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NOTES AND INSIGHTS

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# Synchrotron µXRF imaging reveals elemental distribution in the nickel hyperaccumulator *Odontarrhena muralis* (Brassicaceae) from Serbia

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**Abstract**

*Odontarrhena muralis* (Brassicaceae) is a nickel hyperaccumulator species from the Balkans used as a "metal crop" in nickel phytomining. This study aimed to determine the elemental distribution, focusing on nickel, in fresh-hydrated plant tissue (stems, leaves and inflorescences), to clarify where nickel is localized at the tissue and cellular scale-level and to infer the physiological response to its hypertolerance and hyperaccumulation. For the analysis, intact plant organs of *O. muralis* were subjected to elemental imaging using synchrotron-based micro-X-ray fluorescence (µXRF). The predominant distribution of nickel occurs in the epidermal tissue and at the base of the trichomes, which are also the main sinks for calcium deposition. The obtained results represent a further contribution to the knowledge of the physiological characteristics of this hyperaccumulating "metal crop" species and, consequently, to its application in sustainable metal extraction using phytomining.

**KEYWORDS**

agromining, *Alyssum*, elemental imaging, hyperaccumulator, synchrotron-based micro-X-ray fluorescence (µXRF)

## 1 | INTRODUCTION

Hyperaccumulators are plants that have the ability to concentrate exceptionally high concentrations of particular metals or metalloids in their above-ground organs (Reeves et al., 2018; van der Ent, Baker, Reeves, et al., 2013). In addition to their application in phytoremediation, this specific ability of hyperaccumulator plant species has been used to sustainably obtain target metals, mainly nickel, from traditionally uneconomic resources, including low-grade ultramafic soils, using the commercial technology of phytomining (Rosenkranz et al., 2017; van der Ent et al., 2021; van der Ent, Baker, van Balgooy, & Tjoa, 2013). Nickel is an element whose hyperaccumulation is most common in nature. So far, 532 taxa have been found to take up Ni at concentrations above the provisional threshold of 1000 mg kg$^{-1}$ (Reeves, 2003; van der Ent, Baker, Reeves, et al., 2013). Most of these species belong to the fam. Brassicaceae (Reeves et al., 2018) and *Odontarrhena*, with Ni hyperaccumulation confirmed in more than 50 taxa of this genus (Morrison et al., 1980; Reeves et al., 2018). *Odontarrhena muralis* (Waldst. & Kit.) Endl., formerly known as *Alyssum murale*, together with *O. chalcidica,* is the most intensively studied Ni hyperaccumulator species (Bettarini et al., 2021; Nascimento et al., 2020; Paul et al., 2020) as well as the most important "metal crop" for phytomining in Mediterranean-type climate (Bani et al., 2021; Cerdeira-Pérez et al., 2019). It is predominantly associated with ultramafic soils and in some areas even considered as an indicator for this type of substrate, with Ni concentrations depending largely both on plant characteristics and environmental conditions (Jakovljević et al., 2022). The leaves proved to be the main organ for the deposition of accumulated Ni, and some studies pointed to leaf epidermal tissue and its vacuoles as the hotspot of its distribution (Broadhurst et al., 2004; McNear et al., 2005; Tappero et al., 2007). The sequestration of metals in cells and intracellular compartments is one of the most important plant tolerance mechanisms, alongside precipitation, using redox mechanisms for conversion into the less toxic forms, or binding to the ligands (Leitenmaier & Küpper, 2013). Although the localization pattern of metals is of great importance for the understanding of the underlying mechanisms responsible for metal tolerance and hyperaccumulation, their distribution in the various plant parts is not yet fully understood. Therefore, this study aimed to investigate the distribution of Ni and other elements using synchrotron micro-X-ray fluorescence (μXRF) elemental imaging in fresh-hydrated plant organs of *O. muralis*.


**FIGURE 1** *Odontarrhena muralis* growing in its natural habitat on ultramafic soil (with serpentinite rocks) in Mt. Divčibare, Serbia: (a) habitat; (b) inflorescence; and (c) plant habitus.

## 2 | MATERIALS AND METHODS

### 2.1 | Plant material for the synchrotron investigations

The plant samples used in the experiment were collected in ultramafics of Mt Divčibare, in North-western Serbia (Figure 1). Intact plants were transported to the laboratory and fresh-hydrated organs were subjected X-ray fluorescence microscopy experiments, to obtain the maps of elemental distribution in specific plant organs (van der Ent et al., 2018). The material was identified following the relevant literature (Diklić, 1972), field experience and by examining the herbarium material deposited in BEOU (Herbarium of the University of Belgrade). However, detailed taxonomic analyses of the Serbian *Odontarrhena* populations are still pending, so the investigated taxa should provisionally be referred to as *O. muralis s.l.* The nomenclature was harmonized with the Plants of the World Online Database (POWO, 2023). The voucher specimen was deposited in BEOU (No. 17986).

### 2.2 | Elemental analysis of plant material samples

The plant material was dried in a dehydrating oven at 60°C for three days, ground in an impact mill (IKA Tube-Mill 100 Control) and analyzed with 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 Cu–Se and Hg–Tl–Pb with LODs ranging from 0.009 to 0.025 mg kg$^{-1}$. Samples were analyzed for 30 s in plant mode.

### 2.3 | Synchrotron μXRF experiments

The μXRF experiments were performed at DESY (Deutsches Elektronen-Synchrotron) on the hard X-ray microprobe undulator beamline P06 (Boesenberg et al., 2016) at PETRA III. Detailed information on the setup and instrumental parameters has been provided in van der Ent et al. (2023). The XRF measurements were performed during two separate experiments with KB mirrors as main focusing optics in both cases: The flower was measured with an incident X-ray energy of 16 keV, beam size of 560 nm × 420 nm (h × v) and a photon flux of $3.5 \times 10^{10}$ ph/s, while the leaves and stems were measured at 18 keV with 3.57 μm × 920 nm (h × v) beam size and $1.25 \times 10^{11}$ ph/s photon flux using additional pre-focusing CRLs (Compound Refractive Lenses). A Vortex ME4 in 90° (flower) or 45° (leaves, stems) geometry and a prototype 16-element Silicon Drift Detector (Utica et al., 2021) in 270° (flower) or 315° (leaves, stems) geometry with Xspress 3 pulse processors were used for XRF detection.

### 2.4 | Data processing

Non-linear least-squares fitting of the XRF spectra was performed in PyMCA (Solé et al., 2007), generating 32-bit

**TABLE 1** Elemental concentrations (in mg $kg^{-1}$) in composite bulk samples of *Odontarrhena muralis* analyzed by monochromatic XRF.

| Plant sample | K | Ca | Cr | Fe | Ni | Cu | Zn |
|---|---|---|---|---|---|---|---|
| Leaves | 19,400 | 52,000 | 4.4 | 664 | 3930 | 10.6 | 40 |
| Roots | 6950 | 1890 | 0.8 | 85 | 549 | 3.3 | 13 |
| Stems | 20,600 | 10,800 | 0.9 | 138 | 1670 | 5.2 | 40 |

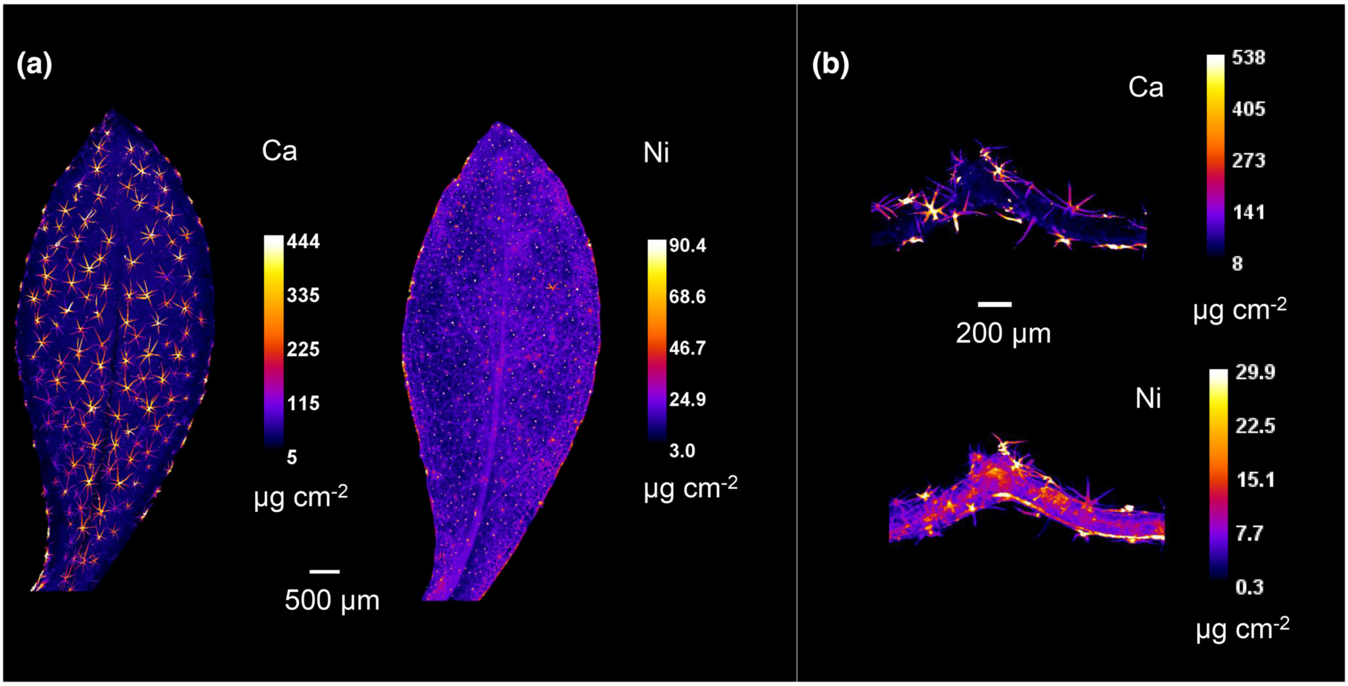


**FIGURE 2** Synchrotron μXRF elemental maps showing the distributions of Ni and Ca in (a) whole leaves (adaxial side); and (b) leaf cross-sections of *Odontarrhena muralis*.

.tiff files with pixel values corresponding to the areal density of the respective element in μg $cm^{-2}$ after quantification using elemental calibration foils. The images were processed with the software ImageJ (Schneider et al., 2012). After changing LUT to "Fire," the maximum values were adjusted, and concentration bars and length scales were added.

## 3 | RESULTS AND DISCUSSION

XRF analysis of elemental distribution in plant leaves shows that Ni, the element taken up in the highest concentrations in *O. muralis*, accumulates mostly in the epidermal tissues, with high concentrations of this element also found at the base of the trichomes (Figure 2). Nickel reaches 3930 mg $kg^{-1}$ in leaves and 549 mg $kg^{-1}$ in roots as determined by monochromatic bulk XRF analysis (Table 1). The vacuoles of most epidermal cells, both on the abaxial and adaxial side, were found to be the main site of deposition of Ni, although high concentrations of other metals are also found here (Broadhurst et al., 2004; McNear et al., 2005; Tappero et al., 2007). Somewhat higher Ni concentrations were found in the basal part of the leaves, but also in the midrib region, as already noted by Tappero et al. (2007). Deposition in the vascular system was less significant, but much higher than in the lamina. The accumulation of Ni in the epidermal tissue and trichome base as the dominant pattern of elemental distribution was even more pronounced as seen in synchrotron μXRF elemental maps of leaf cross-sections (Figure 2b). Strikingly high concentrations of Ca were also found in the leaf samples, with the highest concentrations in the trichomes (Figure 2). The trichomes were already pointed to as the main sink of Ca deposition in the leaves of *O. muralis* with approximately equal measured concentrations of this macroelement (McNear et al., 2005; Nascimento et al., 2020). In contrast to Ni, which is deposited in trichomes primarily as part of a detoxification process (Hauser, 2014), the accumulation of Ca plays a role in protecting plants from predators and increasing resistance to water deficits (McNear & Küpper, 2014). Concentrations of Zn in plant leaves are significantly lower compared with Ni and especially compared with Ca as a macroelement, with some

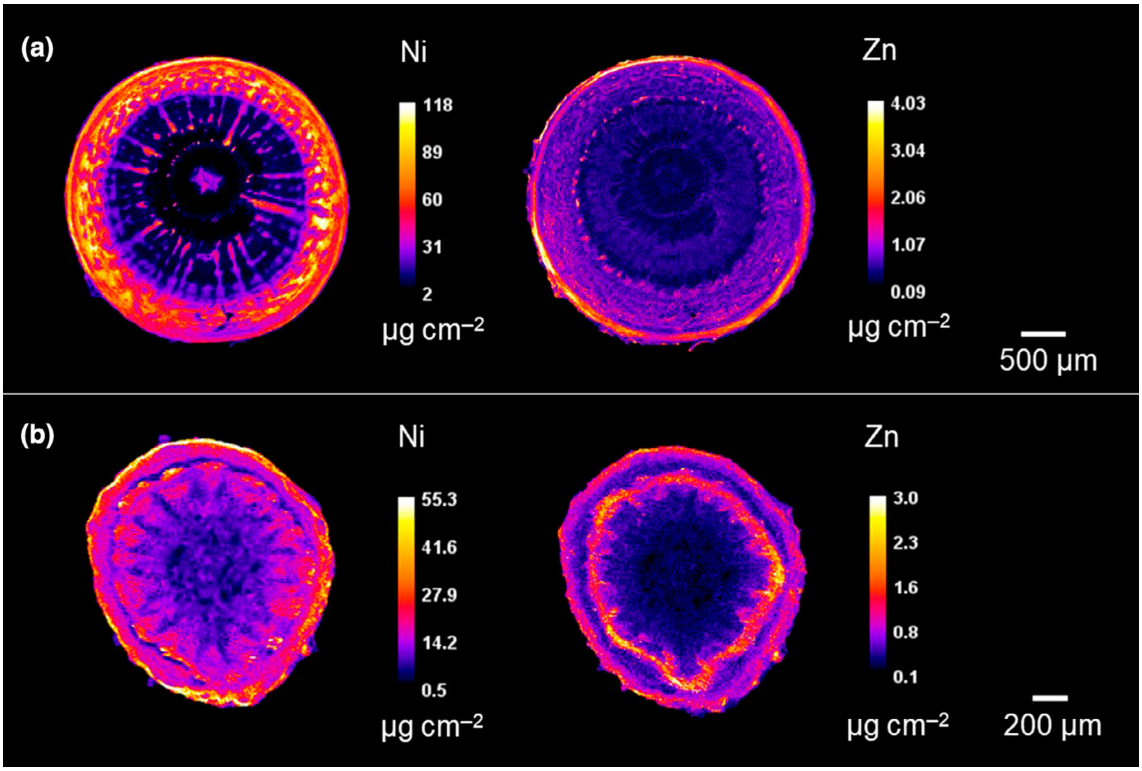


**FIGURE 3** Synchrotron µXRF elemental maps showing the distributions of Ni and Zn in cross-sections of (a) mature stems; and (b) young stems of *Odontarrhena muralis*.

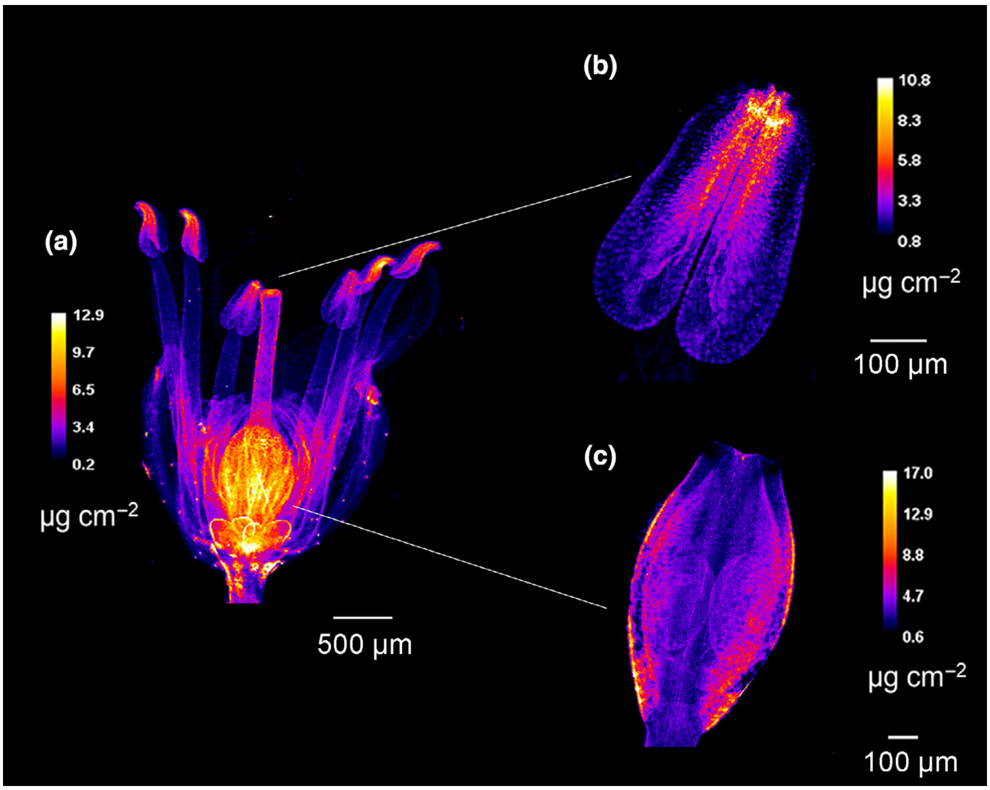


**FIGURE 4** Synchrotron µXRF elemental maps showing the distributions of Ni in (a) flower; (b) anther; and (c) ovary of *Odontarrhena muralis*.

enrichment around vascular bundles and epidermal tissue on the abaxial side of leaves (Figures S1 & S2). Considerably lower Zn concentrations were also found by monochromatic XRF analyses (Table 1).

Patterns of elemental distribution were compared in young and old stems, considering previous evidence that the mechanism of elemental uptake depends largely on the stage of development of the plant (Kashiwabara et al., 2021; Yang et al., 2020). Although the absorbed concentrations of Ni and Zn differ markedly, they are highest for both elements in the epidermal tissues and vascular bundles of young stems, the accumulation of Ni being more pronounced in the epidermis, whereas Zn is predominantly taken up into the vascular system. In mature stems, the redistribution of elements is evident, especially for Zn, where concentrations do not change significantly, but the epidermis becomes the main site of deposition, and the vascular system plays a much weaker role. In the distribution of Ni, the epidermis takes on an even more important role, with intense deposition also observed in the endodermis, and concentrations of this element are notably higher compared to young stems (Figure 3). A redistribution was also found in other analyzed elements (Figure S3). The highest enrichment of Ni in the flowers of *O. muralis* was found in the ovaries, as shown by the synchrotron μXRF elemental maps (Figure 4), with the primary site of Ni deposition being the ovary wall. In the anthers, the highest Ni concentrations are found in their basal parts, whereas the concentration of this element clearly decreases toward the distal parts. A similar enrichment in the ovary walls and anthers was found for Zn, K and Ca (Figure S4).

*Odontarrhena muralis* is one of the most important metal "crops" for Ni phytomining in Mediterranean climate, which puts into focus the molecular and physiological mechanisms involved with the accumulation of metals, especially Ni, as the primary hyperaccumulating element. This study represents a further contribution to the knowledge of the characteristics of the species and thus to its potential application in sustainable metal extraction using phytomining.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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## SUPPORTING INFORMATION

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