treatments (TNiZn), as well as the MF % for Ni (B) and Zn (D) estimated according to Kabala and Singh (2001). Distinct letters indicate significant differences between TNi, TZn and TNiZn compared to TC (p <0.001).
Figure 2

Mobility factor in soil and sum of the absolute concentrations (concentration * biomass) of Ni in leaves, stems and roots of pioneer and non-pioneer species grown in the control (TC), Ni (TNi), Zn (TZn) and Ni+Zn (TNiZn) treatments. Distinct letters indicate significant differences in the sum of the absolute concentrations in plants between treatments.
Figure 3

Mobility factor in soil and absolute concentrations (concentration * biomass) of Zn in leaves, stems and roots of pioneer and non-pioneer species grown in the control (TC), Ni (TNi), Zn (TZn) and Ni+Zn (TNiZn) treatments. Distinct letters indicate significant differences in the sum of the absolute concentrations in plants between treatments.
Figure 4

Heatmap based on the average absolute concentration ratios of nickel (TNi/TC and TNi Zn/TC) and zinc (TZn/TC and TNi Zn/TC) determined in the leaves, stems and roots of pioneer (Pi) and non-pioneer (NPi) species. The ratios were log10 transformed (data available in Table S2, supplementary material). The Blue color in the scale (0 to 1) indicates comparatively low to medium accumulation levels and the red color (>1 to 2) high to very high accumulation levels.

C. floribundus (Pi)
I. sessilis (Pi)
R. ferruginea (Pi)
E. uniflora (NPi)
E. leiocarpa (NPi)
O. odorifera (NPi)
Figure 5

Average Relative Growth Rate (RGR) values for height, number of leaves and total biomass of pioneer (Pi) and non-pioneer (N Pi) species cultivated in the control (TC) and treatments with metals in the soil (TNi, TZn and TNiZn). Distinct letters indicate differences in the TCR of each species between the metal treatments and the control treatment (p <0.05).
Supplementary Files

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