Ecological Research

RESEARCH ARTICLE OPEN ACCESS

Special Feature—Ultramafic Ecology 2025: Proceedings of the 11th International Conference on Serpentine Ecology

Synchrotron Micro-X-Ray Fluorescence Elemental Imaging Reveals Zinc Distribution in the Hyperaccumulator *Sedum plumbizincicola* (Crassulaceae)

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Received: 31 July 2025 | **Revised:** 29 September 2025 | **Accepted:** 17 October 2025

Keywords: elemental localization | hyperaccumulator | intercropping | *Noccaea* | phytoremediation

ABSTRACT

Sedum plumbizincicola is a zinc–cadmium (Zn–Cd) hyperaccumulator native to China with high potential for use in the phytoremediation of contaminated soils in temperate climates. This study aimed to determine the Zn accumulation and distribution in *S. plumbizincicola* tissues grown on soils co-contaminated with Cd, Pb, and Zn. The efficiency of Zn accumulation was assessed in monoculture and intercropping systems with *Noccaea caerulescens*. The samples were analyzed by inductively coupled plasma–atomic emission spectrometry and synchrotron micro-X-ray fluorescence elemental imaging. *Sedum plumbizincicola* grown in monoculture had significantly higher foliar Zn concentrations than the plants grown with *N. caerulescens*, with the leaf tips, petioles and nodes being the main sites of Zn localization in the aerial parts. The highest Zn concentrations were observed in the epidermis and vascular system of both leaves and stems, with the distribution pattern differing between young and mature leaves. This study highlights the Zn localization patterns in *S. plumbizincicola* to improve our understanding of the underlying mechanisms of Zn hyperaccumulation. Growing in monoculture, *S. plumbizincicola* is an effective candidate for Zn agromining or phytoremediation of Zn-Cd contaminated soils, with less promising results when intercropped with *N. caerulescens*.

1 | Introduction

High prevailing metal or metalloid concentrations in the soil can induce various tolerance responses in plants, with exclusion from uptake being the most frequently observed defense mechanism (Bothe 2011). In some plants, however, the concentrations of elements taken up by the roots and translocated to the aerial parts exceed the total concentrations in the soil, and can be 2–3 orders of magnitude higher than those in other plant species growing on the same soils. These extraordinairy species are known as hyperaccumulator plants (Brooks et al. 1977; van der Ent et al. 2021). The concentrations recognized as thresholds for hyperaccumulation are element-specific (van der Ent et al. 2013). To date, hyperaccumulation of nickel (Ni) has been the most frequently recorded (523 taxa; Reeves et al. 2018) and intensively studied in relation to Ni uptake, translocation and sequestration in the aerial parts of plants. In contrast, hyperaccumulation of zinc (Zn) is thus far found in 20 taxa around the world (Reeves et al. 2018), mainly in plants growing on calamine soils, which are soils characterized by high concentrations of cadmium (Cd), lead (Pb), and Zn (Wójcik et al. 2017). Interestingly, Zn hyperaccumulation can also occur in plant species growing on 'normal' soils with background Zn concentrations, which typically range between 10 and 100 $\mu g\, g^{-1}$. This Zn hyperaccumulation as a constitutive trait is best known from *Noccaea caerulescens* and *Arabidopsis halleri*, both model species for hyperaccumulation of not only Zn, but also Cd and Ni in the case of the former (Meyer and Verbruggen 2012; Dinh et al. 2015; Stein et al. 2017; Merlot et al. 2021).

Hyperaccumulation of Zn and Cd, as found in *N. caerulescens* and *A. halleri*, is also known in *Sedum plumbizincicola*, one of only a few examples outside the Brassicaceae family that hyperaccumulate Zn and/or Cd (Li et al. 2018). *Sedum plumbizincicola* is a relatively recently discovered hyperaccumulator species, known only from its type locality in Zhejiang Province, China (Wu et al. 2013), with concentrations of 14,600 $\mu g\,g^{-1}$ Zn and 1470 $\mu g\,g^{-1}$ Cd in shoots in its native habitat (Hu et al. 2015), and more than 18,000 $\mu g\,g^{-1}$ Zn and 7000 $\mu g\,g^{-1}$ Cd in shoots of hydroponically grown plants (Cao et al. 2014). The extremely high Cd and Zn concentrations in the leaves of *S. plumbizincicola* indicate its potential for use in agromining since, at a biomass of 4–12 $t\,ha^{-1}$, the removal efficiency in monoculture was estimated to be 215–515 $g\,ha^{-1}$ Cd and 15–40 $kg\,ha^{-1}$ for Zn (Deng et al. 2016). The multi-contamination tolerance of *S. plumbizincicola*, together with its perennial nature, rapid growth, high biomass production, and easy vegetative propagation from cuttings allowing two–three potential harvests per year, add to the value of this process (Li et al. 2009; Hu et al. 2015; Wu et al. 2021; Song et al. 2022). In China, *S. plumbizincicola* has already been tested in monoculture under different climatic and edaphic conditions; however, trials outside this area are extremely limited. According to the available literature, only one field trial has been carried out in Europe (Angelova 2020). In that study, conducted in sub-alkaline soils (pH 7.7) in Bulgaria, a strong translocation potential for Cd, Pb, and Zn (TF_{Cd} and $TF_{Pb} > 2$, and $TF_{Zn} > 4$) was observed, exceeding the hyperaccumulation thresholds for Cd.

The tolerance of *S. plumbizincicola* to water deficits and shade also enables its cultivation in co-planting systems with crops, such as wheat, maize, rice, sugar cane, and cucumber (Zhao et al. 2011; Deng et al. 2016; Wu et al. 2021). Intercropping is an agronomic strategy that is increasingly used in combination with crop density management practices, plant growth-promoting bacteria and fungi, fertilizers and varietal selection to improve phytoremediation efficiency (Chaney et al. 2007; Kidd et al. 2015; Hossain et al. 2017; Bani et al. 2021; Benizri et al. 2021; Veerapagu et al. 2023; Wan et al. 2023). Phytoextraction efficiency in intercropping can be enhanced by mitigating adverse environmental effects on hyperaccumulator species, by lowering pH and increasing elemental availability, or by overyielding when two hyperaccumulator species with different and complementary ecological niches are grown together (Koelbener et al. 2008). For example, intercropping *S. plumbizincicola* with maize reduced the total concentration of Zn and Cd in the soil by 18.8% and 85.5%, respectively (Deng et al. 2016). In the remediation of multi-contaminated soils, the use of two (hyper)accumulating plant species can be particularly beneficial as the species enable complementary elemental accumulation when targeting different metals (Wang et al. 2022). However, the use of hyperaccumulators of the same elements may lead to accumulation with different efficiency, which is not only species-specific but may also strongly depend on the ecotype used (Jacquet et al. 2025).

Considering the potential of *S. plumbizincicola* for agromining, this study aimed to investigate the accumulation and distribution patterns of Zn in the shoots of plants grown under real field conditions (soils co-contaminated with metals) in temperate climates, that is, outside the species' native range, using synchrotron micro-X-ray fluorescence analysis (μXRF), to better understand the mechanisms of hyperaccumulation. The accumulation capacity and patterns of Zn distribution were additionally analyzed when *S. plumbizincola* was grown in co-culture with *N. caerulescens*, another Zn hyperaccumulator species.

2 | Materials and Methods

2.1 | Plant Culture Conditions

Seeds of *N. caerulescens* (Ganges ecotype, characterized in Gonneau et al. 2014) were collected at a former mining site in southern France in June 2019 and sown under greenhouse conditions in germination trays filled with horticultural compost. Cuttings of *S. plumbizincicola* were propagated at the Ecoplantes Nursery (located in Lunéville, Lorraine, France) from one individual collected at a contaminated site in China. The experiment was conducted under field conditions in a contaminated urban garden in Forest-sur-Marque (near Lille, northern France). *Sedum plumbizincicola* was grown in monoculture (Figure 1) at a density of 36 plants/m^2 for a 10-month cultivation period. To assess the effects of intercropping on Zn accumulation and distribution, *S. plumbizincicola* was intercropped with *N. caerulescens* for the same period. In co-cultivation, *S. plumbizincicola* was planted at 8 plants/m^2 and *N. caerulescens* at 32 plants/m^2. The experiment started from late June to early July 2022, with *S. plumbizincicola* directly planted as 8-cm-tall vegetative cuttings and *N. caerulescens* transplanted as 12-week-old seedlings. Harvesting and sampling took place twice, in September and April of the following year. *Sedum plumbizincicola* was harvested only in the vegetative stage, while *N. caerulescens* was harvested either at the vegetative or flowering stage, depending on the plant.

2.2 | Soil Analysis

Before planting, a composite soil sample was collected from the surface layer (0–20 cm) by mixing five subsamples taken in a cross-shaped pattern. Soil samples underwent air-drying at 40°C for 48 h, followed by sieving through a 2 mm mesh. Pseudo-total metallic trace element (MTE) concentrations were determined following aqua regia digestion and subsequent analysis by inductively coupled plasma-atomic emission spectrometry (ICP-AES, iCAP 6000 series, Thermo Scientific, Cambridge, UK), in accordance with the NF ISO 11464 standard. Five hundred milligrams of soil ground to 250 μm were digested in 6 mL of HCl and 2 mL of HNO_3 at room temperature for 16 h before heating in a DigiPREP system at 105°C for 3 h. The digested samples were filtered through 0.45 μm membranes and diluted to a final volume of 50 mL with ultrapure water. The analysis revealed moderate co-contamination with pseudo-total Zn (350 $\mu g\,g^{-1}$), Pb (120 $\mu g\,g^{-1}$), and Cd (1.5–2 $\mu g\,g^{-1}$). The potential availability of MTEs for hyperaccumulators was assessed based on five additional composite soil samples collected from the surface horizon, following

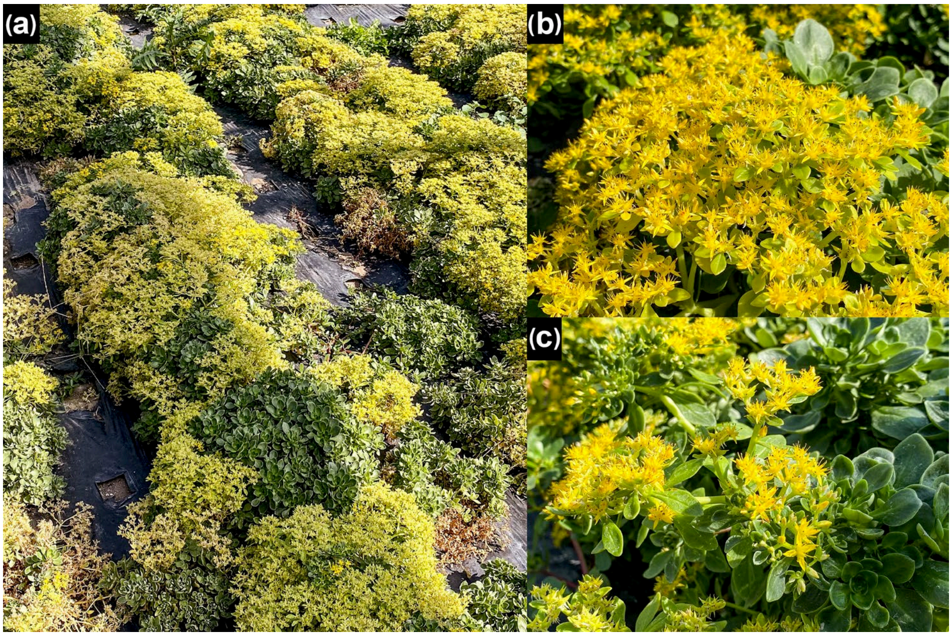

FIGURE 1 | *Sedum plumbizincicola* growing in monoculture at a density of 36 plants m^{-2} (a) with details on the inflorescences (b) and leaves (c).

the same protocol. This was evaluated by measuring soil water pH (NF ISO 10390) and the concentrations of MTEs extractable with a DTPA solution (diethylenetriaminepentaacetic acid, NF ISO 14870) using ICP-AES. DTPA-extractable Zn levels ranged from 13 to 40 μg g^{-1}, exceeding the possible toxicity threshold for standard crops (> 10 μg g^{-1}; Lindsay and Norvell 1978), and soil water pH varied from 6.0 to 6.5, indicating a chemical potential for Zn phytoextraction. DTPA-extractable Cd and Pb concentrations were below the ICP-AES quantification limits.

2.3 | Bulk Elemental Analysis of Plant Samples

After 3 and 10 months of cultivation, whole plants of *N. caerulescens* and *S. plumbizincicola* were collected from each treatment and bulked to make composite samples. Prior to analysis, the aerial parts of the plants were carefully washed with tap water and rinsed with deionized water to remove soil dust and particles. The plant material was dried in an oven at 60°C for at least 48 h. Plant organs were ground to a fine powder (< 200 μm) in an impact mill and weighed at 50 ± 5 mg in 15 mL polypropylene tubes. These samples were pre-digested with 1 mL HNO_3 (70%) and 2 mL H_2O_2 (30%) for 16 h and then digested in a block heater (DigiPREP MS, SCP SCIENCE) for 3 h (ramped up and held at 95°C). Samples were then diluted to 10 mL with ultrapure water (Millipore 18.2 MΩ cm^{-1} at 25°C) and filtered through 0.45 μm syringe filters before analysis by ICP-AES.

2.4 | Synchrotron μXRF Experiments

The synchrotron micro-X-ray fluorescence analysis was performed at PETRA III (Deutsches Elektronen-Synchrotron DESY), a 6 GeV synchrotron radiation source, specifically at the hard X-ray microprobe undulator beamline P06 (Boesenberg et al. 2016). Beamline P06 is equipped with a cryogenically cooled double-crystal monochromator with Si (111) crystals and the X-ray beam can be focussed down to the sub-micrometer range using different focussing optics. An ion chamber upstream of the sample is used to monitor the incoming flux, while a 500 μm thick Si PIPS diode with an active area of 19 mm diameter (PD300-500CB, Mirion Technologies (Canberra) GmbH, Germany) downstream of the sample can be used to record the transmitted X-ray intensity in order to extract absorption data. Multiple XRF detectors enable the measurement of X-ray fluorescence data. The incident X-ray energy was 18 keV throughout the experiment and the beam was focused to 3.57 μm × 920 nm ($h \times v$) using KB mirrors and prefocusing compound refractive lenses (CRLs), resulting in a flux of approximately 1.25^{11} ph/s at the focus. For XRF detection, both a Vortex ME4 in 45° geometry and a prototype 16-element SDD Ardesia detector 800 μm thick chip with a 324 mm^2 combined active area for all 16 elements, Politecnico Milano, Italy (Utica et al. 2021) in 315° geometry with Xspress 3 pulse processors were used. The fresh/live plant specimens were brought from the experimental field (Forest-sur-Marque, France) to the beamline at DESY in Hamburg. At the beamline, the plant organs were sectioned by hand using a steel razor blade ("dry knife method"), and mounted between two layers of thin film of 4.0 μm thickness (Cole-Parmer SamplePrep 3525) stretched over a plastic frame in a tight sandwich. The μXRF elemental imaging then took place within 15 min. of mounting the sample.

2.5 | Data Processing

Data acquisition was managed by a custom workflow (Garrevoet 2025), and the XRF spectra were processed using non-linear least squares fitting as implemented in PyMCA (Solé et al. 2007). After calibration using metal foils, this produced 32-bit .tiff files with pixel values corresponding to the μg cm^{-2} areal density of each element. Neither Pb nor Cd could be analyzed, as Pb was below the detection limit for μXRF analysis and Cd could not be excited at the interference-free K-line at the incident energy used in this experiment. The figures were prepared in ImageJ (Schneider et al. 2012) by changing the LUT to "Fire," adjusting the maximum values and adding concentration bars using the "calibration" tool, and including length scales.

3 | Results

Synchrotron μXRF analysis of the aerial part of *S. plumbizincicola* from monoculture revealed overal distributional patterns for Zn accumulation, with the highest Zn concentrations occurring in the leaf tips, petioles and nodes (Figure 2). Zinc accumulation in epidermal parts was observed in both leaf and stem cross-sections, with higher concentrations in the upper leaf epidermis, especially towards the tip. In the mesophyll, Zn concentrations were considerably lower and evenly distributed in the palisade and spongy tissues (Figure 3A). Besides the epidermis, most of the Zn was localized in the vascular bundles of the stem, but only in alternating ones, while the others were lower in Zn. Much lower concentrations of Zn were observed in the pith and cortex (Figure 3B).

Large differences in foliar Zn accumulation in *S. plumbizincicola* were observed between plants from monoculture and those intercropped with *N. caerulescens*, but also between young and mature leaves (Figure 4). Mature leaves, especially those from the monoculture, had the highest concentrations of Zn. The leaf tips, petioles (partially shown in Figure 4) and nodes were the main areas of Zn enrichment in all of the groups analyzed, with preferential sequestration in vascular tissues of leaves exhibiting higher Zn concentrations. Unlike the vascular system, which had accumulation of Zn at high concentrations, Zn

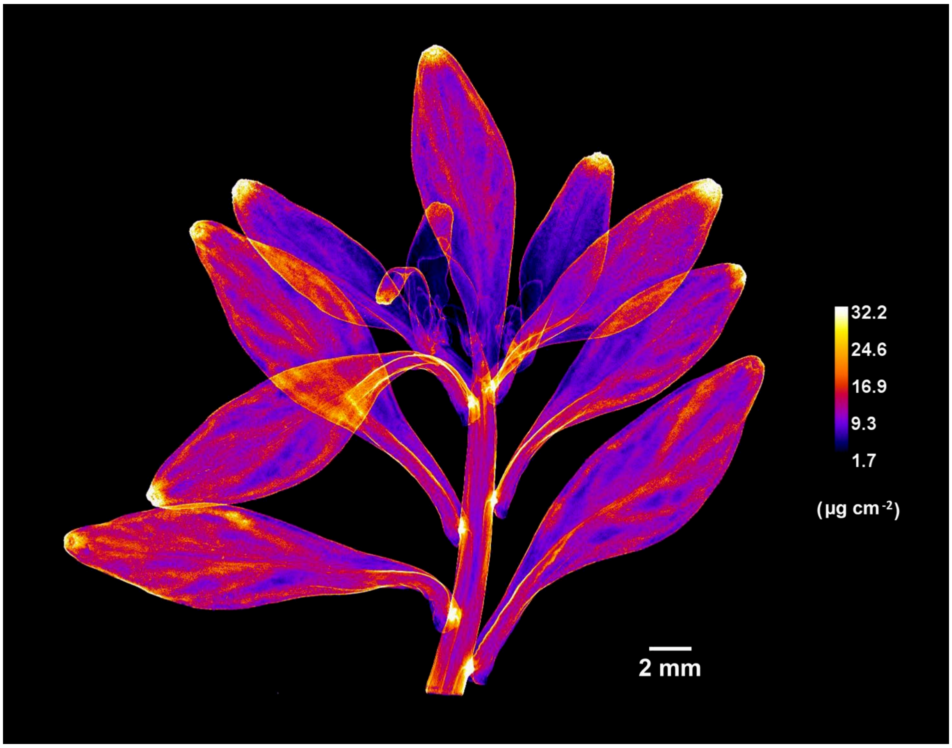

FIGURE 2 | Synchrotron micro-X-ray fluorescence analysis (μXRF) elemental maps showing the distribution of Zn in shoots of *Sedum plumbizincicola* grown for 10 months in monoculture on a moderately contaminated soil (see Section 2 for details).

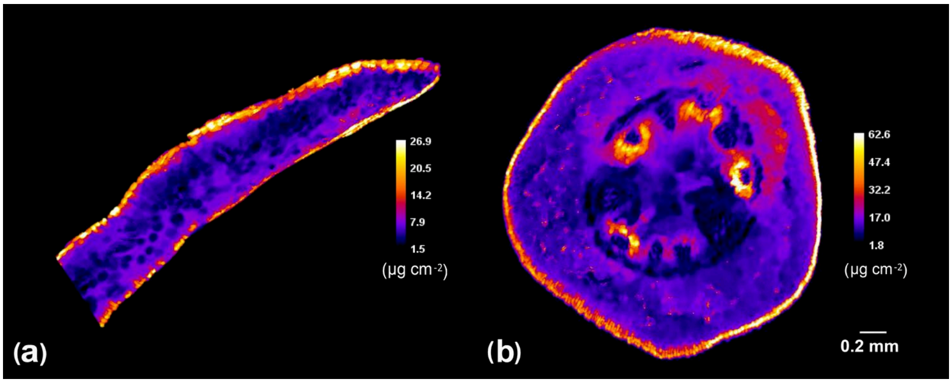

FIGURE 3 | Synchrotron micro-X-ray fluorescence analysis (μXRF) elemental maps showing the distribution of Zn in cross-sections of (a) leaves and (b) stems of *Sedum plumbizincicola* grown for 10 months in monoculture on a moderately contaminated soil.

localization in the epidermis was found in all groups analyzed, although with varying intensity. In co-culture with *N. caerulescens*, young leaves of *S. plumbizincicola* had significantly lower Zn concentrations in the vascular tissue than in the epidermis, whereas this effect was less pronounced in mature leaves.

The bulk ICP-AES analyses confirmed the differences observed by synchrotron μXRF analysis between monoculture and co-cultivated *S. plumbizincicola* plants. In contrast to the 626 $\mu g\, g^{-1}$ Zn found in plants grown in association with *N. caerulescens* for 3 months, foliar Zn concentrations in *S. plumbizincicola* monoculture reached 1660 $\mu g\, g^{-1}$. In the *N. caerulescens* monoculture, Zn concentrations of 2980 $\mu g\, g^{-1}$ were found in foliar tissues, whereas up to 4430 $\mu g\, g^{-1}$ were detected in the leaves of the co-cultivated plants (Table 1). After 10 months, the monocultured plants of *S. plumbizincicola* accumulated more Zn than those in co-culture, with higher Zn concentrations reported—3900 $\mu g\, g^{-1}$ in the monocultured plants, and 1730 $\mu g\, g^{-1}$ in the plants co-cultivated with *N. caerulescens*. However, in *N. caerulescens*, not only a decrease in foliar Zn concentration was observed at the end of the experiment, but also a reversal of the trend, as higher concentrations (2910 $\mu g\, g^{-1}$) were measured in the monocultured plants compared with those grown in co-culture (1900 $\mu g\, g^{-1}$). More subtle differences were found in Cd and Pb concentrations in both species (Table 1).

4 | Discussion

The pattern of Zn localization in *S. plumbizincicola* was found to differ between young and mature leaves and was mainly related to differences in elemental concentrations, which were much higher in the latter group. In plants grown with *N. caerulescens*, which had significantly lower foliar Zn concentrations, this element accumulated predominantly in the epidermal tissues. In contrast, in plants grown in monoculture, where foliar

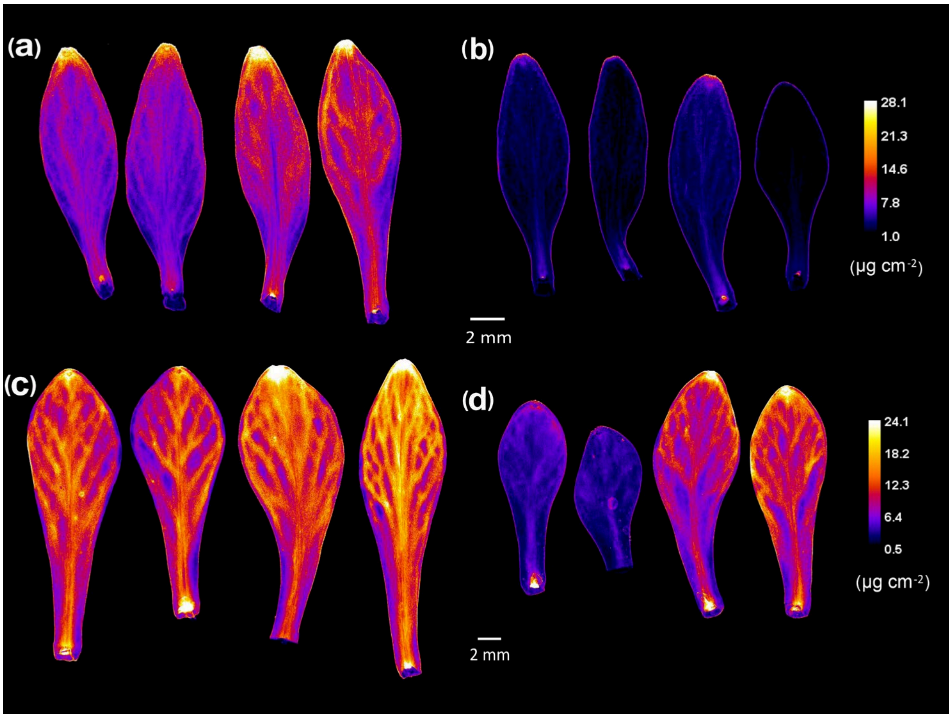

FIGURE 4 | Synchrotron micro-X-ray fluorescence analysis (μXRF) elemental maps showing the distribution of Zn in young leaves of *Sedum plumbizincicola* from monoculture (a) and intercropping with *Noccaea caerulescens* (b), and mature leaves from monoculture (c) and intercropping with *N. caerulescens* (d) after 10 months of cultivation on a moderately contaminated soil. For easier comparison (a) and (b) have the same calibration bar, as do (c) and (d). Four repetitions were performed for each treatment.

TABLE 1 | Concentrations of Zn, Cd, and Pb (in $\mu g\, g^{-1}$) in foliar tissues of *Sedum plumbizincicola* and *Noccaea caerulescens* after 3 and 10 months in monoculture and co-culture.

		3 months			10 months		
Species	**Type of cultivation**	**Zn**	**Cd**	**Pb**	**Zn**	**Cd**	**Pb**
Sedum plumbizincicola	Monoculture	1660	6.4	0.5	3900	24.3	1.5
S. plumbizincicola	Co-culture	626	9.6	0.3	1730	17.9	4.3
Noccaea caerulescens	Monoculture	2980	102.0	0.2	2910	46.5	< DL
N. caerulescens	Co-culture	4430	189.0	0.3	1900	47.3	0.4

Zn concentrations exceeded $3000\,\mu g\,g^{-1}$, Zn was evenly distributed between the epidermis and the vascular system (both central and secondary veins). The predominant localization of Zn in the epidermis is a common strategy in hyperaccumulator species (Scheckel et al. 2007; van der Ent et al. 2019, 2022), considering the lower metabolic activity in these tissues, especially compared to the mesophyll. The epidermal tissues of the leaves and stems were also found to be important sites of Zn localization in *Sedum alfredii*, another hyperaccumulator of Cd–Zn (Tian et al. 2009), although the taxonomical status of its hyperaccumulating genotype in relation to *S. plumbizincicola* is uncertain. In hydroponically grown *S. plumbizincicola*, however, the mesophyll was reported to be almost equally important for Zn accumulation, especially in younger leaves with higher Zn concentrations, which is in contrast to the results of this study (Cao et al. 2014). The significant contribution of the mesophyll to Zn distribution was also confirmed by the work of Hu et al. (2015), in which more than 50% of the Zn in mature leaves was found in this tissue layer. A similar finding was made for Zn in *Arabidopsis halleri* (Küpper et al. 2000) and for Cd in *S. alfredii* (Tian et al. 2011), when the supply of these elements was high, but the epidermal cells and their vacuoles were not large enough to store the excess metal (Küpper et al. 1999). In both hyperaccumulator *Sedum* species, the xylem also proved to be rich in Zn, indicating an efficient transport of Zn into the upper parts of the plant. In *S. alfredii* this enrichment was only observed in the hyperaccumulator ecotypes, but not in the non-accumulator ones (Tian et al. 2009), while the phenomenon of high Zn concentrations in alternating vascular bundles, which was also observed in *S. plumbizincicola* using the micro-PIXE technique (Hu et al. 2015) has not yet been clarified. High Zn concentrations in the nodes and petioles, preferentially in monocultured plants, additionally suggest efficient Zn xylem loading and intensive translocation to the epidermal tissue, where it was predominantly sequestered. A similar pattern was also observed for Zn in *Viola allchariensis* (Jakovljević et al. 2023) and in *Paulownia tomentosa* (Azzarello et al. 2012), and for Cd in the Zn–Cd hyperaccumulator *Potentilla griffithii* (Qiu et al. 2011).

Intercropping, one of the most promising methods for improving metal extraction in agromining, has shown differing results depending on the species or ecotype involved, even when two (hyper)accumulator species were used (Hu et al. 2019; Cao et al. 2021). When grown with *N. caerulescens*, a striking decrease in foliar Zn concentrations was observed in *S. plumbizincicola* compared to monocultured plants, with concentrations falling below the hyperaccumulation threshold ($3000\,\mu g\,g^{-1}$; van der Ent et al. 2013), although concentrations increased with longer exposure time. A similar finding, a strong decrease in shoot Zn concentration when co-cultivated with *N. caerulescens*, was reported for *Salix dasyclados* (Fuksová et al. 2009), indicating higher efficiency of monoculture in phytoremediation practice. In *N. caerulescens* this trend largely depended on the exposure time, as after the initial predominant foliar accumulation of Zn, a reduction in concentration was observed in plants in co-culture, and after 10 months more Zn was accumulated in monoculture. Beyond the stronger affinity of *N. caerulescens* for Zn uptake, which must have depleted available Zn concentrations in the soil, this could also be attributed to its morphological characteristics, notably its well-developed root system and the better yield of aboveground tissue, making it significantly more competitive than *S. plumbizincicola*. Root exudates are another factor that strongly influence the efficiency of element uptake in co-cultures, primarily by altering pH and modifying element availability, while significant effects were found in the rhizospheric microbiome. Being species-specific and with abundance depending on soil elemental characteristics, bacterial communities in the rhizosphere represent an important segment of phytoextraction strategies, that should be considered when designing intercropping systems.

Author Contributions

J.J., C.S., and A.v.d.E. designed the study. J.J. collected the samples, and J.J. and C.S. collected and analyzed the data. A.v.d.E. conducted the synchrotron μXRF experiment and D.B. processed the data. K.J., J.J., and A.v.d.E. wrote the original draft of the manuscript. All authors contributed to the final version of the manuscript.

Acknowledgments

We acknowledge DESY (Hamburg, Germany), a member of the Helmholtz Association HGF, for the provision of experimental facilities. Parts of this research were carried out at Parts of this research were carried out at PETRA III. Data was collected using beamline P06 operated/provided by DESY Photon Science. Beamtime was allocated for proposal I-20220755 EC. This research was supported in part through the Maxwell computational resources operated at Deutsches Elektronen-Synchrotron DESY, Hamburg, Germany. We would like to thank Gerald Falkenberg and Jan Garrevoet for assistance in using beamline P06. The authors would like to thank the Pocheco (France) and Econick (France) companies for their collaboration in the Permagromine project. In particular, we acknowledge Guillaume Echevarria, Gabrielle Michaudel, Fanny Vautrin, Julien Verny, Pierre Vanden Berghe and their teams for growing the hyperaccumulators, maintaining the experiment and sampling. K.J. acknowledges the support of Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grant No 451-03-136/2025-03/200007).

Funding

The authors have nothing to report.

Conflicts of Interest

K.J. and A.v.d.E. serve as Guest Editors of this special feature, but had no involvement in the peer review and decision-making processes for this paper. The other authors declare no conflicts of interest.

Data Availability Statement

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

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