

RESEARCH PAPER

Habitat-specific allocations of elements in *Atriplex lentiformis* seeds indicate adaptation to metal toxicity

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Abstract

Self-sustaining vegetation in metal-contaminated areas is essential for rebuilding ecological resilience and community stability in degraded lands. Metal-tolerant plants originating from contaminated post-mining areas may hold the key to successful plant establishment and growth. Yet, little is known about the impact of metal toxicity on reproductive strategies, metal accumulation, and allocation patterns at the seed stage. Our research focused on the metal tolerant *Atriplex lentiformis*. Specifically, we examined the effects of toxic metal(loid) concentration in soils on variability in its reproductive strategies, including germination patterns, elemental uptake, and allocation within the seeds. We employed advanced imaging techniques like synchrotron X-ray fluorescence microscopy (2D scans and 3D tomograms) combined with inductively coupled plasma mass spectrometry to reveal significant differences in metal(loid) concentration and distribution within the seed structures of *A. lentiformis* from contrasting habitats. Exclusive Zn hotspots of high concentrations were found in the seeds of the metallicolous accession, primarily in the sensitive tissues of shoot apical meristems and root zones of the seed embryos. Our findings offer novel insights into phenotypic variability and metal tolerance and accumulation in plants from extreme environments. This knowledge can be applied to enhance plant survival and performance in land restoration efforts.

Keywords: Germination, heavy metals, imaging, metal adaptation, metallophyte, mine tailings, phytoremediation, seed, synchrotron X-ray fluorescence microscopy (XFM), zinc.

Abbreviations: BCF, bioconcentration factor; DTPA, diethylenetriaminepentaacetic acid; ICP-MS, inductively coupled plasma mass spectrometry; M, metalliferous; NM, non-metalliferous; SAM, shoot apical meristem; ROI, region of interest; XFM, X-ray fluorescence microscopy; XRF, X-ray fluorescence.

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Introduction

Industrial and mining activities have led to toxic levels of metals in soils across the world (Liu *et al.*, 2018; Briffa *et al.*, 2020; Wang *et al.*, 2021), often leaving these areas unsuitable for re-use or habitation. Moreover, dust migration from these metal-contaminated sites constitutes a hazard for neighboring ecosystems and communities (Sultan *et al.*, 2022). Remediation of such sites through vegetation restoration (phytoremediation) is an economical and eco-friendly solution for stabilizing and reducing the spread of contaminants (Yan *et al.*, 2020). Phytoremediation technologies employ plants to stabilize (phytostabilization), detoxify (phytotransformation), or extract (phytoextraction) metal(loid)s from contaminated waste materials (Arthur *et al.*, 2005). Yet, the success of phytoremediation depends on understanding and overcoming plant toxicity associated with elevated metal concentrations (Kranter and Colville, 2011; Seneviratne *et al.*, 2019). Further, a large proportion of metal-contaminated sites are a result of mining operations that crush and process ores to harvest metal, producing mine tailings as a waste material. In particular, legacy mine tailings are commonly characterized by high metal content, low nutrient availability, minimal microbial biomass, and acidic pH (Mendez and Maier, 2008), although some tailings may be alkaline or neutral (Krzaklewski and Pietrzykowski, 2002; Escarré *et al.*, 2010). These factors together can negatively affect seed germination, plant metabolism and biomass production, limiting the effectiveness of phytoremediation (Nagajyoti *et al.*, 2010; Rastogi *et al.*, 2017).

Research has shown that metal-tolerant plant populations originating from metal-contaminated areas are adapted to metal toxicity (Baker, 1981; Bradshaw and McNeilly, 1991; Reeves *et al.*, 1995; Macnair *et al.*, 1999; Meyer *et al.*, 2016; Babst-Kostecka *et al.*, 2018) and thus may have greater success in phytoremediation applications on legacy mine sites (Dietrich *et al.*, 2021; Nandillon *et al.*, 2021). This suggests that high levels of metals in soil trigger the evolution of defense mechanisms in plants necessary for successful germination and development in metal-contaminated lands. Such strategies may involve structural modifications of plant organs, metabolic changes, hormone balance alterations, or the sequestration of metals in plant tissues (Bothe and Słomka, 2017; Yadav *et al.*, 2021).

Seed germination is the first crucial stage of metal tolerance, and some plants have evolved strategies to maintain viable and healthy seeds in metal contaminated sites (Kranter and Colville, 2011; Yadav *et al.*, 2021). In particular, plants employ various mechanisms to prevent metal(loid)s from entering the seed embryo. One protective mechanism observed in some metal-tolerant and hyperaccumulating species is that the endosperm serves as a barrier, preventing excessive metal accumulation in the embryo (Mesjasz-Przybyłowicz *et al.*, 1999; Vogel-Mikuš *et al.*, 2007; Babst-Kostecka *et al.*, 2020b).

This study focuses on *Atriplex lentiformis* (Torr.) S. Watson, commonly known as the big saltbush. This perennial halophyte,

native to semiarid regions of southwestern USA and northern Mexico, belongs to the *Amaranthaceae* family which is known to possess specific adaptations to thrive in stressful environments, such as extreme drought, high soil salinity, and metal tolerance (Eissa, 2015; Todorović *et al.*, 2022). Such plants can play a pivotal role in restoration efforts, especially in regions where evaporation rates exceed precipitation (Mendez and Maier, 2008). *Atriplex lentiformis* is known to tolerate elevated concentrations of metals in soil, including Zn, Cd, As, and Pb (Mendez *et al.*, 2007; Solís-Dominguez *et al.*, 2012; Eissa and Abeed, 2019). While an increased uptake of Zn, Cd, and Pb into shoot tissues was reported in plants grown in the soil amended with compost (Eissa, 2017; Kumar *et al.*, 2022; Kushwaha *et al.*, 2023), other studies suggest limited metal uptake by *A. lentiformis* (Mendez *et al.*, 2007).

Therefore, gaining deeper insight into metal uptake strategies of this species represents a crucial step in better understanding of metal tolerance and accumulation processes. In particular, little is known about the composition, allocation, and concentration of beneficial and potentially toxic elements in *A. lentiformis* seeds. This contributes to the research gap in metal(loid) distribution patterns within the reproductive tissues of metal tolerant plants from arid environments—where further complexity comes from drought and hot conditions—impeding the understanding of metal(loid) tolerance and allocation strategies unique to these particular species or ecotypes (Mesjasz-Przybyłowicz *et al.*, 1999; Babst-Kostecka *et al.*, 2020a). Furthermore, the associations between seed tissue metallome and the concentrations of metal(loid)s in the soil have rarely been investigated. This can be attributed to the small size of seeds in metal-tolerant species and to the limitations of available techniques for elemental analysis, each susceptible to specific biases. Additionally, a comprehensive understanding of the reproductive strategies of both tolerant and native plant species is imperative for the self-sustenance of phytoremediation efforts and to facilitate the development of self-sustaining ecosystems through plant colonization.

In this study we investigate the effect of toxic metal(loid)s on *A. lentiformis* reproductive strategies, including germination patterns, elemental uptake, and allocation within the seed. We compared seeds from two contrasting habitats (metal-contaminated versus non-contaminated), providing an ideal setting for investigating responses triggered by metal stress. Specific questions we ask include: (i) How does prolonged exposure to soil metal(loid) toxicity affect the metallomic composition, allocation patterns, and structural characteristics of *A. lentiformis* seeds? (ii) Does the presence of elevated metal(loid) levels lead to the exclusion or enhanced accumulation of specific elements within the plant system compared with strategies observed in plants from non-contaminated site? (iii) Do plants from metal-contaminated and non-contaminated locations differ in germination rates and seed viability? The results

of this research will contribute to the understanding of the spatial distribution of metal(loid)s within seeds, offering insights into metal tolerance and accumulation processes in plants from extreme environments.

Materials and methods

Study sites and plant material collection

Atriplex lentiformis was sampled at two study sites (Supplementary Fig. S1). The first is an uncontaminated (non-metalliferous, NM-PHX) site located within a nature reserve of Rio Salado Audubon Center in Phoenix, AZ, USA (33°25′09.7″N 112°04′20.9″W) characterized by native vegetation and in close proximity to an artificial pond and the Salt River. The second site is a metalliferous 60 ha mine tailings pile that is part of the Iron King Mine and Humbolt Smelter Superfund site (M-IK) ~150 km north of Phoenix (34°50′07.32″N 112°25′25.12″W). The mine operated between the end of the 19th century and 1969, producing Cu, Ag, Au, Pb, and Zn (Creasey, 1952), and the resulting mine tailings are highly acidic (pH 2–3) and contain As, Pb, Zn, and Fe, with an average concentration of ~4000, 2400, 6100, and 106 000 mg kg⁻¹, respectively, in the top 0.5 m layer of substrate (Root *et al.*, 2015). These tailings remain devoid of vegetation to this day. However, an area of the tailings (60 × 30 m) was the subject of a compost-assisted direct planting revegetation trial from 2010 to 2017 (Gil-Loaiza *et al.*, 2016, 2018). In 2010, the top 30 cm of the tailings field trial site was amended with 10–20% compost (immediately increasing the pH to 5–7, depending on the amount of compost added) and seeded with six native species including *A. lentiformis*. Over time, as a result of heavy rains and erosion, much of the field site has re-acidified, but at the time of sampling for this study (2018) there still remained a small number of localized fertility islands including some with five mature *A. lentiformis* plants, investigated in this study.

Ripe seeds, leaves, stems, and roots were simultaneously sampled from five *A. lentiformis* plants at each of the two sites. The distance between sampled plants was ~10 m, a spacing resembling the sparse vegetation distribution of the US Southwest. The seeds from the five sampled individuals were proportionally pooled to create one homogenized bulk seed sample per site. Soil samples were collected from each plant, at a distance of 0.5 m from the plant, at a depth of 10–15 cm, after the removal of the top organic layer and avoiding areas with visible plant roots. Soil samples were composited to provide one mixed and homogenous soil sample per site and were stored at room temperature prior to geochemical analysis.

Chemical analyses of soil and plant samples

Triplicate soil samples were sieved through a 1 mm mesh, ground, oven-dried at 110 °C, and stored in a desiccator. Samples (0.1 g) were pre-digested with 1 ml concentrated nitric acid (HNO₃, Omni-trace, EMD Chemicals) at room temperature for 4 h, followed by the addition of 1 ml ultrapure water (18 Ω). For samples containing large fractions of organic material, an aliquot of hydrogen peroxide (30%, Suprapure H₂O₂, EMD) was added instead of ultrapure water. The vessels were kept at room temperature overnight. After the H₂O₂ reaction diminished, the vessels were capped and samples were digested in a microwave oven (CEM Model MARS6 microwave, Matthews, NC, USA, including a standard reference material (NIST 2711; Supplementary Table S1) in the batch. Digested soil samples were further analysed by inductively coupled plasma mass spectrometry (ICP-MS) to determine total concentrations of Li, Be, Na, Mg, Al, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Ag, Cd, In, Sn, Sb, Cs, Ba, Tl, Pb, Bi, and U. These analyses were performed by the Arizona Laboratory for Emerging Contaminants (ALEC), at the University of Arizona, Tucson, AZ, USA. Additionally, analyses of diethylenetriaminepentaacetic acid (DTPA)-extractable Mn, Fe, Cu, and Zn, as

well as organic matter were conducted by an external commercial laboratory following cited references (Lindsay and Norvell, 1978; Gavlak *et al.*, 2005; Schulte and Hopkins, 2015).

The soils were additionally examined for total C, N, and S concentration using an elemental combustion analyser (ECS 4010, CNS Unit, Costech Analytical Technologies, USA). Soil samples were sieved through a 1 mm mesh, dried at 60 °C for 1 h, and ~50 mg of each sample was weighed in triplicate. Limits of detection were 100 mg kg⁻¹ for N and C, and 400 mg kg⁻¹ for S. The device calibration was performed using sulfanilamide and LECO Corp. (St Joseph, MI, USA) sample standards including soil, corn gluten, and cysteine.

Soil pH was measured in a 1:1 (w/v) suspension of soil in ultrapure water (18 Ω; Mclean, 1982). Electrical conductivity was determined from the supernatant of a 2:1 water soil suspension following 30 min of shaking (Gavlak *et al.*, 2005).

The samples of leaves (*n*=5 biological replicates per location), stems (*n*=5 biological replicates per location), and roots (*n*=5 biological replicates per location) were washed three times with deionized water, and oven dried at 70 °C for 72 h. After drying, the samples were cooled in a vacuum desiccator, then packed into 4.5 ml tubes with ceramic spheres (TallPrep Lysing Matrix M, 4.5 ml tube; MP Biomedicals), and finely ground to a homogenous powder using a FastPrep-24 5G homogenizer (MP Biomedicals) following the manufacturer's recommended settings. Air-dried seeds (60 per location) were separated from their corresponding seed achenes, ground, oven-dried at 60 °C, and stored in a vacuum desiccator prior to weighing (*n*=1 analytical replicate). A 0.1 g sample of ground plant material was pre-digested with 1 ml concentrated HNO₃ (Ari-Star Plus HNO₃, BDH Chemicals) at room temperature for 4 h, followed by the addition of 1 ml H₂O₂ (30% Suprapure H₂O₂, EMD). After the H₂O₂ reaction subsided, 1 ml of ultrapure water (18 Ω) was added, the vessels were capped, and samples were subjected to microwave digestion (CEM Model MARS 6, Matthews, NC, USA). The digestion program used for plant samples is a slow ramp over 20–25 min to 200 °C, followed by 10 min at 200 °C. Controls including an acid blank and a certified reference material (NIST #1515 Apple Leaves; Supplementary Table S1) were similarly digested. Bulk elemental analysis was performed on all samples using ICP-MS (7700x, Agilent Technologies, Santa Clara, CA, USA) for Li, Be, Na, Mg, Al, K, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Ag, Cd, In, Sn, Sb, Cs, Ba, Tl, Pb, Bi, and U.

Seed germination test

One hundred seeds were randomly selected from each location, soaked in ultrapure water, stored at 4 °C in dark for 48 h, and then placed on filter paper moistened with ultrapure water in glass Petri dishes. Seeds from each location were distributed across 10 Petri dishes, with each dish containing 10 seeds. Incubation conditions were 25 °C during the day and 20 °C during the night with a photoperiod of 14 h of light (100 μmol photons m⁻² s⁻¹ irradiance). Petri dish arrangement was randomized and seed germination was assessed over a 20-day period by visual detection of radicle emergence. The germination percentage and time course for each location were calculated and plotted as described previously (Murawska-Włodarczyk *et al.*, 2022).

Two-dimensional synchrotron X-ray fluorescence microscopy

Seeds from M-IK and NM-PHX, 54 and 35 respectively, were randomly selected for 2D X-ray fluorescence microscopy (XFM) analysis. This microanalytical method, due to its high resolution and sensitivity for elemental mapping and quantification, is highly effective for trace element analysis in biological samples, facilitating precise data extraction from selected micro-areas of the studied tissues (Kopittke *et al.*, 2018; van der Ent *et al.*, 2018). Seeds were sectioned longitudinally with a stainless-steel razor blade to obtain thin (300–400 μm) slices of the mid-section of each

seed, preserving the embryo axis. The thickness of each slice was assessed using a digital micrometer (Mitutoyo 293-185-30 Quantumike Coolant Proof Micrometer, Mitutoyo, Japan) with a resolution of 1 μm . The sections were fixed to Kapton tape (polyimide, 20 μm thick) and mounted on a glass microscope slide. Additionally, three intact seeds from each location, along with their corresponding seed achenes, were prepared for analysis and mounted on a microscope slide. The slides were covered with 4 μm Ultralene film (SPEX Certiprep, Matuchen, NJ, USA) until XFM analysis, to prevent drying and contamination of the samples.

Elemental maps of seed samples were collected at the X-ray fluorescence (XRF) beamline installed at the 3 BeV Australian Synchrotron (Clayton, Australia), using a Kirkpatrick–Baez mirror microprobe, which produces a focal spot of 2 $\mu\text{m} \times 2 \mu\text{m}$ full width at half-maximum. The latest description of all details of this beamline and microprobe can be found in Howard *et al.* (2020). A three-element Hitachi Vortex silicon drifted detector (SDD), with large solid angle and excellent energy resolution ($\sim 135 \text{ eV}$), was used to obtain high-definition $\mu\text{-XRF}$ of plant material without artefacts due to peak overlaps. In this study only XRF signals from the central large area (80 mm^2) element of the vertical three-element array of SDDs was employed. Incident energy of 15.9 keV was used to map the distribution of elements (As, Zn, Pb, Se, S, P, K, Ca, Mn, Fe, Rb, and Cu) and subsequently examine elemental correlations. Additionally, smaller subsets of seed samples were selected for more detailed analysis by decreasing the scan area, increasing the dwell time, and decreasing the pixel size, thereby increasing the resolution of the images. Specifically, 13 seeds from the M-IK location and 10 seeds from the NM-PHX location were scanned with a dwell time per pixel of 1.25 ms. Within this subset, one randomly selected seed per location was additionally scanned with a per-pixel dwell time of 2.0 ms. Intact seed achenes (three and six for the NM-PHX and M-IK location, respectively) were analysed with a dwell time of 2.0 ms.

Two-dimensional X-ray fluorescence tomography and three-dimensional scanning

Tomographic single-slice cross-section scans were generated to visualize the elemental distribution in the transverse plane of intact seeds and achenes. The images obtained in this plane enabled a more precise determination and confirmation of structures and elemental allocations without compromising the seed's integrity. Additionally, one seed from the M-IK location was scanned for 3D reconstruction, to provide insight into the spatial distribution of elements within the seed. The images were obtained at the 6 GeV synchrotron radiation source PETRA III (Deutsches Elektronen-Synchrotron; DESY, Hamburg, Germany), using the undulator of the hard X-ray microprobe beamline P06 (Boesenberg *et al.*, 2016). This beamline is equipped with a cryogenically cooled double-crystal monochromator that uses Si (111) crystals. An ion chamber positioned upstream of the sample monitored the incoming flux, while a 500 μm thick Si PIPS diode with a 19 mm diameter active area (PD300-500CB, Mirion Technologies (Canberra) GmbH, Germany) located downstream of the sample recorded the transmitted X-ray intensity, providing absorption data. The incident X-ray energy was 16 keV, focused to a beam size of 600 $\times$ 500 nm for 3D measurements ($h \times v$) using K/B mirrors, which resulted in a flux of approximately 1.5×10^{10} photons s^{-1} in the focus. XRF detection was performed using a four-element Vortex SDD (ME4, 350 μm thick chip with 170 mm^2 combined active area for all four elements, Hitachi High-Tech, USA) and a prototype 16-element SDD Ardesia detector (800 μm -thick chip with 324 mm^2 combined active area for all 16 elements; Politecnico, Milano, Italy) in 90° and 270° geometry, respectively, where the forward direction of the X-ray beam was defined as 0°. Xspress 3 pulse processors (Quantum Detectors, UK) were used as readout electronics for both XRF detectors. The tomographic measurements were performed in single-slice tomography mode, with multiple slices stacked vertically to create a 3D volume.

Data processing and statistical analysis

The differences in element concentrations in soils, leaves, stems, and roots (ICP-MS data), as well as in mean germination time between the two locations studied were determined using the non-parametric Wilcoxon test. Bioconcentration factors (BCFs), were determined to evaluate the efficiency of a plant in accumulating a metal into its leaves from the surrounding environment. BCF was calculated by dividing the average elemental concentration of the target metal in leaves by the total elemental concentration of the same metal in soil ($C_{\text{leaves}} : C_{\text{soil}}$ ratio; where C is the concentration of the target metal; Gal *et al.*, 2007; Zhuang *et al.*, 2007). Similarly, translocation factors were calculated to evaluate the efficiency of each plant in translocating the accumulated metal from its roots to shoots. The translocation factor was derived by dividing the average elemental concentration in leaves by that in roots ($C_{\text{leaves}} : C_{\text{roots}}$ ratio; Padmavathiamma and Li, 2007).

The 2D XFM data were analysed using the GeoPIXE software package (Ryan *et al.*, 1990; Ryan, 2000) and projected using the Dynamic Analysis method. This method allows overlap-resolved and subtracted background images to be generated that are quantitative [concentration in $\mu\text{g g}^{-1}$ dry weight (dry weight unit)] elemental maps (Ryan and Jamieson, 1993). The matrix composition was formulated as cellulose ($\text{C}_6\text{H}_{10}\text{O}_5$) with a density of 1.1 g cm^{-3} (van der Ent *et al.*, 2018) and with consideration of variation in sample thickness. The Compton scatter maps were used to correct for local areal density variations. The error estimates were extracted from the error matrix generated in the fit, and the minimum detection limits were calculated using the Currie equation (Currie, 1968). Based on the seed morphological structures that were visible on 2D images, six regions of interest (ROI) were selected and distinguished: (i) root tip zone, (ii) shoot apical meristem (SAM), (iii) cotyledons, (iv) perisperm, (v) procambium, and (vi) seed coat (Fig. 1A–C). At the site level, the differences in mean concentration of elements among ROIs were assessed using the Wilcoxon test.

The 3D XFM data were analysed using non-linear least squares fitting as implemented in PyMca (Solé *et al.*, 2007). After alignment using tomographic consistency methods to correct for sample movement in the horizontal direction, the datasets were reconstructed using a maximum-likelihood expectation-maximization (MLEM) algorithm (Bruyant, 2002). The resulting tomograms were calibrated and quantified using calibration foils with known area densities. 3D visualization and animation of the seed volume was performed using the open-source software Drishti (<https://github.com/nci/drishti>; Limaye, 2012).

Results

Soil chemical characteristics

Soil pH and electrical conductivity indicated that the NM-PHX soil was alkaline ($\text{pH } 8.63 \pm 0.04$) and non-saline, while the M-IK soil was acidic ($\text{pH } 6.13 \pm 0.09$) and slightly saline (electrical conductivity 3.19 ± 0.06) (U.S. Department of Agriculture, Natural Resources Conservation Service, 2023) (Supplementary Table S2). In terms of essential nutrients, total C was 31-fold higher in the NM-PHX soil while there was no difference in total N content among the soils. Sulfur was much higher in the M-IK soil (46 400 mg kg^{-1} versus below detection). Metal(loid) concentrations were significantly different in the two soils except for Mg. The NM-PHX soil displayed higher concentration of K (5-fold; corresponding to $\sim 2700 \text{ mg kg}^{-1}$ difference), and Mn (2-fold; $\sim 250 \text{ mg kg}^{-1}$ difference), indicating a richer profile for these essential nutrients.

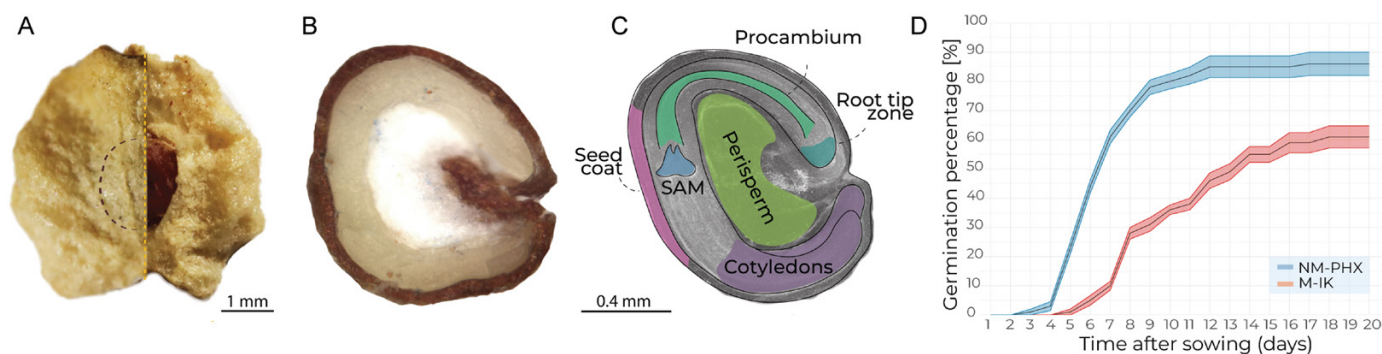


Fig. 1. *Atriplex lentiformis* seeds and their structures, alongside a germination graph for two populations. (A–C) Photograph of the achene of *Atriplex lentiformis* with seed (A), longitudinal section of seed (B), and schematic representation of the six seed structures used in the interpretation of X-ray fluorescence microscopy images, namely seed coat, shoot apical meristem (SAM), cotyledons, procambium, root tip zone, perisperm (C). (D) Mean cumulative germination percentage (solid line) over time $\pm$ SE (shaded areas) for *Atriplex lentiformis* seeds from a non-metalliferous (NM-PHX, blue) and a metalliferous (M-IK, red) site ($n=100$ per site).

Conversely, the M-IK soil was found to have higher levels of Ca (2-fold; $\sim 24\,800\text{ mg kg}^{-1}$ difference), Fe (6-fold; $\sim 80\,870\text{ mg kg}^{-1}$ difference), Cu (4-fold; $\sim 70\text{ mg kg}^{-1}$ difference), and Zn (21-fold; $\sim 1675\text{ mg kg}^{-1}$ difference). Regarding non-essential elements, the NM-PHX soil showed elevated levels of Rb (10-fold; 13 mg kg^{-1} difference), Li (4-fold; 17 mg kg^{-1} difference), Be, and Ba (4-fold; 0.6 and 110 mg kg^{-1} difference, respectively) compared with the M-IK soil. In contrast, the M-IK soil had significantly increased concentrations of As (240-fold; $\sim 2500\text{ mg kg}^{-1}$ difference), Pb (20-fold; 2450 mg kg^{-1} difference), and Cd (15-fold; 5 mg kg^{-1} difference). It is noteworthy that concentrations of S, Ca, Fe, Cu, Zn, As, Cd, and Pb at the M-IK site exceeded the ranges considered normal or optimal for unpolluted soils (Thornton and Webb, 1980; Chesworth *et al.*, 2008; Hossner *et al.*, 2008). The NM-PHX ranges were within the normal limits, with a deficiency of S. DTPA-extractable concentrations of elements of the M-IK soil revealed higher concentrations of Fe (103 mg kg^{-1}), Cu (5.6 mg kg^{-1}), and Zn (184 mg kg^{-1}) compared with NM-PHX soil (51-, 4-, and 61-fold, respectively), while Mn did not vary between the sites.

Elemental composition of leaves, stems, and roots

Reflecting the higher concentrations of As, Pb, Zn, Cd, and Fe in the M-IK soil, concentrations of these five elements were significantly higher in M-IK plant roots, stems, and leaves (except for Pb uptake into roots, which was not significant) (Table 1; Supplementary Table S3). Notably, the mean concentration of Zn surpassed $\sim 830\text{ mg kg}^{-1}$ in leaves and exceeded $\sim 270\text{ mg kg}^{-1}$ in M-IK plant stems and roots. Although Cu was also higher in the M-IK soil, there was no significant difference in uptake into plant tissues.

There was a more complex pattern for metal(loid)s that were at higher concentration in the NM-PHX soil. Both Na and Ba were at higher concentrations in the NM-PHX soils as well as in plant roots, stems, and leaves. But for Rb, although its

concentrations were higher in the NM-PHX soil, this element was at higher concentration in the M-IK plant tissues (except for uptake into stems, which was not significantly different between the two sites). Both concentrations of Li and Mn were higher in the NM-PHX soil, but there were no major differences in uptake into plant tissues from the two sites (except Mn was at higher concentration in the NM-PHX roots).

Overall, all metal(loid)s except Zn had concentrations within the established normal range for plant physiology (Kabata-Pendias, 2011; Stanton *et al.*, 2022). Essential macro- and micronutrients in leaves, e.g. K, Ca, Mn, and Cu, maintained levels within the normal range (Campbell and Plank, 2000; Jatav *et al.*, 2020), and no significant variations in tissue uptake of these elements were detected between locations. Conversely, Mg concentrations in leaves greatly surpassed the generally recognized optimum range at the M-IK site (1.0%; Ishfaq *et al.*, 2022) and were notably high (0.7%) at the NM-PHX site.

Interesting patterns were observed regarding elemental concentration variation within plant tissues, as well as the corresponding concentrations of each element in the soil. Fe, As, and Pb were retained and accumulated in the roots rather than in leaves, as indicated by translocation factor <1 (Fig. 2). Furthermore, BCF values for these elements were either within the typical range or even lower (Kabata-Pendias, 2011). In contrast, the translocation factors for Zn and Rb were greater than 1 in both locations. Additionally, the BCF for Zn at the M-IK site surpassed the generalized threshold, with values reaching 12.6 ± 1.4 . Other macronutrients such as K, Na, and Mg, along with micronutrients (Li, Mn, and Cu), were also present at higher concentrations in leaves compared with roots (Table 1). These trends were consistent across both locations, except Ca at the NM-PHX location, with similar concentrations in both leaves and roots.

Bulk elemental concentration in seeds and achenes

Seeds (60 per location) and their corresponding achenes were combined into two separate samples and analysed with

Table 1. Elemental concentrations in roots, stems, leaves, seeds, and achenes of *Atriplex lentiformis* originating from non-metaliferous (NM-PHX) and metalliferous (M-IK) locations

	Roots			Stems			Leaves			Seeds			Achenes		
	NM-PHX	M-IK	P	NM-PHX	M-IK	P	NM-PHX	M-IK	P	NM-PHX	M-IK	P	NM-PHX	M-IK	P
Essential elements	Mg	2318 ± 839	2623 ± 417	ns	3502 ± 779	3360 ± 649	ns	7321 ± 1552	10 991 ± 3900	ns	2287	2552	7699	2552	12 875
	K	6718 ± 4089	20 282 ± 2111	**	29 623 ± 4207	16 021 ± 4452	**	24 115 ± 4280	31 730 ± 16 279	ns	6254	7298	44 310	44 310	46 483
	Ca	5547 ± 3064	2390 ± 612	ns	5907 ± 749	8640 ± 2241	*	5916 ± 1105	13 061 ± 4881	ns	2048	1668	15 027	15 027	19 789
	Mn	20 ± 1.2	11 ± 3.3	**	13 ± 1	12 ± 5.2	ns	53 ± 6.9	43 ± 29	ns	23	21	50	21	55
	Fe	116 ± 28	526 ± 281	**	32 ± 10	109 ± 25	**	80 ± 21	225 ± 89	**	109	108	355	108	1039
Non-essential elements	Cu	4.7 ± 2.4	5.3 ± 1.3	ns	9.1 ± 3	5 ± 1.6	ns	6.3 ± 1.3	6.9 ± 1.9	ns	9	6.5	18	6.5	11
	Zn	21 ± 11	269 ± 58	**	42 ± 9.3	276 ± 103	**	57 ± 10	827 ± 208	**	66	150	70	150	529
	Mo	0.3 ± 0.2	0.3 ± 0.2	ns	0.3 ± 0.2	0.3 ± 0.4	ns	0.6 ± 0.1	1.4 ± 0.5	**	0.5	0.3	1.2	0.3	0.9
	Li	2.1 ± 1.7	1.4 ± 1.2	ns	0.8 ± 0.2	1.2 ± 0.3	ns	18 ± 3.6	13 ± 6.3	ns	4	10	85	10	184
	Na	7668 ± 3857	1020 ± 524	**	21 637 ± 2291	2950 ± 960	**	43 731 ± 3133	8073 ± 1996	**	2502	1622	56 992	1622	30 989
	Al	131 ± 29	251 ± 165	ns	29 ± 15	52 ± 20	ns	44 ± 16	125 ± 55	**	8	5	343	5	292
	As	0.5 ± 0.3	22 ± 16	**	0 ± 0	2 ± 0.8	*	0.1 ± 0	3.6 ± 1.5	*	0.3	0.4	0.25	0.4	30
	Rb	1.3 ± 0.5	14 ± 2.4	**	8.4 ± 2.9	10 ± 1.7	ns	12 ± 4.4	23 ± 6.3	*	30	162	131	162	529
	Cd	0.1 ± 0.1	0.2 ± 0.1	*	0 ± 0	0.2 ± 0.1	*	0.1 ± 0	0.3 ± 0.1	*	0.05	0.11	0.76	0.11	0.85
	Ba	10 ± 5.3	0.9 ± 0.4	*	11 ± 2.4	1.2 ± 1	**	3.8 ± 0.5	1 ± 0.4	*	0.9	0.07	11	0.07	2
	Pb	3.5 ± 2.1	10 ± 6.8	ns	0.1 ± 0	1.5 ± 0.5	*	0.1 ± 0	2.7 ± 1.5	*	0.71	0.35	18	0.35	14

Elemental concentrations analysed using ICP-MS, mg kg⁻¹, mean ±SD, n=5. Due to low biomass of individual seed and achene, tissues were subjected to bulk analyses (60 per location). The P-values indicate the significance level of differences between the locations (ns, P>0.05; *P≤0.05; **P≤0.01; ***P≤0.001); unpaired Wilcoxon rank sum test; ns: not significant.

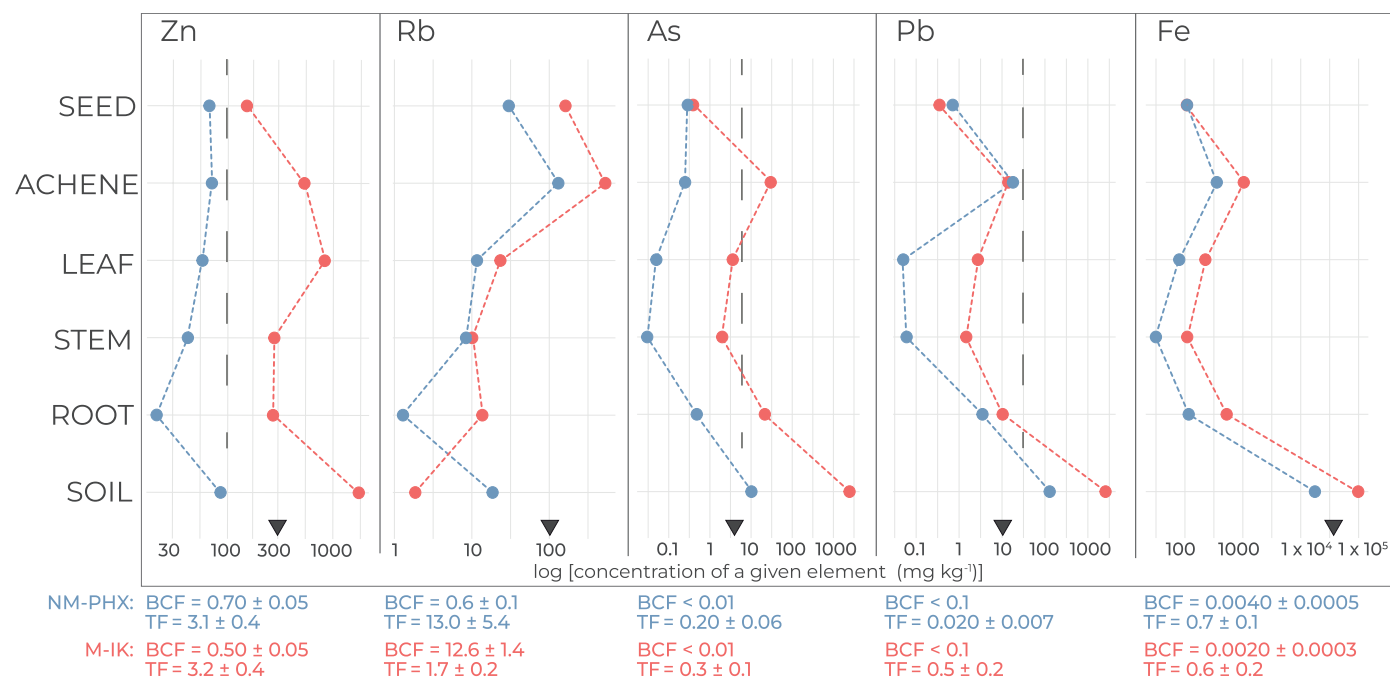


Fig. 2. The mean concentration values (mg kg⁻¹) of selected elements in the soil (total concentrations) and various tissues of *Atriplex lentiformis* (roots, stems, leaves, achenes, and seeds) from a non-metalliferous (NM-PHX; blue) and metalliferous (M-IK; red) site. Bioconcentration factor (BCF) and translocation factor (TF) were calculated for each metal(loid) (mean ± SE). Grey dashed lines indicate the average threshold at which an element is considered to be in excess or toxic for most plant species (Zn: 100 mg kg⁻¹, As: 5 mg kg⁻¹, Pb: 30 mg kg⁻¹) (Kabata-Pendias, 2011; White and Broadley, 2011). The thresholds for Rb and Fe in plant tissues have not been clearly defined in the current literature. The average optimal elemental levels in soil are indicated with a ▼ (Buckman and Brady, 1969; Kabata-Pendias, 2011; Lindsay, 1972). Refer to Table 1 and Supplementary Table S2 for the corresponding standard error values. Note that the connecting lines were added to facilitate pattern recognition and do not represent a direct link between the data points.

ICP-MS, to determine elemental concentrations. Overall, results showed higher concentrations of Zn, Li, and Rb in the M-IK seeds (2.3-fold, 2.7-fold, and 5.4-fold, respectively), whereas Pb was twice as high in NM-PHX seeds (Table 1). Note that the concentrations of the latter element were relatively low in both populations, indicating an inhibited translocation of Pb to seeds. Concentrations of the macronutrients Na and Ca were higher in the NM-PHX seeds (1.5-fold and 1.2-fold, respectively). All elements were at higher concentrations in achenes than in the corresponding seeds. Achenes from the M-IK had higher concentrations of several essential (Mg, Ca, Fe, and Zn) and non-essential (As, Li, and Rb) elements than achenes from the NM-PHX.

Seed germination percentage and mean germination time

Seed germination percentages from the NM-PHX and M-IK locations were 86 ± % and 61 ± 1 %, respectively (Fig. 1D), with mean germination times of 166 ± 53 h (NM-PHX) and 246 ± 74 h (M-IK). The mean germination times also differed significantly between the locations, with shorter germination time in the NM-PHX ($P \leq 0.001$; unpaired Wilcoxon sum rank test). Overall, the observed time course curves showed faster

and more uniform germination of a higher total percentage of viable seeds for the NM-PHX accession, while the seed germination of the M-IK accession was delayed and more steadily distributed over time, with more non-viable or dormant seeds.

Elemental distribution and quantification in seed structures

The 2D XFM images were used to investigate the spatial distribution of macro- and micronutrients in *A. lentiformis* seeds, including Zn, Rb, As, Fe, Cu, Mn, Ca, K, P, and S. The XFM results for some elements are not reported, because their X-ray lines were too weak to separate them with confidence from the background (Si, Cl, and Ti), or from the overlapping stronger X-ray lines of other elements (Cr, Co, Ni, Br, Se, Cd, Hg, and Pb).

A comparison of NM-PHX and M-IK seeds revealed significantly higher levels of Zn in all ROIs for the M-IK seeds and showed that Zn distribution is associated with distinct allocation patterns within the M-IK seed structures, so called ‘Zn hotspots’ (Figs 3, 4; Supplementary Table S4; Supplementary Fig. S2; Supplementary Video S1). These hotspots are characterized by distinctly bright and uniform aggregations of Zn, covering areas of at least 0.1 mm × 0.1 mm within the ROI,

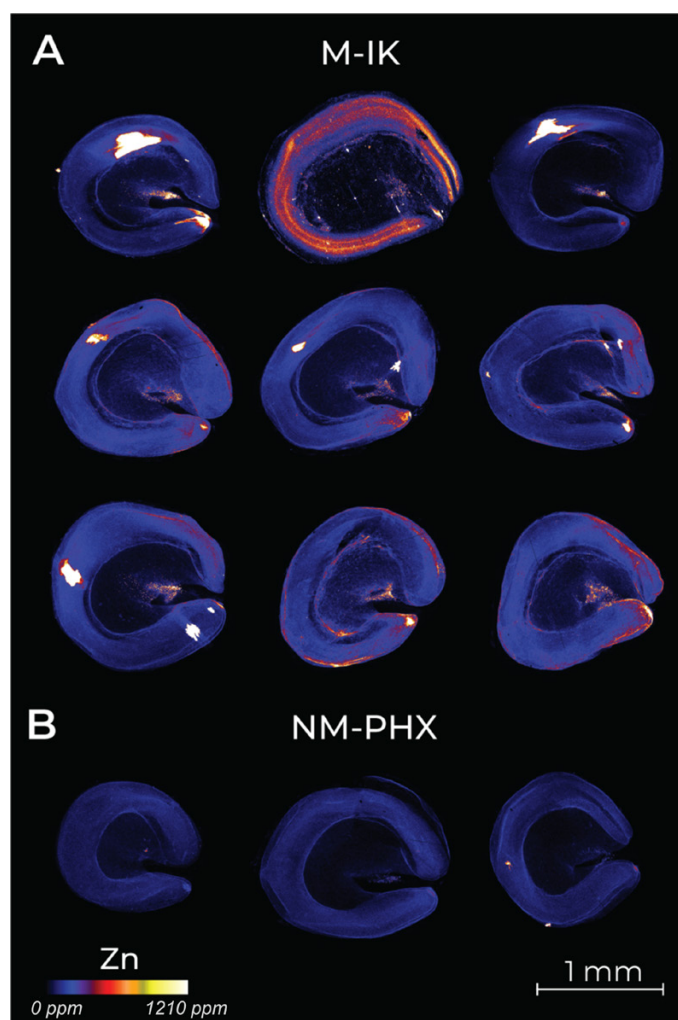


Fig. 3. X-ray fluorescence microscopy (XFM) elemental maps showing the distribution of Zn in *Atriplex lentiformis* seed cross-sections. Seeds originated from metalliferous (M-IK) (A) and non-metalliferous (NM-PHX) (B) locations. Zinc hotspots were observed in the shoot apical meristem and in the root tip zone of the seeds from metalliferous site. The images were obtained at an incident energy of 15.9 keV and dwell time per pixel of 1.25 ms.

and they stand in high contrast to the overall Zn signal in a given sample. In particular, Zn hotspots (which were present exclusively in the M-IK seeds) consistently appeared in the root tip zone (9 out of 13 seeds) with Zn concentrations ranging from 275 to 2650 mg kg⁻¹ and an average of 1255 mg kg⁻¹. Additionally, hotspots found in the shoot apical meristem (5 out of 13 seeds) exhibited Zn concentrations ranging from 1570 to 4360 mg kg⁻¹, with a mean value of 2327 mg kg⁻¹. In contrast, very little Zn was observed in the NM-PHX seeds.

Other differences that were observed between the two accessions include significantly higher Ca concentrations in all structures of the NM-PHX seeds (except for the seed coat) compared with the M-IK seeds. Significant differences were also found in root tip zone (for S, K, Mn, and Fe),

procambium (for Ca and Mn), cotyledons (for Ca and Mn), perisperm (for P, Ca, Cu, and As), and seed coat (for P, S, Cu, and As). Levels of P, K, Cu, and Fe were comparable for most seed structures of both locations (Supplementary Table S4; Supplementary Fig. S3).

To investigate the proportions of K, Ca, Mn, Fe, Cu, and Zn distributed across seed structures between the accessions for each element, we calculated the seed structure attributable fractions (Fig. 5). The most interesting differences were observed for Ca and Zn. The biggest fraction of Ca was allocated to the seed coat of both accessions, with 53% and 31% of total Ca found in the seed coats of the M-IK and NM-PHX seeds, respectively. This resulted in lower fractions of this element in the procambium, cotyledons, and SAM regions. This pattern was stronger in the M-IK seeds. For Zn, a disproportionately high fraction (37%) was allocated in the root tip zone of the M-IK seeds, compared with the NM-PHX seeds (26%). The preferential allocation of Zn in the root tip zone resulted in lower fractions of this element in the procambium, cotyledons, and SAM of the M-IK seeds. The fractions of K, Mn, Fe, and Cu revealed similar patterns of distribution among seed structures and between the two accessions.

The 2D XFM images of elemental composition of the achenes showed higher concentrations of Ca, K, Zn, and Rb in samples from the M-IK site (Supplementary Fig. S4). These observations were in line with the results of the bulk chemical analyses (Table 1). Calcium was primarily distributed in areas adjacent to the seed, specifically in the central part of the achene. In contrast, K, Zn, and Rb were distributed more evenly throughout the achene volume. Notably, higher concentrations of Zn were also accumulated in the achene veins (Supplementary Fig. S4).

XRF tomographic images of seeds and achenes

To conduct a more precise investigation of the distribution of Zn between the achene and seed tissues, we captured 2D tomographic images of seeds enclosed in achenes in the transverse plane (one seed per location; Fig. 6). The images indicate that Zn was mainly localized in the distal parts of the achene, but it was also present near the inner sheath surrounding the seed (internal lay of fruiting bracts) in the form of clusters of elevated concentrations.

Discussion

This study uncovered striking differences in the concentration and distribution of macro- and micronutrients within seed structures of *A. lentiformis* growing at contrasting metalliferous versus non-metalliferous habitats. Particularly interesting were Zn hotspots, found exclusively in the seeds of the metalliferous location, mainly in the sensitive tissues of SAMs and root zones of the seed embryos. In contrast to Zn, the translocation

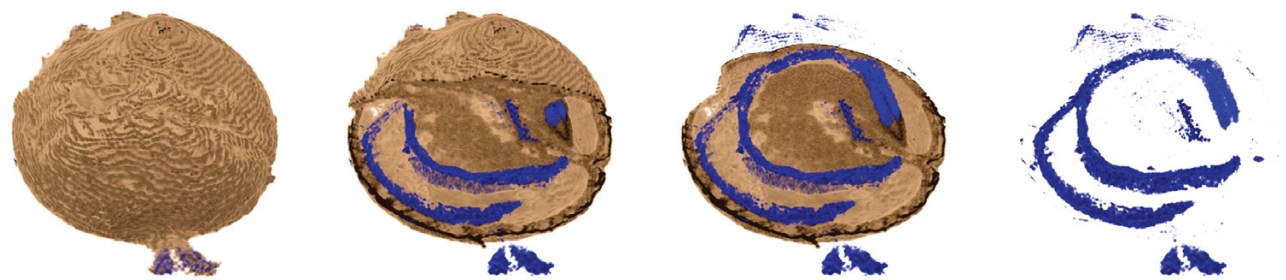


Fig. 4. 3D XFM tomographic reconstruction renderings of an *Atriplex lentiformis* seed from the metalliferous (M-IK) location (absorption + Zn shown in blue). Supplementary Video S1 is an animated model of the reconstructed seed volume.

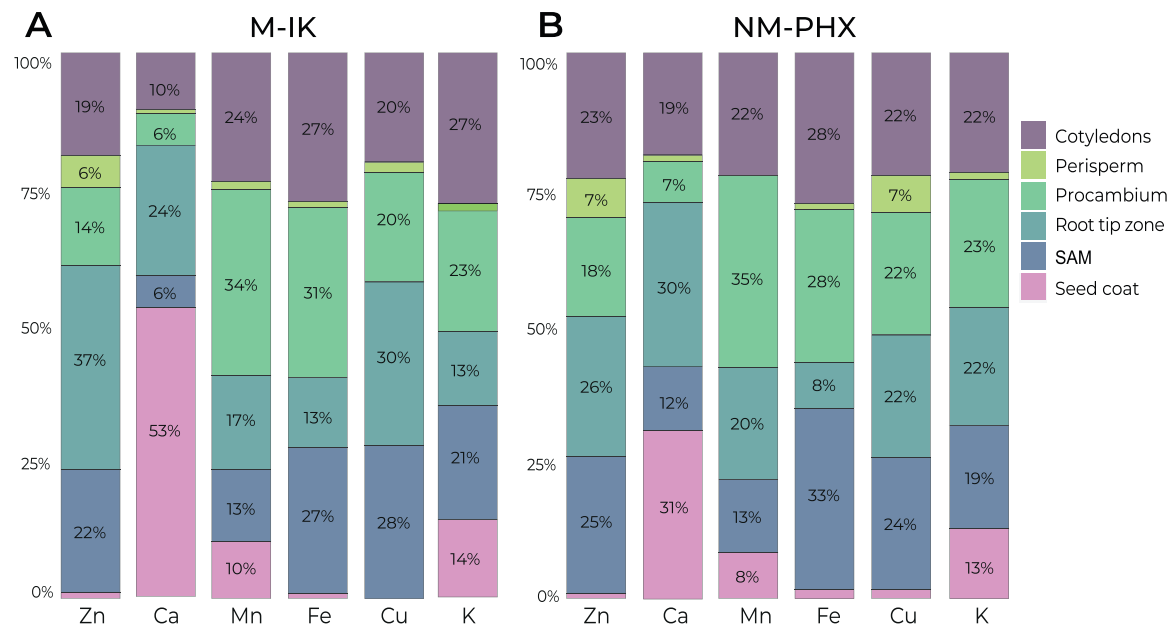


Fig. 5. Bar plots displaying fractions of K, Ca, Mn, Fe, Cu, and Zn distributed among six structures of *Atriplex lentiformis* seeds collected from metalliferous (M-IK) (A) and non-metalliferous (NM-PHX) sites (B). Each color corresponds to a given seed structure. Fractions with values <2% are not signed in the figure. SAM, shoot apical meristem.

of other elements—such as Fe, As, and Pb—into the inner seed structures was limited. Indeed, the latter elements were predominantly sequestered in the achene, suggesting that this structure acts as a protective sink and prevents the excessive accumulation of potentially toxic metal(loid)s within the seed itself. Here we discuss these findings considering associations with the plant’s edaphic origin, soil metal(loid) contamination, and local adaptations to metal-rich environments.

Metal accumulation patterns in metallicolous and non-metallicolous accessions of *Atriplex lentiformis*

Total concentrations of both essential and non-essential elements (e.g. Zn, As, Cd, Pb, Cu, and Fe) in the soil of the M-IK site largely exceed the thresholds considered non-toxic for plants (Kabata-Pendias, 2011). Furthermore, given the slightly acidic pH, substantial fractions of these elements are likely to

be more plant-bioavailable. This was reflected in the higher DTPA-extractable Zn, Cu, and Fe concentrations found at the M-IK site, indicating conditions conducive to increased metal(loid) uptake into plant tissues (Wang et al., 2006; Zeng et al., 2011; Gupta et al., 2016). These results indicate that M-IK *A. lentiformis* plants have been continuously exposed to extreme metal-stress pressure for 8 years. Metal pollution is known to favor plasticity and contribute to metal homeostasis in extreme environments (Bradshaw, 1965; Dechamps et al., 2007), and thus is likely leading to phenotypic adjustments of specific metal-tolerance mechanisms in M-IK plants to improve their performance and survival under these harsh conditions. Indeed, despite this exposure, all macro- and micronutrients and metal(loid)s in plant tissues, except Mg and Zn, had concentrations within the established physiological range in plants (Kabata-Pendias, 2011), showing that the M-IK plants possess effective mechanisms regulating processes of uptake, storage

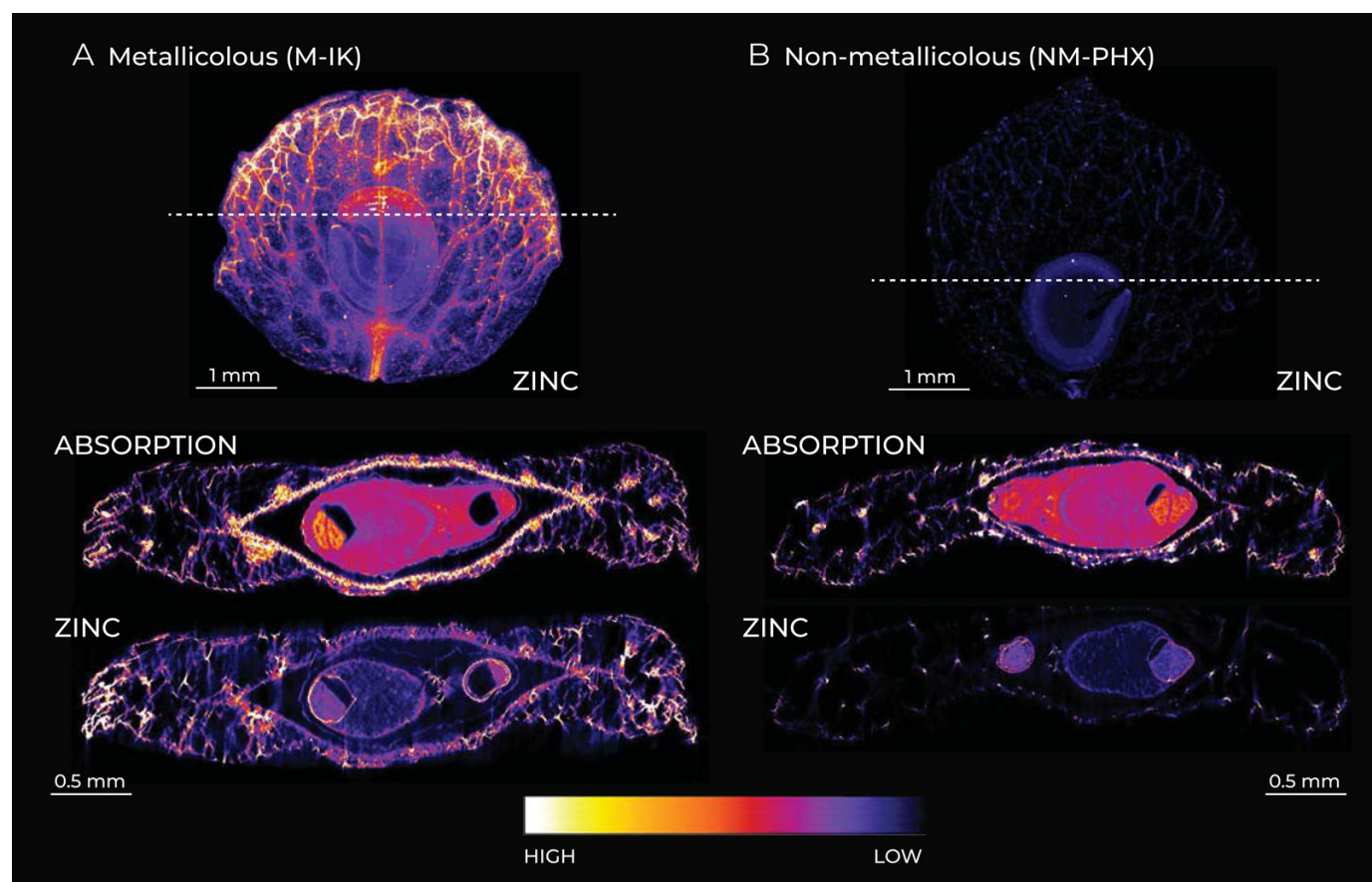


Fig. 6. Single-slice X-ray fluorescence microscopy tomograms of *Atriplex lentiformis* seeds within the achene revealing the presence of Zn in intact tissues, collected from metalliferous (M-IK) (A) and non-metalliferous (NM-PHX) (B) locations. Absorption images were included to present the density and structure of the sample. The transverse slices were scanned at the heights indicated by the dashed line.

and sequestration of metal(loid) ions. While some elements had comparable concentrations in roots and leaves (Cd, Ba, Cu), others were primarily translocated to the aboveground tissues (Zn, Rb, Mn, Li, Na, Mg, K, Ca). Still others, including As and Fe were mainly allocated in the roots with no transport or accumulation into the leaves. Remarkably, despite lower soil concentrations at the M-IK site compared with the NM-PHX site, plant tissues in the former exhibited elevated Rb uptake. This can be attributed to the similarity between Rb and K as chemical analogues, allowing plants to absorb and translocate Rb in place of K under specific soil conditions, e.g. K deficiency and elevated Rb concentrations (Lauchli and Epstein, 1970; Drobner and Tyler, 1998; Shtangeeva *et al.*, 2021).

Notably, only Zn was taken up in concentrations surpassing toxic thresholds in all investigated organs of M-IK plants, including leaves, achenes, and seeds (830, 530, and 150 mg kg⁻¹, respectively). The elevated Zn concentration in aboveground organs of metallicolous *A. lentiformis* plants indicates an enhanced capacity for Zn accumulation, aligning with recent work by Kushwaha *et al.* (2023) in which Zn accumulation was reported under greenhouse conditions. Our field investigation also suggests that despite the overall higher Zn uptake

by the M-IK plants, there is considerable variability in Zn uptake among the sampled individuals and plant organs.

One potential mechanism supporting a defense response that has been reported to stabilize physiological processes in plants under abiotic stress conditions involves elevated concentrations of Ca in leaves (Xu *et al.*, 2022; Feng *et al.*, 2023). Indeed, metallicolous plants in our study had twice as high concentrations of Ca in leaves compared with their counterpart from the non-metalliferous site. Another mechanism suggested by our findings indicates the strategy of increased accumulation of toxic elements within the achenes of metallicolous seeds. These achenes not only provided physical protection against seed damage but also served as an alternative sink, preventing harmful metal(loid) effects for seeds at the M-IK site. Indeed, our study suggests that multiple elements (including Fe, Zn, Li, As, Rb, and Pb) might be sequestered in the achenes, highlighting an important role of this structure in *A. lentiformis* regulation of metal(loid) translocation from the mother plant to the seed. Further, such detailed analysis, where achenes and seeds are considered separately, supports the typical pattern of translocation of elements within plant tissues, where metal content in reproductive parts is expected to be lower than in vegetative

parts, and allocation into above-ground plant organs follows the sequence leaves>stem>flower/fruit/seed (Bhatia *et al.*, 2003; Gupta *et al.*, 2016). Plastic responses to harsh local conditions at the metalliferous M-IK site can be adaptive (Bradshaw, 1965; Ackerly *et al.*, 2000), and future work should involve reciprocal transplant experiments *in situ* to test for local adaptations. Such follow-up investigations are needed to assess the extent to which the patterns and variability between seeds from NM and M locations identified in our study are attributable to phenotypic variability, genetic background, ongoing microevolutionary changes, and/or environmental heterogeneity.

Zinc hotspots in the shoot apical meristem and root tip zone in seeds from the metalliferous location

The key findings from our study were the unique Zn distribution and allocation patterns that we observed in the seed structures of *A. lentiformis* from the M-IK site. A survey of over 60 XFM images revealed that 78% of the examined seeds displayed distinct Zn hotspots in the shoot apical meristem, root tip zone, or cotyledons. A detailed breakdown revealed that 25% of the hotspots were exclusive to the root tip zone, 19% were present in both the root tip zone and shoot apical meristem, 13% were found in the root tip zone and cotyledons, 13% were solely in the shoot apical meristem, and 8% occurred only in the cotyledons. Interestingly, average Zn concentrations were higher in the hotspots identified in the SAM than in the root tip zone (Supplementary Table S4). Intriguingly, a mere 12% of all examined M-IK seeds did not exhibit any specific Zn hotspots. Instead, this small subset of samples had uniformly elevated Zn levels throughout the entire seed volume. In contrast, no Zn hotspots were observed in the non-metallicolous (NM-PHX) seeds.

Previous studies have noted elevated metal(loid) concentrations near the root tip zone and ascribed this to a compartmentalization strategy to protect sensitive tissues from exposure to metal(loid) concentrations (Vogel-Mikuš *et al.*, 2007). For Zn, this presents an interesting conundrum since it is an essential element that plays a pivotal role in plant protein synthesis, as an integral component of enzymes like dehydratase, dehydrogenase, protease, and peptidase. It also participates in the metabolism of carbohydrates, nucleic acids, and lipids (Takahashi *et al.*, 2009; Kabata-Pendias, 2011). Given the high demand for Zn in the metabolic processes of the developing seed (Sadeghzadeh, 2013), the presence of this element in the shoot apex zone, including shoot apical meristem, was expected. These rapidly growing tissues require a substantial influx of nutrients, including Zn, to sustain healthy cell development and ensure optimal plant productivity (Kitagishi and Obata, 1986; Ondrasek *et al.*, 2019). Yet, to the best of our knowledge, there is no previous report of Zn hotspots in essential embryo structures, and the processes and mechanisms underlying this strategy are yet to be resolved. While the Zn hotspot in the shoot apical meristem region of metallicolous

A. lentiformis could be attributed to increased plant demand for Zn, this phenomenon may also serve as a mechanism for the sequestration of excessive Zn. Indeed, Zn concentrations in all observed hotspots exceed the concentrations considered optimal for plant tissues (estimated at around 100 ppm; Kabata-Pendias, 2011; White and Broadley, 2011; Gupta *et al.*, 2016). Considering that excessive Zn levels can cause toxicity and induce oxidative stress and cellular damage, the occurrence of Zn hotspots in the shoot apical meristem and other tissues in the M-IK seeds warrants further investigation under controlled conditions to elucidate the following: what drives elevated Zn allocation into the seed embryo; the role of Zn hotspots; phenotypic plasticity versus evolutionary mechanisms that mediate individual plant responses to Zn; and the plant genes that may regulate the presence of Zn in the shoot apex of *A. lentiformis* seeds from the metalliferous location. It is noted that the M-IK plants have only been exposed to strong selection pressures inherent to the highly toxic environment at the Iron King site for 8 years (Gil-Loaiza, 2016; Gil-Loaiza *et al.*, 2016). We hypothesize that the unique distribution and allocation of Zn within the seeds, identified as hotspots, could be associated with adaptive plasticity in response to Zn contamination. Such an adaptive hypothesis aligns with the findings from controlled conditions experiment on a short-living pseudometallophyte, *Nocca caerulea* (Nowak *et al.*, 2018), illustrating an evolutionary mechanism allowing plants to enhance their Zn tolerance or accumulation capabilities under metal stress. Additionally, such adaptive responses could lead to increased divergence among and within populations, which may be reflected by the variability in Zn hotspot occurrence in seeds from metalliferous location in our study. Yet, testing this hypothesis requires a factorial experiment in which the fitness of each phenotype is assessed in each environment.

Edaphic origin on seed germination rate

Seed traits, such as size and embryo structure, often closely align with a species' ecological niche (Vandelook *et al.*, 2021). Moreover, the transport of metals to seeds is strongly related to metal uptake by the mother plant (Kranter and Colville, 2011). Thus, understanding the influence of origin and edaphic factors, particularly soil metal concentrations, on seed germination is pivotal for successful revegetation of contaminated sites. Our study indicates that the seeds of *A. lentiformis* from the metalliferous location exhibited both a reduced germination percentage and prolonged mean germination time in comparison with the non-metallicolous one. In metallicolous plants, reduced seed germination rate is often a negative consequence of the parental plant's exposure to the elevated soil metal(loid) concentrations. Indeed, alterations in the seed germination process might be a consequence of a 'cost of tolerance' phenomenon, indicating physiological or metabolic constraints driven by additional energy requirements needed to withstand metal(loid) stress (Tyler *et al.*, 1989). Alternatively, the lower

germination rate observed for M-IK plants could be attributed to markedly higher Zn concentrations within the entire seed volume and/or to the presence of specific Zn hotspots. However, establishing a direct link between reduced germination and hotspot occurrence remains challenging due to the destructive nature of synchrotron-based analytical methods.

Similarly, elevated levels of As, Cd, Cu, and Pb in the soil have been linked to delayed germination or extended seed dormancy (Sethy and Ghosh, 2013; Dresler *et al.*, 2017; Shanying *et al.*, 2017). Such delays can be associated with plant defense mechanisms that aim to promote offspring survival and resilience under adverse environmental conditions (Bothe and Slomka, 2017; Penfield and MacGregor, 2017). Given the relatively short time (20 days) of the seed germination test in this study (but see Li *et al.*, 2005; Aflaki *et al.*, 2017; Yáñez-Espinosa *et al.*, 2020), it is possible that germination of the M-IK seeds was simply delayed in comparison with the non-metalliferous accession, not actually reduced.

It is noted that lower germination percentage of seeds from metalliferous locations compared with non-metalliferous ones has been reported for other metallophytes, when tests were performed under favorable and undisturbed conditions (Vogel-Mikuš *et al.*, 2007; Dresler *et al.*, 2017; Murawska-Włodarczyk *et al.*, 2022). Conversely, germination tests using water solutions with gradually increasing metal(loid) concentrations have shown greater metal toxicity tolerance in seeds of *A. halimus* and *Helichrysum microphyllum* collected from metal-contaminated regions (Marquez-García *et al.*, 2013; Boi *et al.*, 2019). These findings suggest an overall higher metal(loid) tolerance and potential local adaptations of metalliferous populations, as reported in these studies. Overall, this calls for further experiments under controlled conditions, which will be crucial for discerning the factors influencing differences in germination rates between *A. lentiformis* accessions from metalliferous and non-metalliferous locations and identifying potential trade-offs.

In summary, *A. lentiformis* is a promising plant for use in phytoremediation of metal-contaminated sites in arid and semi-arid regions of the world. The results of this study, which compared plants and seeds of *A. lentiformis* grown in both low- and high-metal content soils, suggest that metal contamination leads to phenotypic adjustments and the acquisition of specific metal-tolerance traits in both mature *A. lentiformis* plants and seeds. Such information can be used to improve the performance and survival of these plants under harsh conditions.

Supplementary data

The following supplementary data are available at [JXB online](#).

Fig. S1. Map with geographic locations of study sites.

Fig. S2. The XFM elemental distribution maps of Ca, Zn, and Mn in the *Atriplex lentiformis* seed cross-sections.

Fig. S3. The XFM elemental maps showing the distribution of K, Ca, Mn, Fe, Cu, and Rb in *Atriplex lentiformis* seed cross-sections.

Fig. S4. XFM elemental maps revealing Zn, Ca, K, and Rb distribution in empty achenes of *Atriplex lentiformis*.

Table S1. Total elemental concentrations for NIST 2711 and NIST 1515 standards.

Table S2. Soil chemical properties.

Table S3. Elemental concentrations in roots, stems, leaves, seeds, and achenes of *Atriplex lentiformis*.

Table S4. Elemental composition of *Atriplex lentiformis* seed cross-sections.

Video S1. Animated model of the 3D XFM tomographic reconstruction of *Atriplex lentiformis* seed.

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Author contributions

KMW and ABK: conceptualization; AVDE: methodology; TW: resources; KMW, DP, AVDE, and DB: formal analysis; KMW, TW, CR, DP, AS, and AVDE: investigation; RM and ABK: funding acquisition; ABK and AVDE: supervision; KMW, AVDE, and DB: visualization; KMW: writing—original draft preparation; ABK, RM, WJP, JMP, AVDE, TW, DB, DP, and CR: review and editing.

Conflict of interest

The authors declare they have no conflict of interest.

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Data availability

All data supporting the findings of this study are available within the paper and within its supplementary data.

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