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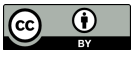
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NOTES AND INSIGHTS

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Elemental localization in inflorescences of the hyperaccumulators *Noccaea praecox* and *Noccaea caerulescens* (Brassicaceae)

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Abstract

Noccaea praecox and *Noccaea caerulescens* (Brassicaceae) are nickel and zinc hyperaccumulators, native to Europe. To date, most studies have focused on metal accumulation in the leaves, whereas the distribution of metals in the inflorescences of hyperaccumulator plants remains largely unexplored, but of great interest in the context of adaptation to fertility and (insect) pollination. Samples of *N. praecox* from an ultramafic site and *N. caerulescens* from an industrially contaminated site were used for synchrotron-based micro-X-ray fluorescence (μXRF) analysis. The results showed that nickel and zinc in the flowers of *N. praecox* are mainly distributed in the receptacle, ovary, and anthers, but at different concentrations. Similar results were found in *N. caerulescens*, with the greatest accumulation in the receptacle and ovary, especially in the walls, however at lower levels in the anthers. Although the leaves of *N. praecox* and *N. caerulescens* are the main deposition sites for nickel and zinc, significant concentrations of these elements were deposited in the flowers, especially in the pistils and anthers, indicating possible negative effects on fertility and pollinator species.

KEYWORDS

anthers, flowers, hyperaccumulator, Noccaea, pollination

1 | INTRODUCTION

Soil is the most important source of mineral elements for plants, especially when it comes to those essential for their growth and development. Depending on their role in the plant's physiological processes and their concentration, plants use different strategies to control the accumulation of metals. The main organs for deposition are roots and leaves, and depending on the metal concentration, plants are categorized as either excluders or accumulators (Baker, 1981). The latter are species that are hypertolerant to high metal concentrations, which they take up and translocate to above-ground tissues without significantly impairing metabolic processes. Plants that can accumulate elements in concentrations 100–1000 times or even higher than non-accumulator species in

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the same location without showing symptoms of toxicity are called hyperaccumulators, with the leaves being the main deposition site. To date, this phenomenon has been confirmed in more than 700 taxa for several different elements (As, Cd, Co, Cu, Mn, Ni, Se, Tl, Zn; Dalla Vecchia et al., 2023; van der Ent et al., 2013; Reeves et al., 2018; Jakovljević et al., 2022). With 523 known hyperaccumulators, Ni is the element that is most frequently accumulated in concentrations above the hyperaccumulation threshold (Reeves et al., 2018). The main mechanisms of metal tolerance in (hyper)accumulator plant species include compartmentalization in specific cell types and complexation by binding of metals to ligands, such as thiols (Leitenmaier & Küpper, 2013). The vacuoles of leaf epidermal cells are the most important sites for the deposition of metals, as this prevents their toxic effect from inhibiting photosynthesis. However, with increasing concentrations, an excess of metals is transported into the mesophyll, which has a negative effect on photosynthesis (Küpper et al., 2001) and limits the hyperaccumulation potential of the species.

In contrast to the leaves, the flowers are rarely strongly enriched with metals and metalloids and are therefore underrepresented in the analysis of hyperaccumulation. However, in *Stanleya pinnata* and *Astragalus bisulcatus*, both Se hyperaccumulators, Se has been shown to be predominantly deposited in the flowers, which led to a more intensive investigation of the absorption mechanism and chemical speciation (Quinn et al., 2011; Valdez Barillas et al., 2012). The reproductive organs were found to be important deposition sites for Ni in *Noccaea fendleri* subsp. *glauca* with no major differences in concentrations between plant parts (Meindl et al., 2014), and in *Streptanthus polygaloides*, with up to 5300 mg kg^{-1} Ni in the flowers (Sánchez-Mata et al., 2014), which clearly exceeds the hyperaccumulation threshold (1000 mg kg^{-1}; van der Ent et al., 2013). Metal concentrations above the hyperaccumulation threshold were also detected in the flowers of *Hybanthus floribundus* (5000 mg kg^{-1}; Severne, 1974), *Odontarrhena* (formerly *Alyssum*) *bertolonii* (7940 mg kg^{-1}; Gabbrielli et al., 1997), and *Stackhousia tryonii* (8400 mg kg^{-1}; Batianoff et al., 1990), but the distribution of elements within the floral parts remained unknown. Considering their abundance, an analysis of the elemental distribution in the flowers of Ni hyperaccumulator species, mainly belonging to the genera *Noccaea* and *Odontarrhena* (Reeves et al., 2018), would be of particular interest. In addition, the simultaneous hyperaccumulation of at least two elements has also been confirmed in *Noccaea* species, although their interdependence is not always unambiguous (Deng et al., 2019; Kozhevnikova et al., 2021). *Noccaea caerulescens*, a model species for studying metal tolerance, frequently takes up Zn, Cd, and Ni above the thresholds for hyperaccumulation, but with considerable differences between populations (Assunção, Schat, & Aarts, 2003; Deng et al., 2019; Kozhevnikova et al., 2020; Sterckeman et al., 2017). The hyperaccumulation of Zn in *N. caerulescens* is a constitutive trait at the species level (Deng et al., 2019; Seregin et al., 2021) with foliar concentrations of up to 53,000 mg kg^{-1} (Reeves et al., 2001), whereas Ni and Cd are accumulated differently on contrasting substrates (Assunção et al., 2008; Kozhevnikova et al., 2020) with concentrations of up to 16,200 and 3410 mg kg^{-1}, respectively (Reeves et al., 2001). *Noccaea praecox* also inhabits metalliferous areas with excessive concentrations of Ni, Zn, Cd, and Pb and has been shown to hyperaccumulate Ni, Zn and Cd. The accumulation of Ni is mainly associated with ultramafic populations (up to 21,000 mg kg^{-1}; Jakovljević et al., 2022), in contrast to Zn whose uptake is inherited (Mišljenović et al., 2020), whereas Cd has been shown to hyperaccumulate in the seeds of plants inhabiting Cd-rich soils (Vogel-Mikuš et al., 2007).

Considering the highly efficient metal uptake, especially of Ni and Zn, the aim of this study is to assess for the first time the elemental distribution in the flowers of *N. praecox* and *N. caerulescens* using synchrotron-based micro-X-ray fluorescence (μXRF) elemental images. These data are complemented by previously unknown elemental localization in the leaves of *N. praecox*.

2 | MATERIALS AND METHODS

2.1 | Plant specimens collected for the synchrotron experiments

The samples were collected in the flowering stage, *N. praecox* was collected in the ultramafics on Mt. Maljen in Serbia (N 44.12229°, E 20.01494°), and samples of *N. caerulescens* were collected at an industrially contaminated site near Lille in France (N 50.6346111°, E 3.1860278°), where they were planted as part of a phytoremediation project carried out by Econick and Pocheco. At both sites, whole living plants were carefully extracted from the soil, wrapped in moist paper, and transported to the synchrotron facility for further analysis, as described below. Prior to analysis, the plant organs were carefully washed with distilled water and blotted dry. The soils associated with *N. praecox* and *N. caerulescens* at the sites of the collection were characterized in previous studies (Mišljenović et al., 2018).

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2.2 | Elemental analysis of plant material samples

For the complimentary bulk elemental analysis, the plant material was dried in a dehydrating oven at 60°C for at least 48 h and then ground in an impact mill (IKA Tube-Mill 100 Control) to a fine powder (<200 μm) and 0.5 g subsamples were inserted into custom-made XRF sample holders and covered with an 6 μm thin polypropylene film (Chemplex Industries Inc.) for XRF analysis. The XRF analysis of the plant powdered material was performed using a Z-Spec JP500 instrument (Z-Spec Inc.). The instrument uses monochromatic X-ray fluorescence excitation at 17.48 keV to analyze elements $Z = 15$ (P) to $Z = 39$ (Y) on the K-lines and up to $Z = 92$ (U) on the L-lines with optimum sensitivity for elements As-Cu–Se and Hg-Tl-Pb-Zn, with the detection limits (LODs) ranging from 0.009 to 0.030 mg kg^{-1}. Samples were analyzed for 30 s in the plant mode setting.

2.3 | Synchrotron-based μXRF experiments

One individual of each species was subjected to synchrotron-based μXRF experiments, carried out at PETRA III, at the X-ray microprobe beamline P06 (Boesenberg et al., 2016), within the DESY (Deutsches Elektronen-Synchrotron). The instrumental parameters and setup were described in detail in van der Ent et al. (2023). Inflorescences were measured with an incident X-ray energy of 18 keV for the entire experiment and the X-ray beam was focused to 3.57 μm × 920 nm (h × v) using KB mirrors, which resulted in a flux of approximately 1.25×10^{11} ph/s at the focus. For the detection of XRF, a Vortex ME4 in 45° geometry was used together with a prototype 16-element Silicon Drift Ardesia detector (Utica et al., 2021) in a geometry of 315° and Xspress 3-pulse processors.

2.4 | Data processing and statistical analyses

To process the XRF spectra non-linear least-squares fitting was used, as implemented in PyMCA (Solé et al., 2007). In combination with the data from elemental calibration foils, 32-bit .tiff images were created with pixel values corresponding to the areal density of the respective element in μg cm^{-2}. The ImageJ software (Schindelin et al., 2012) was used to prepare the figures. For the visualization, the look-up tables (LUT) were first changed to 'Fire' and then the maximum values were adjusted, and concentration bars and length scale were added.

3 | RESULTS AND DISCUSSION

The distribution of Ni and Zn in the flowers and inflorescences of *N. praecox* shows a similar pattern (Figure 1). The highest concentrations of both elements were found in the pedicels, particularly in the lower and older parts of the inflorescence, with Ni being more evenly distributed compared to Zn. In the younger parts, however, the highest concentrations were found in the upper parts of the pedicels and receptacles (Figure 1a,b). This suggests that the receptacles are one of the hotspots for the localization of Ni and Zn, with redistribution occurring through the pedicel as the plant grows and accumulates a higher concentration of the elements. Another important hotspot for localization are the anthers, both for Ni and Zn, with a greater accumulation in the pollen sacs of flowers from the lower parts of the inflorescences. The ovaries were also rich in Ni, especially in the walls and ovules (Figure 1c). The distribution of Zn in the flowers of *N. praecox* was similar, with slightly lower concentrations as the main difference. This was particularly noticeable in the anthers and the walls of the ovary, whereas the concentrations in the receptacle were quite similar. In contrast to Ni, slightly higher Zn concentrations were found in the style of the pistil, increasing toward the stigma (Figure 1d).

Synchrotron μXRF elemental maps showed that the highest Ni concentrations in the flowers of *N. caerulescens* were found in the ovules and the ovary walls, although elevated concentrations were also found in the peduncle (Figure 2a). A strong accumulation of Zn was also observed in the pistils, especially in the ovaries and their walls, and to a lesser extent in the style and stigma, whereas the role of the anthers in the localization of Ni and Zn was not as pronounced as in *N. praecox* (Figure 2b). Higher elemental concentrations were found in the stamens in the lower parts of the inflorescence, which is particularly evident in Ni, where the differences between younger and older parts are more pronounced. Compared to the other floral parts, Ni and Zn were underrepresented in the petals and sepals. This is in contrast to the Ni distribution pattern in *S. polygaloides*, where the petals and sepals were found to be the most important localization sites in the flowers, with sepals dominating in the yellow samples, and petals and stamens dominating in the purple individuals (Sánchez-Mata et al., 2014). The contrasting pattern in accumulation was also shown for Se in hyperaccumulators of this element. In *A. bisulcatus*, more Se was found in the petals

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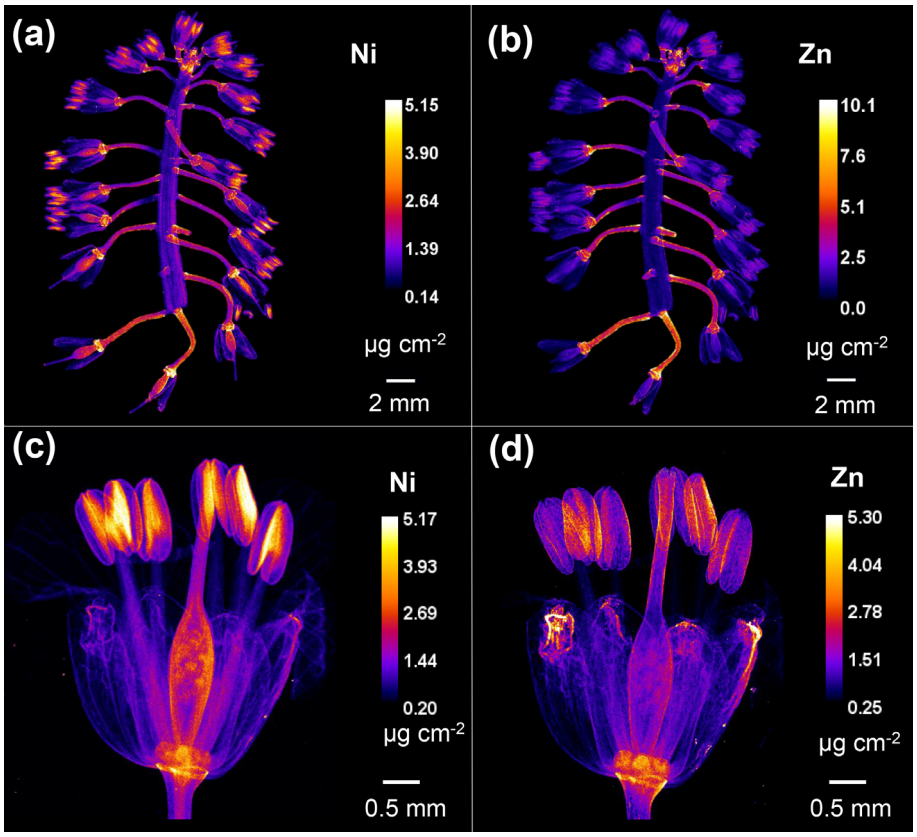


FIGURE 1 Synchrotron µXRF elemental maps showing the distribution of Ni and Zn in the inflorescences (a & b); and in the flowers (c & d) of *Noccaea praecox*.

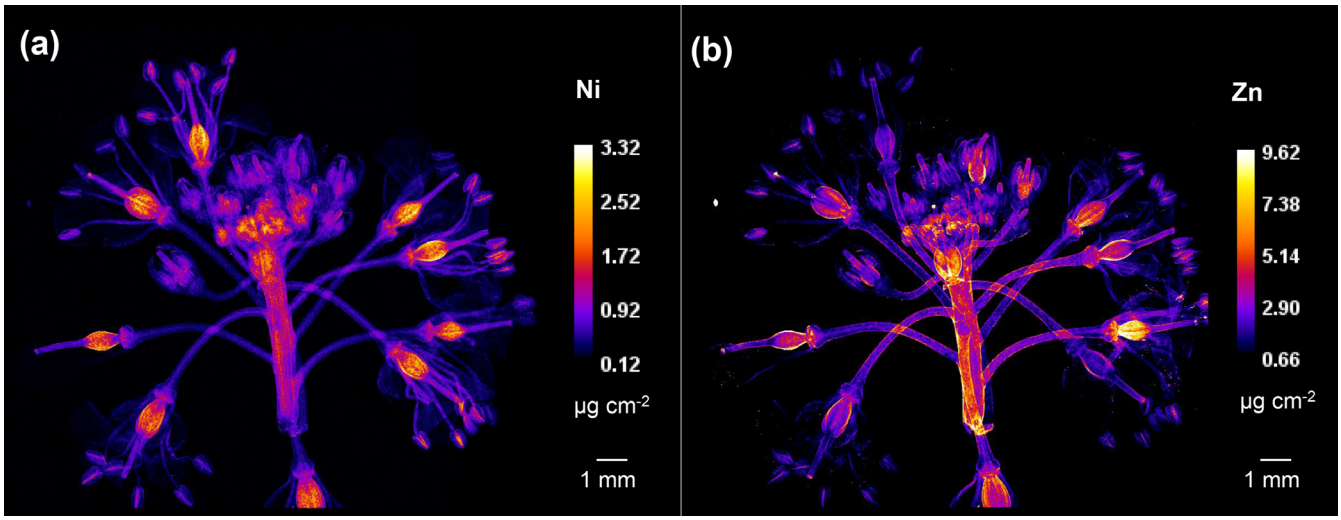


FIGURE 2 Synchrotron µXRF elemental maps showing the distribution of Ni (a) and Zn (b) in the inflorescences of *Noccaea caerulescens*.

and sepals than in the pistils and stamens, with increased concentrations also found at the base of the anthers (Valdez Barillas et al., 2012), whereas in *S. pinnata* the ovules of the pistil and the pollen grains at the anther tips are the primary sites of Se localization (Quinn et al., 2011). The anthers also proved to be important for Ni deposition in *Noccaea fendleri* subsp. *glauca*, which at 3000 mg kg^{-1} Ni is well above the hyperaccumulation threshold for this element (Meindl et al., 2014). The allocation of metals and metalloids in the reproductive organs, pistils and anthers, could be important for reproductive efficiency and influence the interaction with pollinators. An increased concentration of Ni and Zn can have a favorable effect on hyperaccumulators of these elements, as they may exhibit higher fitness on metalliferous soils, whereas it can significantly reduce

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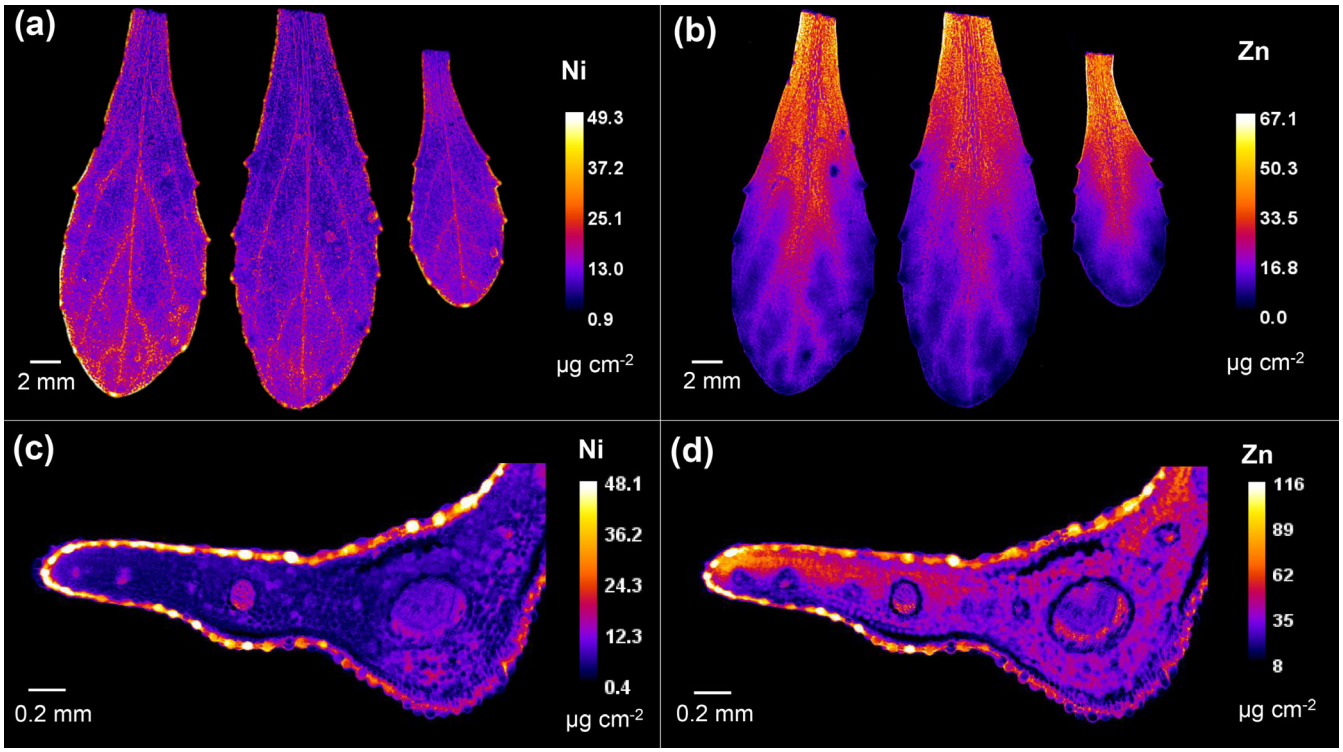


FIGURE 3 Synchrotron μXRF elemental maps showing the distribution of Ni and Zn in the leaves (a & b) and leaf cross-sections (c & d) of *Noccaea praecox*.

reproductive success in non-tolerant species (Meindl & Ashman, 2017). Beneficiary effect was shown in increased flowering (including number and size of the inflorescences; Ghasemi et al., 2014), as well as in pollen and seed germination (Meindl et al., 2014). However, the effect of metals on the reproductive organs of plants depends not only on the metal concentration but also on the speciation of the elements, i.e. on the toxicity of a particular form, as a correlation between higher tolerance and less toxic metal forms in plant flowers has been demonstrated in plants such as *Stanleya pinnata* (Quinn et al., 2011). Although it is a useful defense against herbivory, excessive metal concentrations in nectar can affect the health of pollinators and associated agents in the food chain. Hence, pollen analyses in *Orobanche nowackiana* (Pavlova & Bani, 2019) and several *Odontarrhena* species (Pavlova et al., 2016) showed significantly lower Ni concentrations compared to other parts of the stamens, thus protecting the reproductive potential of the species. In line with this, Ni concentrations in the seeds of two subspecies of *Odontarrhena serpylifolia* from the Iberian Peninsula were significantly lower compared to those in the vegetative organs (de la Fuente et al., 2007). In addition to the impairment of pollinators, the germination rate can also be affected by metal toxicity (Quinn et al., 2011).

In both *N. praecox* and *N. caerulescens*, Ni and Zn were mainly deposited in the leaves (Mišljenović et al., 2020; Nascimento et al., 2021; Regvar et al., 2013). The results of the chemical analyses of *N. caerulescens* leaf samples showed that the concentrations of Ni were in the range of 37–102 mg kg^{-1} (mean is 68 mg kg^{-1}, $n = 4$) and Zn varied from 5180 to 12,000 mg kg^{-1} (mean is 9160 mg kg^{-1}, $n = 4$). Foliar Ni concentrations in *N. praecox* ranged from 4486 to 9600 mg kg^{-1} (mean is 6250 mg kg^{-1}, $n = 5$), while Zn concentrations varied from 1180 to 7480 mg kg^{-1} (mean is 3390 mg kg^{-1}, $n = 5$). Whereas Ni accumulation is mainly related to the ultramafic substrate, the hyperaccumulation of Zn in *N. caerulescens* proved to be a constitutive trait unrelated to soil concentration (Assunção et al., 2003b). In this study, hyperaccumulation of Zn of up to 12,000 mg kg^{-1} was observed while growing on a substrate with only 360 mg kg^{-1} Zn (Jacquet, unpublished). In *N. praecox*, Zn hyperaccumulation was found at sites highly enriched with Zn (Vogel-Mikuš et al., 2005), in contrast to the sample site on Mt. Maljen, where foliar concentrations of up to 7480 mg kg^{-1} were found on the soil with <100 mg kg^{-1} pseudo-total Zn (Mišljenović et al., 2018). *Noccaea praecox* attained Ni concentrations of up to 9600 mg kg^{-1} while growing in soils containing 1280 mg kg^{-1} Ni (Mišljenović et al., 2018). The highest Ni enrichment was found at the leaf margins of *N.*

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praecox (Figure 3a). This accumulation corresponded to the age of the leaf, with the Ni accumulation being highest in the oldest leaves, and a redistribution of the element into the surrounding tissue. A similar distribution with the leaf margins and the tips of the leaf blade as the main site of Ni deposition was also found in the leaves of *N. caerulescens* (Nascimento et al., 2021). The leaf cross-section showed the most extreme Ni concentrations in the epidermal tissue of *N. praecox*, which were more pronounced on the abaxial side. Slightly lower concentrations were found in the vascular system, both in the midrib and in the lateral veins and veinlets, which stood out as sites of increased accumulation in the cross-section (Figure 3c). In addition, the enrichment points were scattered over the entire leaf blade, being most pronounced in the upper leaf area. As their distribution does not correspond to elements that are strongly represented in soil particles, such as Fe and Ca (Figure S1), these localized Ni enrichments could be the result of guttation rather than foliar contamination, as has been observed in some other hyperaccumulator plant species such as *Noccaea japonica* or *Odontarrhena muralis* (McNear et al., 2005; Mizuno et al., 2003). The distribution pattern of Zn is similar to that of Ni in the leaf cross-section of *N. praecox*. The strongest enrichment was found in epidermal cells and more on the abaxial side, but there was also some accumulation in the vascular bundles. In contrast to Ni, however, conspicuously high concentrations of Zn were also found in the spongy mesophyll and were highest in the leaf blades (Figure 3d). Significant differences were also observed in the lamina, where Zn uptake occurred mainly in the basal parts, whereas concentrations in the distal part were insignificant, and Zn was only present in the leaf veins, decreasing toward the upper part of the leaf (Figure 3b). The highest enrichment of Zn around the central and secondary veins was also found in the leaves of *N. caerulescens* (Nascimento et al., 2021). The vascular system proves to be the main transport pathway for both Ni and Zn, with the epidermis being the sink tissue for Ni, where its excessive concentrations are deposited, whereas Zn is mainly retained in the basal part of the leaf.

4 | CONCLUSIONS

The leaf epidermal tissue and its vacuoles have been shown to be the main deposition site for Ni in several, mainly hyperaccumulator plant species from the Brassicaceae family, with *Noccaea* spp. and *Odontarrhena* spp. being the most abundant among the Ni-hypertolerant species (Broadhurst et al., 2004; Küpper et al., 2001; Tappero et al., 2007), suggesting that compartmentalization is one of the main mechanisms for detoxification of the metal. However, high concentrations of Zn and Ni were also found in the flowers, especially in the pistils and anthers, with possible negative effects on fertility and pollinators.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest relevant to the content of this manuscript.

DATA AVAILABILITY STATEMENT

The data that support this study will be shared upon reasonable request to the corresponding author.

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