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RESEARCH ARTICLE

The effect of L-histidine on nickel translocation and the activities of antioxidant enzymes in hyperaccumulator (*Odontarrhena inflata*) and non-accumulator (*Aurinia saxatilis*) plants

Soraya Soleymanifar · Ali Akbar Ehsanpour · Rasoul Ghasemi · Henk Schat

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Abstract

Background and Aims The role of L-histidine (L-His) in nickel (Ni) hyperaccumulation is not well known. The present study aimed to understand the impact of L-His on Ni translocation and Ni tolerance in *Odontarrhena inflata* and *Aurinia saxatilis*.

Methods To determine the impact of L-His and L-alanine (L-Ala) on the shoot Ni accumulation, we quantified the Ni concentration in plants pretreated with amino acids for 4 h and then exposed to Ni for 8 h. Hydrogen peroxide (H_2O_2) and antioxidant enzymes activities were determined after 4 h of pretreatments and 48 h of Ni treatments.

Results L-histidine increased Ni translocation to shoots in *O. inflata* and *A. saxatilis*. Ni increased the activity of POD, APX, and CAT in both species, but the activities of APX and CAT were higher in *O. inflata*. Ni exposure increased the H_2O_2 concentration in *A. saxatilis*. L-His pretreatment did not decrease the H_2O_2 concentration in Ni-treated plants, but decreased the activity of POD, APX, and CAT only at 300 µM Ni in *O. inflata*. In *A. saxatilis,* L-His pretreatment, decreased the activity of CAT, but increased POD activity at 150 and 300 µM Ni. Pretreatment with L-Ala decreased POD and APX activity but had no significant impact on H_2O_2 concentration and CAT activity.

Conclusion L-Histidine promoted root-to-shoot Ni translocation and alleviated Ni toxicity, possibly through inducing the activities of antioxidant enzymes, in hyperaccumulator and non-accumulator plants. Histidine might not only facilitate the Ni translocation, but also contribute to Ni tolerance, at least in *A. saxatlilis*.

Keywords *Odontarrhana* · Histidine · Antioxidative enzymes · Hyperaccumulation · Nickel translocation

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Introduction

A minority of plant species, called hyperaccumulators (about 0.2% of the total of angiosperms), are capable to grow and reproduce on metal-enriched soils and to accumulate metals to normally toxic concentrations in their foliage (Reeves et al. 2018; van der Ent et al.

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2020). Approximately 500 out of the 720 hyperaccumulator plant species known to date hyperaccumulate nickel, and are endemic to serpentine soils. They are able to accumulate and tolerate Ni at more than 1000 μg Ni g^{-1} dry weight in their shoots (Broadhurst and Chaney 2016; Reeves et al. 2018). About 50 species of the genus *Odontarrhena* (formerly *Alyssum*) from the Brassicaceae family are Ni hyperaccumulators, and are among the best candidates for investigating Ni accumulation in plants (Baker and Brooks 1989; Koch and Al-Shehbaz 2009).

Non-accumulating plants retain the absorbed heavy metals via vacuolar sequestration mainly in their root cortical cells, thereby decreasing their availability for radial transport toward the stele via the cytoplasmic pathway (Kozhevnikova et al. 2014a, b; Richau et al. 2009). In contrast, in hyperaccumulator plants, a much bigger portion of the metal taken up is transported to the shoot where it is mainly sequestered in the vacuoles of leaf epidermal cells (Brooks et al. 1977; Corso and de la Torre 2020; Lasat et al. 2000; Richau et al. 2009; Tappero et al. 2007).

In plants, the Ni ion usually does not form complexes with higher-molecular-weight sulfur-based chelators, such as phytochelatins and metallothioneins, but mainly with low-molecular-weight (LMW) polar oxygen- or nitrogen-based ligands such as organic acids and amino acids (Kachenko et al. 2010). Histidine has been suggested to be the most important chelating agent of Ni in plants, at least in plants with Ni hyperaccumulation capacity (Kerkeb and Krämer 2003; Krämer et al. 1996; Richau et al. 2009). This amino acid serves as a tridentate ligand that forms a Ni complex [$Ni(His)_2$] with a high stability constant at physiological pH (Callahan et al. 2006; Krämer et al. 1996; Valenti et al. 2006). Ni–histidine complexes have been identified in the roots, xylem sap, and shoots of *Noccaea caerulescens* and in the plant sap and vascular tissue of *Alyssum* (presently *Odontarrhena*) *murale* (McNear et al. 2010; Ouerdane et al. 2006). It seems that a constitutively high free His concentration in roots is a common property of Ni accumulators within the Brassicaceae family, including *Alyssum lesbiacum*, *Alyssum serpyllifolium*, *Noccaea goesingense* and *Noccaea caerulescens* (Ingle et al. 2005; Krämer et al. 1996; Persans et al. 1999; Richau et al. 2009). Next to L-His, nicotinamine (NA) might function as a LMW chelator of Ni, at least in Ni hyperaccumulators, although its precise role is poorly understood yet (Seregin and Kozhevnikova 2023).

Excessive Ni concentrations can disrupt iron (Fe) homeostasis and subsequently cause Fe chlorosis (Ghasemi et al. 2009), and inhibit photosynthesis through disruption of the electron transport chain, chloroplast structure, chlorophyll biosynthesis, or trough replacing cofactors in the active sites of metalloenzymes (Hassan et al. 2019; Seregin and Kozhevnikova 2006; Singh and Pandey 2011). Induction of oxidative stress and stimulation of plant antioxidant systems have also been reported under Ni stress (Gajewska and Skłodowska 2007; Rao and Sresty 2000). Therefore, in the cytoplasm of plant cells, free Ni ions should be rapidly chelated and detoxified to prevent Ni toxicity (Corso and de la Torre 2020; Merlot et al. 2018), to this end, high-affinity ligand molecules are employed to facilitate metal chelation, transport, and sequestration (Clemens 2019).

When exogenously supplied, via the nutrient solution, L-His pretreatment has been shown to promote Ni translocation in many Brassicaceae species, both in Noccaea Ni hyperaccumulaors, but also in several non-accumulators (Kozhevnikova et al. 2021b; Seregin et al. 2022), though often not in Odontarrhena Ni hyperaccumulators (Seregin et al. 2019). The latter might be attributable to the strong induction of L-His biosynthesis in roots upon Ni exposure (Kerkeb and Krämer 2003; Krämer et al. 1996), which might obscure any effect of pretreatment with exogenous L-His. In any case, enhanced L-His concentrations in the xylem itself, such as found upon Ni exposure in the xylem fluid of plants that had been grown in hydroponics without added Ni (Kerkeb and Krämer 2003; Krämer et al. 1996), are apparently lacking in plants that had been grown under high Ni from germination onward (Centofanti et al. 2013), suggesting that the role of L-His in Ni hyperaccumulation, if any, does not primarily lie in the chaperoning of Ni during its transport through the xylem itself, but rather in its inhibiting effect on the vacuolar sequestration of Ni (or Zn) in root cortical cells, such as suggested by uptake experiments with root tonoplast vesicles obtained from *Noccaea caerulescens* and *Thlaspi arvense* (Richau et al. 2009; Kozhevnikova et al. 2014a). If so, then it seems that the loading of Ni (or Zn) into the xylem may be more often limited by these metals' vacuolar retention in the root cortex or, consequently, their radial transport from

the root surface toward the stele, than by the plants' capacity to load them into the xylem as such, at least among many hyperaccumulators, but also in at least several non-hyperaccumulator Brassicaceae (Seregin et al. 2022).

Next to the apparent facilitation of Ni or Zn root-to-shoot translocation, L-His might conceivably affect Ni toxicity in accumulating and non-accumulating plants. Therefore, the present study aims at understanding the role of L-His in Ni translocation, accumulation, and detoxification. We investigated the effect of exogenous L-His on Ni translocation to the shoots, H_2O_2 accumulation, and the activities of antioxidant enzymes in a Ni hyperaccumulator, *Odontarrhena inflata*, originating from serpentine soils of west Iran, and the non-accumulator, *Aurinia saxatilis*.

Materials and methods

Seedling growth

Seeds of *Odontarrhena inflata* (formerly *Alyssum inflata* Nyár.) were harvested from an Iranian ultramafic region, Baneh (Ghaderian et al. 2007). *Aurinia saxatilis* seeds were acquired from Horti Tops-Tuinplus, Heerenveen, Netherlands. Since, both plants species (*A. saxatilis* and *Alyssum inflate*) belong to the same tribe (Alysseae) of the family Brassicaceae was selected as model plants for heavy metal accumulation studies (Hendriks et al. 2023; Li et al. 2015; Španiel et al. 2015). Seeds were sown on Perlite and watered using ultrapure water for the first week, then the germinated seeds were supplied with a one-quarter-strength Hoagland type solution (1.5 mM $Ca(NO_3)_2$, 0.28 mM KH_2PO_4, 0.75 mM $MgSO_4$, 1.25 mM KNO_3, 0.5 µM $CuSO_4$, 2.5 µM $ZnSO_4$, 5 µM $MnSO_4$, 25 µM H_3BO_3, 0.1 µM Na_2MoO_4, 50 µM KCl, 5 µM Fe–EDDHA (ethylenediamine-N,N′-bis(2-hydroxyphenylacetic acid), 3 mM MES, with pH 5.7, adjusted using KOH). Three-week-old seedlings were transferred to 400 mL hydroponic culture vessels supplemented with the same Hoagland solution. The solution was continuously aerated and replaced every 7 days by a freshly prepared one. All experiments were performed in a growth chamber with a 16-h illumination/8-h dark day/night cycle (150 µmol m^{-2} s^{-1} light intensity) and a 24/18°C (day/night) temperature regime. Experiment 1 To characterize the effect of exogenously supplied L-histidine on root-to-shoot Ni translocation, 8 weeks-old plants were transferred to a 2-m*M* Mes/KOH-buffer supplemented with 1 mM L-histidine or L-alanine (pH 5.5). A subset of plants not undergoing this pretreatment was used as a control. After 4 h of pretreatment, the roots of plants were thoroughly rinsed with ultrapure water, then transferred to a fresh nutrient solution supplemented with 300 µM $NiSO_4$. Finally, after 8 h of Ni treatment, the shoots of plants were harvested, rinsed with ultrapure water, blotted dry with paper towels, and then snap-frozen in liquid nitrogen, and stored at − 80°C.

Experiment 2 Preliminary experiments showed that 48 h was the optimum time for measuring catalase activity in response to Ni treatments (data not shown). To measure the activities of antioxidant enzymes in the shoots, 8 weeks old plants were transferred to a MES/KOH buffered medium containing L-His or L-Ala (0,150, 300, 600, and 1000 µM, pH 5.5) for 4 h. A subset of plants not pretreated with amino acids was kept as a control. Then, the roots of the pretreated plants were rinsed in ultrapure water and then the plants were transferred to the same one-quarter-strength Hoagland solution containing $NiSO_4$ (0,150, and 300 µM) for 48 h. A subset of plants pretreated only with amino acids were rinsed with ultrapure water and were transferred to a nutrient solution without Ni for 48 h. Then the shoots of all the plants were harvested, rinsed twice with ultrapure water, and dried carefully between paper towels. Shoot samples were weighted and immediately snap-frozen in liquid nitrogen and then stored at -80° C.

Activities of catalase, ascorbate peroxidase and peroxidase

Aliquots of 100 mg frozen leaf tissue were homogenized with a cooled mortar and pestle after adding 1 mL of cold enzyme extraction buffer containing 100 mM potassium phosphate, 2% (w/v) polyvinylpyrrolidone (PVP), 4 mM DTT and 1 mM EDTA (pH 7.8). The homogenized tissues were centrifuged at 15,000 g, 4°C for 20 min. Supernatant aliquots of approximately 100 µl were collected and used to determine the activities of different enzymes. The protein concentration was measured according to Bradford (1976). All enzymatic assays were conducted using a UV–VIS spectrophotometer (BioTek

Synergy HTX). Catalase activity was determined based on the Aebi and Lester (1984) method. Catalase activity was initiated by adding 10 µl of 10 mM H_2O_2 to 160 µl of reaction mixture containing 50 mM potassium phosphate buffer, pH 7.0, and 5 µl protein extract. The absorbance was recorded at 240 nm. One unit of CAT activity was defined as the required amount of enzyme for the decomposition of 1 µmol H_2O_2 in 1 min at 240 nm at 25 °C using an extinction coefficient of 0.036 mM^{-1} cm^{-1}.

For the ascorbate peroxidase (APX) activity, the decrease in absorbance at 290 nm, as a consequence of ascorbate oxidation during the reaction, was measured (Nakano and Asada 1981). The assay reaction solution was a mixture of 5 µl leaf protein extract, 165 µl of 50 mM potassium phosphate buffer, pH 7, containing 0.2 mM EDTA and 0.5 mM ascorbic acid. The APX mediated reaction was started by adding 5 µl of 10 mM H_2O_2 to the reaction solution. APX activity was calculated using an extinction coefficient of 2.8 mM^{-1} cm^{-1} for ascorbate. One unit of APX enzyme activity was defined as the oxidation of 1 µmol /min ascorbic acid at 25 °C. The activity of peroxidase (POD) was quantified through measuring the rate of tetragaiacol formation at 470 nm. The reaction solution was a combination of 100 mM potassium phosphate buffer, pH 7.0, 10 mM guaiacol, 10 mM H_2O_2, 0.1 µM EDTA, and protein extract. POD activity was calculated using the extinction coefficient of tetraguaiacol (26.6 mM^{-1} cm^{-1}) (Plewa et al. 1991).

Analysis of hydrogen peroxide (H_2O_2)

Hydrogen peroxide was determined according to the Velikova et al. (2000) method. Approximately, 100 mg of fresh leaf biomass was homogenized with 1 ml of 0.1% (w/v) trichloroacetic acid (TCA) on ice. The homogenate was centrifuged for 15 min at 12,000 g at 4 °C. Subsequently, 500 µl of 10 mM potassium phosphate buffer, pH 7, and 1 mL of 1 M potassium iodide (KI) were added to 500 µl of the supernatant. The concentration of H_2O_2 was calculated from the absorbance at 390 nm, using an extinction coefficient of 0.28 μM^{-1} cm^{-1}, and expressed as µmol H_2O_2 g^{-1} FW.

Quantification of amino acids

Approximately 200 mg of frozen homogenized leaf tissue powder per sample was mixed with 400 µl of 80% (v/v), ethanol in a 2.5 mM HEPES buffer, pH 7.5 (Scheible et al. 1997). Tissue extracts were obtained through vortexing and incubating the samples in buffer at 80 °C for 20 min in a heating block. After centrifugation at 20,800 g for 10 min, the supernatants were transferred to new tubes. To extract all L-His and L-Ala from the tissues, the pellets were centrifuged two more times, as described above, after resuspension in 400 µl of 50% (v/v) ethanol in 2.5 mM HEPES buffer (pH 7.5) and then in 200 µl of 80% (v/v) ethanol. The three supernatants were collected and stored at -20°C for further analysis. The concentrations of L-His and L-Ala in plant extracts were measured through LC-coupled MSE-based quantification using high-performance liquid chromatography (HPLC).

Elemental Analysis

Shoot aliquots were freeze-dried overnight (Alpha 1–4 LDplus, Martin Christ, Osterode, Germany), equilibrated at room temperature for 1 day, and weighed. Dry shoot samples were digested in 3 ml of 65% nitric acid overnight at room temperature. Thereafter, sample-containing tubes were incubated in a water bath at 90°C for 4 h, followed by cooling, and the addition of 1 ml of 30% (v/v) H_2O_2 per sample, before the tubes were placed again at 90°C for 1 h. Eventually, the final solution volumes were brought to 10 ml with ultrapure water. Element concentrations were quantified by atomic absorption spectrophotometry (AAS-6200, Shimadzu Corporation, Chiyoda-ku, Tokyo, Japan).

Statistical analysis

All experiments were carried out in a completely randomized design with at least three replications. Data were analyzed using two-way ANOVA and differences between individual means were tested using Duncan's test at a significance threshold of $P < 0.05$ (SPSS software version 22, for Windows).

Results

The effect of exogenously supplied L-histidine and L-alanine on root-to-shoot Ni translocation under short-term Ni treatment (8 h).

Pretreatment with L-His significantly increased the Ni concentration in the shoots of both *O. inflata* and *A. saxatilis* by 1.4 = and 3.9 = fold, respectively, compared to the control plants, while the L-Ala pretreatment was without a significant effect in *A.saxatilis*, or slightly deceased the shoot Ni concentration in *O. inflata* (Fig. 1a).

In both plant species, exogenous L-His pretreatment increased the shoot L-His concentration significantly (Fig. 1b). Pretreatment with amino acids had little effect on the concentrations of Fe, Zn (Fig. 1a) and other micro- and macro-nutrients in shoots (Data not shown).

The effect of exogenous L-histidine and L- alanine pretreatments (4 h) and subsequent Ni supply (48 h) on antioxidant enzymes activity in shoots.

The shoot H_2O_2 concentration was significantly higher in *A. saxatilis* than in *O. inflata*. Increasing the Ni concentrations in the nutrient solution increased the H_2O_2 concentration in the shoots of *A. saxatilis*, though only significantly at 300 µM Ni. However; the H_2O_2 concentration remained unchanged in *O. inflata* (Fig. 2a). In the absence of Ni, and in both species, exogenous L-His and L-Ala were without significant effects on the shoot H_2O_2 concentrations (Fig. 2b, d), except for a small concentration-independent increase for exogenous L-Ala in *A. saxatilis* (Fig. 2d). L-His ptretreatment did not significantly affect the shoot H_2O_2 concentration in *A. saxatilis*, except for a modest, but a significant increase was observed in plants treated with 1000 µM L-His and 150 µM Ni, or with 1000 µM L-His and 300 µM Ni, compared to the non-L-His pretreated plants (Fig. 2c). In *O. inflata* L-His pretreatment modestly raised the shoot H_2O_2 concentration, though only significantly in plants treated with 1000 µM L-His and 150 µM Ni, or with 600 or 1000 µM L-His and 300 µM Ni, compared to the non-L-His pretreated plants (Fig. 2c). Compared to exclusively Ni-exposed plants, there were no significant effect of the L-Ala pretreatments on the shoot H_2O_2 concentrations in *O. inflata and A. saxatilis* at 150 and 300 µM Ni (Fig. 2e).

In both species the Ni treatment caused a significant increase of the peroxidase (POD) activity in the shoot (Fig. 3a). Interestingly, also the L-His pretreatment alone (not followed by Ni treatment) significantly increased the shoot POD activity in both species, but much more in the hyperaccumulator, *O. inflata*, (up to more than 29-fold at 1000 µM L-His), while, the activity in *A. saxatilis* was only slightly, but significantly, increased, (± twofold at 600 and 1000 µM L-His) (Fig. 3b).

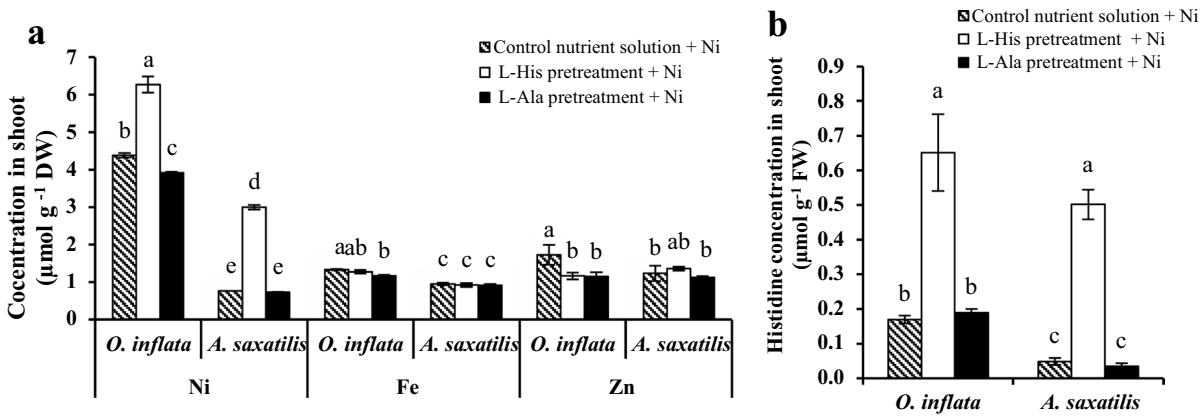
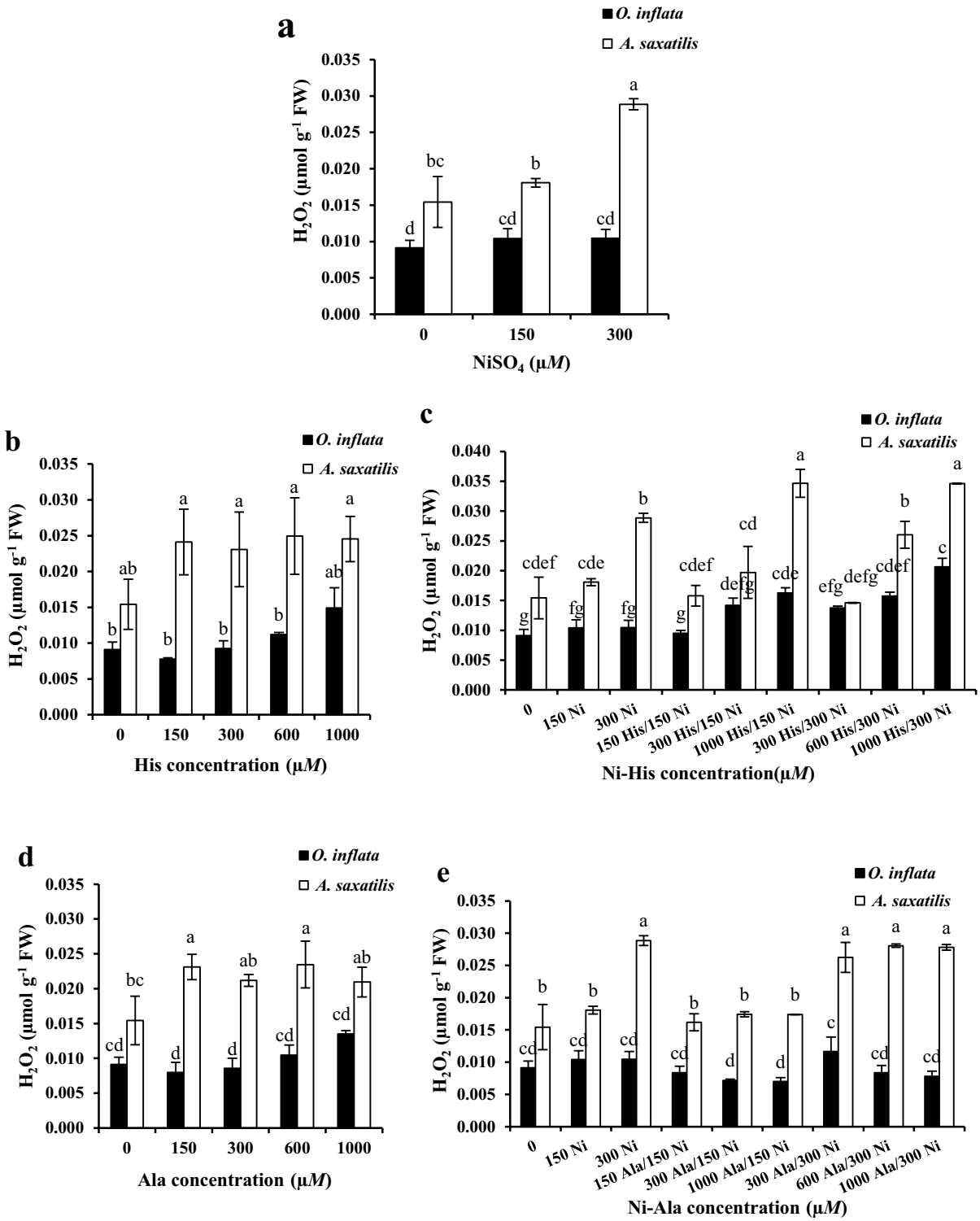


Fig. 1 The effects of exogenously supplied L-His and L-Ala on the Ni, Fe, and Zn (**a**) and His (**b**) concentration in the shoots of *O.inflata* and *A. saxatilis* after short-term Ni exposure (8 h). Eight-week-old plants were pretreated with 1 mM L-His (open bars), 1 mM L-Ala (closed bars), or control nutrient solution (dashed bars) for 4 h and subsequently treated with 300 µM Ni (8 h). Values represent means ± SE of three replicates. Different letters show significant differences ($P < 0.05$) based on Duncan's test


a
O. inflata
A. saxatilis
H2O2 (µmol g-1 FW)
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
d
bc
cd
b
cd
a
0
150
300
NiSO4 (µM)
b
O. inflata
A. saxatilis
H2O2 (µmol g-1 FW)
ab
b
a
b
a
b
a
ab
a
0
150
300
600
1000
His concentration (µM)
c
O. inflata
A. saxatilis
H2O2 (µmol g-1 FW)
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
g
cdef
fg
cde
fg
b
g
cdef
defg
cd
cde
a
efg
defg
cdef
b
c
a
0
150 Ni
300 Ni
150 His/150 Ni
300 His/150 Ni
1000 His/150 Ni
300 His/300 Ni
600 His/300 Ni
1000 His/300 Ni
Ni-His concentration(µM)
d
O. inflata
A. saxatilis
H2O2 (µmol g-1 FW)
cd
bc
d
a
d
ab
cd
a
cd
ab
0
150
300
600
1000
Ala concentration (µM)
e
O. inflata
A. saxatilis
H2O2 (µmol g-1 FW)
cd
b
cd
b
cd
a
cd
b
d
b
d
b
c
a
cd
a
cd
a
0
150 Ni
300 Ni
150 Ala/150 Ni
300 Ala/150 Ni
1000 Ala/150 Ni
300 Ala/300 Ni
600 Ala/300 Ni
1000 Ala/300 Ni
Ni-Ala concentration (µM)

◂**Fig. 2** The effect of different concentrations of Ni (**a**), L-His (**b**), L-His pretreatment and Ni (**c**), L-Ala (**d**), and L- Ala pretreatment and Ni (**e**) on H_2O_2 concentrations in the shoot of *O. inflata* (closed bars) and *A. saxatilis* (open bars). The roots of 8-week-old plants were pretreated with different concentrations of L-His or L-Ala for 4 h, then supplemented with Ni (48 h). Values represent means ± SE of three replicates. Different letters show significant differences ($P < 0.05$) based on Duncan's test

Also L-Ala pretreatment alone (without Ni exposure) significantly increased the shoot POD activity in both species, but again, more in *O. inflata* than in *A. saxatilis* (Fig. 3d).

The species responded clearly distinctly to the combined Ni and L-His treatments. In *O.inflata* Ni exposure consistently decreased the shoot POD activity, whereas in *A. saxatilis* Ni exposure consistently increased the shoot POD activity in L-His pretreated plants (Fig. 3c), in comparison with plants that were pretreated with L-His alone (Fig. 3b). Also in comparison with the plants that were exposed to Ni alone (without L-His pretreatment), the POD activities were decreased, generally significantly, in L-His pretreated *O. inflata*, but unaffected, or further increased in *A. saxatilis*, except for the combination of 300 µM L-His with 300 µM Ni (Fig. 3c).

In comparison with the L-Ala pretreatment alone (without Ni exposure), Ni significantly moderated the increase in shoot POD activity in both species, at least at 300 µM Ni, in plants pretreated with 600 or 1000 µM L-Ala (Fig. 3d, e). Similarly, in comparison with the Ni treatment alone, the L-Ala pretreatment moderated the Ni-induced increase in shoot POD activity in both species, at least at the 300 µM Ni exposure level (Fig. 3e).

The activity of APX in the shoots of both species was enhanced by increased Ni concentrations in the medium and in the hyperaccumulator, *O. inflata*, this increase was bigger than in the non-accumulator, e.g. up to 1.8-fold times at 300 µM Ni (Fig. 4a). Pretreatment with L-His alone (without subsequent Ni exposure) significantly increased the APX activity in *O. inflata*, but not in *A. saxatilis* (Fig. 4b). Similarly, pretreatment with L-Ala increased the shoot APX activity in *O. inflata*, but not in *A. saxatilis* (Fig. 4d). In *O. inflata*, L-His pretreatments (300, 600 and 1000 µM), prior to Ni exposure (300 µM Ni), decreased the APX activity, compared to the control plants. In contrast to the L-His pretreatment, both plant species pretreated with L-Ala, showed a major decrease in APX activity at 300 µM Ni, compared to the plants treated with Ni alone (Fig. 4e).

The activity of CAT was increased by Ni exposure, by approximatly 45% and 38% at 300 µM Ni, in *O. inflata* and *A. saxatilis,* respectively, compared to the control plants (Fig. 5a). Without subsequent Ni exposure, both the L-His and L-Ala pretreatments had no significant impact on CAT activity in both species (Fig. 5b, d). The CAT activity in Ni-exposed *O. inflata* was decreased (by 52%) by L-His pretreatment, though significantly in the 300, 600 and 1000 µM L-His pretreatments, followed by exposure to 300 µM Ni. In Ni-exposed *A. saxatilis,* exogenous L-His dramatically decreased the activity of CAT, both at 150 and 300 µM Ni, compared with the plants treated with Ni only (Fig. 5c). Unlike POD and APX, the activity of CAT was not significantly affected by the exogenous L-Ala pretreatment, compared to the plants exposed to Ni only (Fig. 5e).

As expected, increasing the Ni concentration in the treatment solution resulted in elevated Ni concentrations in the shoots, with a remarkably higher Ni concentration in *O. inflata,* than in *A. saxatilis* (more than a 5-fold higher in *O. inflata* at 300 µM Ni, Fig. 6a). At both Ni concentrations (150 and 300 µM Ni), L-His pretreatments significantly increased the Ni concentration in the shoots of both species, compared with unpretreated plants. In *O. inflata*, pretreatment with 1000 µM L-His increased the shoot Ni concentrations, both at 150 and 300 µM Ni, by approximately 40% and 20%, respectively, but in *A. saxatilis* by more than 72% and 73%, respectively (Fig. 6b). Pretreatment of plants with L-Ala resulted in a slight decrease of shoot Ni concentrations in both species, compared to the plants treated with Ni only (Fig. 6c).

The 48-h Ni treatments (150 and 300 µM Ni, without prior exposure to L-His or L-Ala) significantly increased the L-His concentrations in the shoots of both *O. inflata* and *A. saxatilis*, compared to the control plants (Fig. 7a). However, the shoot L-Ala concentrations of both species were not affected by Ni exposure (Fig. 7d). In both species the L-histidine pretreatments (without and with subsequent Ni exposure, Fig. 7b, c) increased the shoot L-His concentrations in a concentration-dependent way, compared to the non-Ni-exposed control plants. A similar pattern was observed for the shoot L-Ala concentration (Fig. 7e). Ni exposure did not further enhance

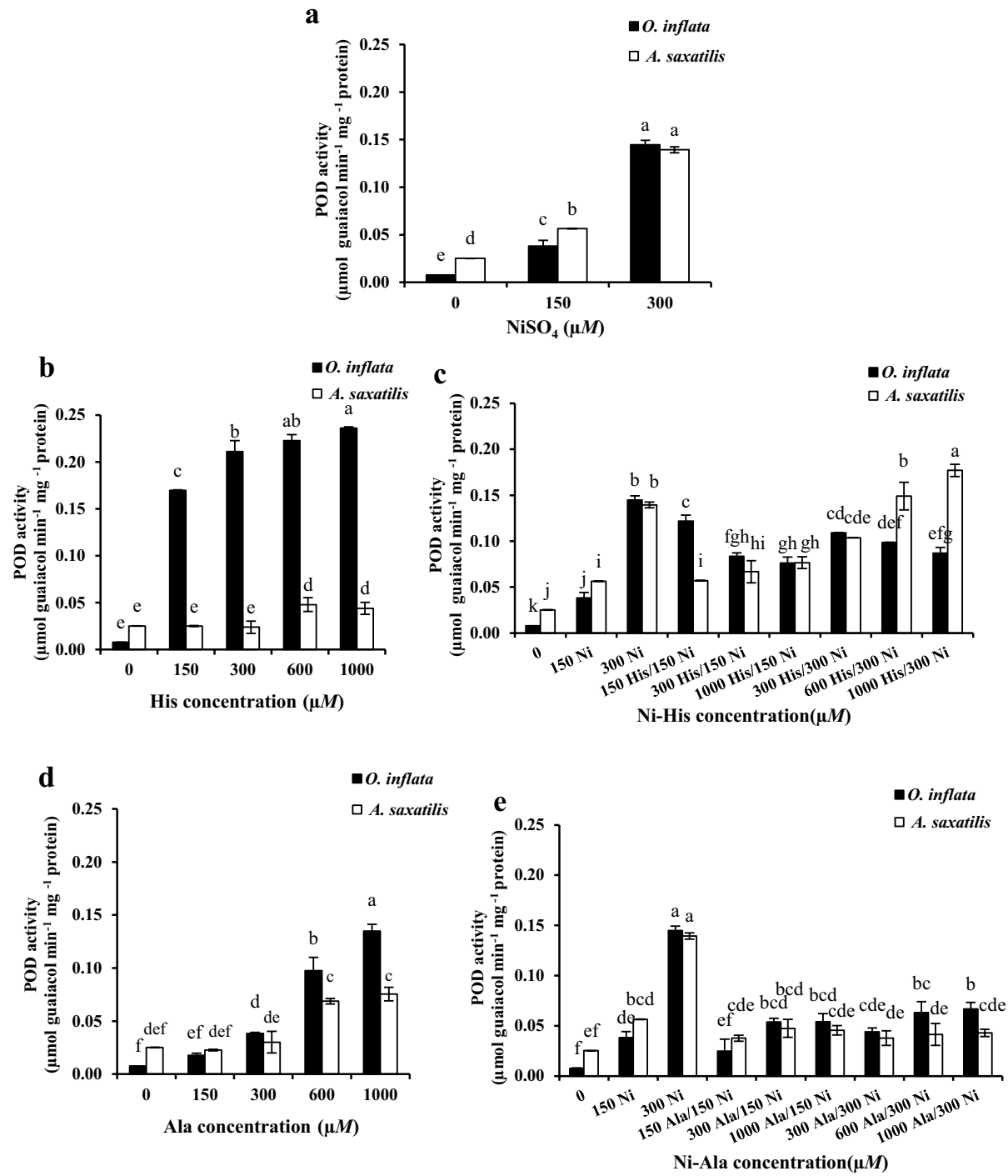


Fig. 3 The effect of different concentrations of Ni (**a**), L-His (**b**), L-His pretreatment and Ni (**c**), L-Ala (**d**), and L- Ala pretreatment and Ni (**e**) on the activity of peroxidase in *O. inflata* (closed bars) and *A. saxatilis* (open bars). Assays were conducted using total soluble protein extracts from shoots of 8-week-old plants pretreated with L-His or L-Ala for 4 h and then supplemented with Ni (48 h). Values represent means ± SE of three replicates. Different letters show significant differences ($P < 0.05$) based on Duncan's test

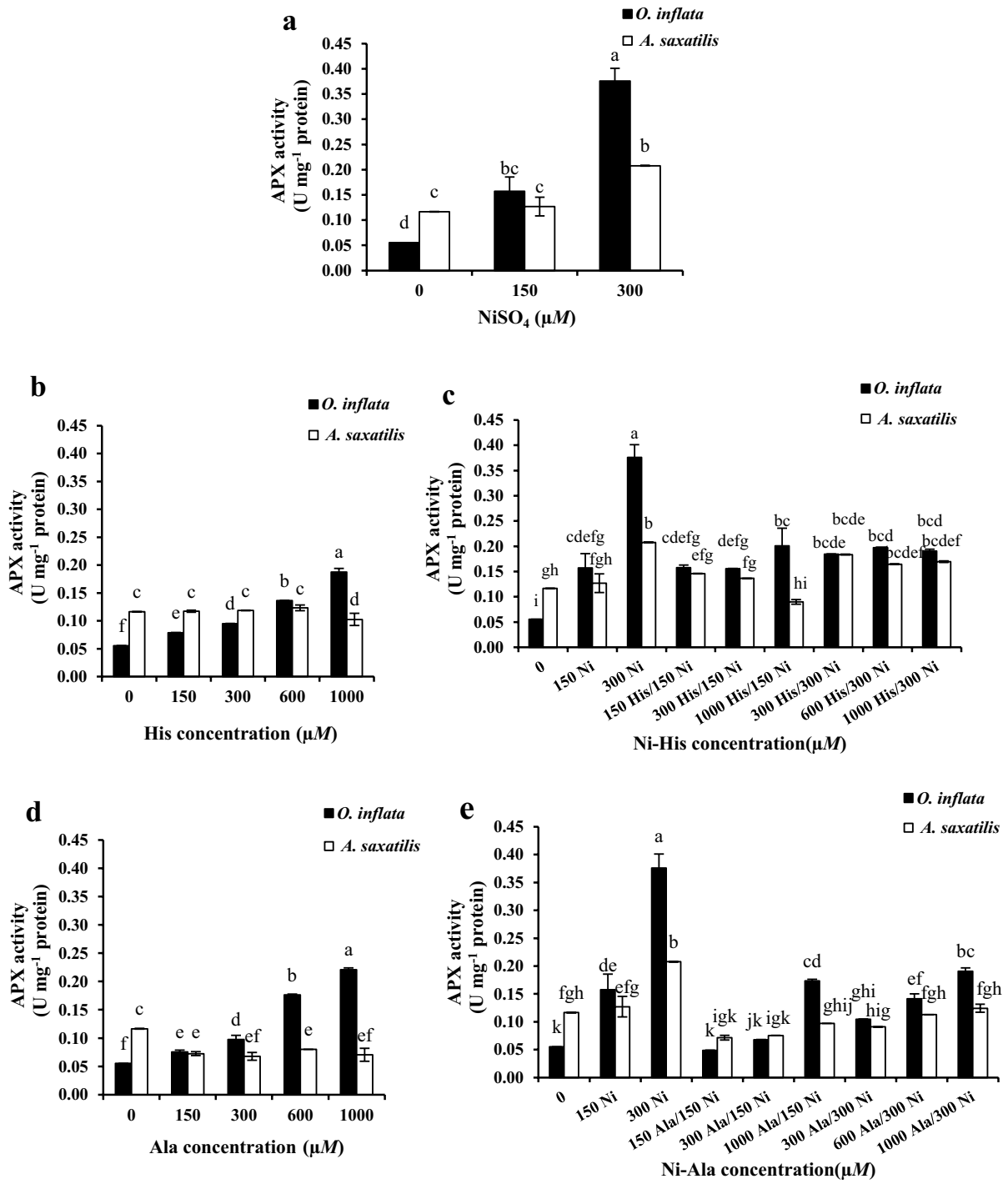


Fig. 4 The effect of different concentrations of Ni (**a**), L-His (**b**), L-His pretreatment and Ni (**c**), L-Ala (**d**), and L- Ala pretreatment and Ni (**e**) on the activity of ascorbate peroxidase in *O. inflata* (closed bars) and *A. saxatilis* (open bars). Assays were conducted using total soluble protein extracts from shoots of 8-week-old seedlings pre-exposed to L-His and L-Ala for 4 h and then supplemented with Ni (48 h). Values represent means ± SE of three replicates. Different letters show significant differences ($P < 0.05$) based on Duncan's test, 1 U (µMol/min)

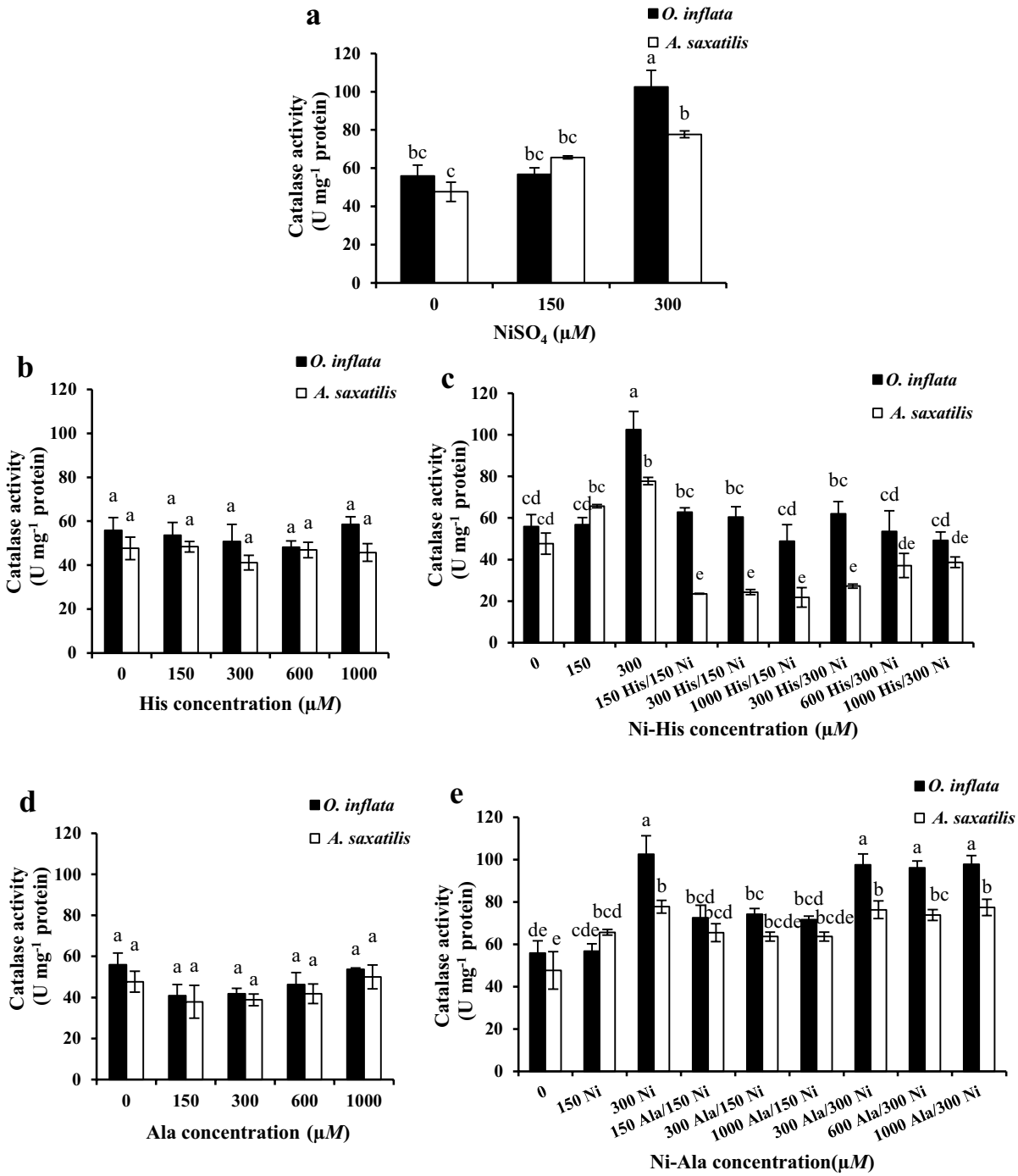


Fig. 5 The effect of different concentrations of Ni (**a**), L-His (**b**), L-His pretreatment and Ni (**c**), L-Ala (**d**), and L- Ala pretreatment and Ni (**e**) on the activity of catalase in *O. inflata* (closed bars) and *A. saxatilis* (open bars). Assays were conducted using total soluble protein extracts from shoots of 8-week-old plants pre-exposed to L-His and L-Ala for 4 h and then supplemented with Ni (48 h). Values represent means ± SE of three replicates. Different letters show significant differences ($P < 0.05$) based on Duncan's test

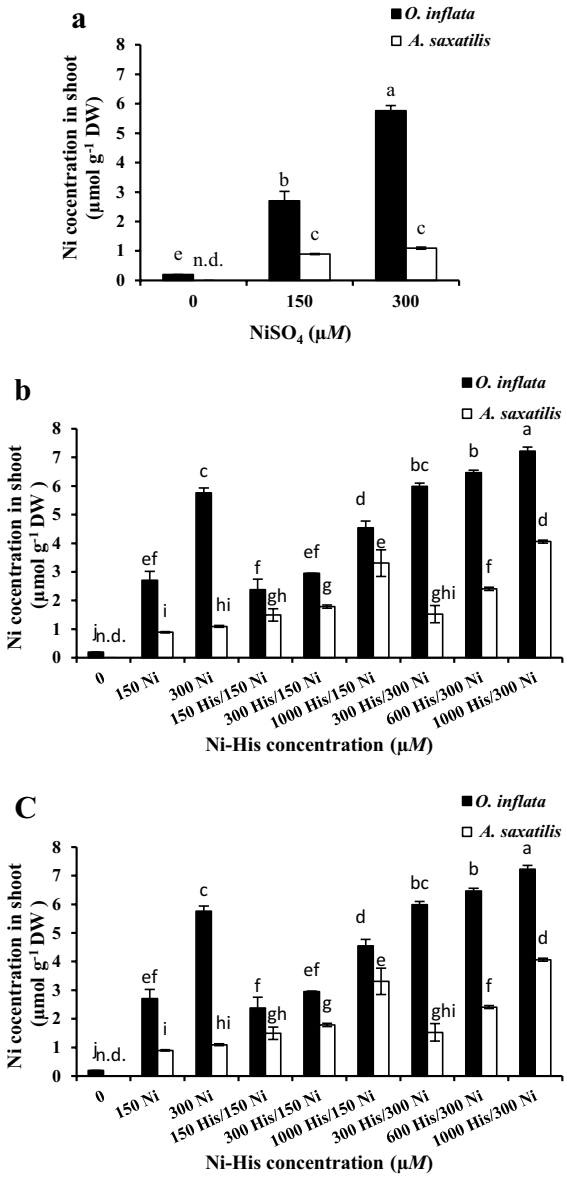


Fig. 6 The effect of different concentrations of Ni (**a**), L-His pretreatment and Ni (**b**), and L- Ala pretreatment and Ni (**c**) on shoot Ni concentrations of *O. inflata* (closed bar) and *A. saxatilis* (open bars). The nickel concentration was determined in shoots of 8-week-old plants pre-exposed to L-His or L-Ala for 4 h and then supplemented with Ni (48 h). Values represent means ± SE of three replicates. Different letters show significant differences ($P < 0.05$) based on Duncan's test. n.d. = not detectable

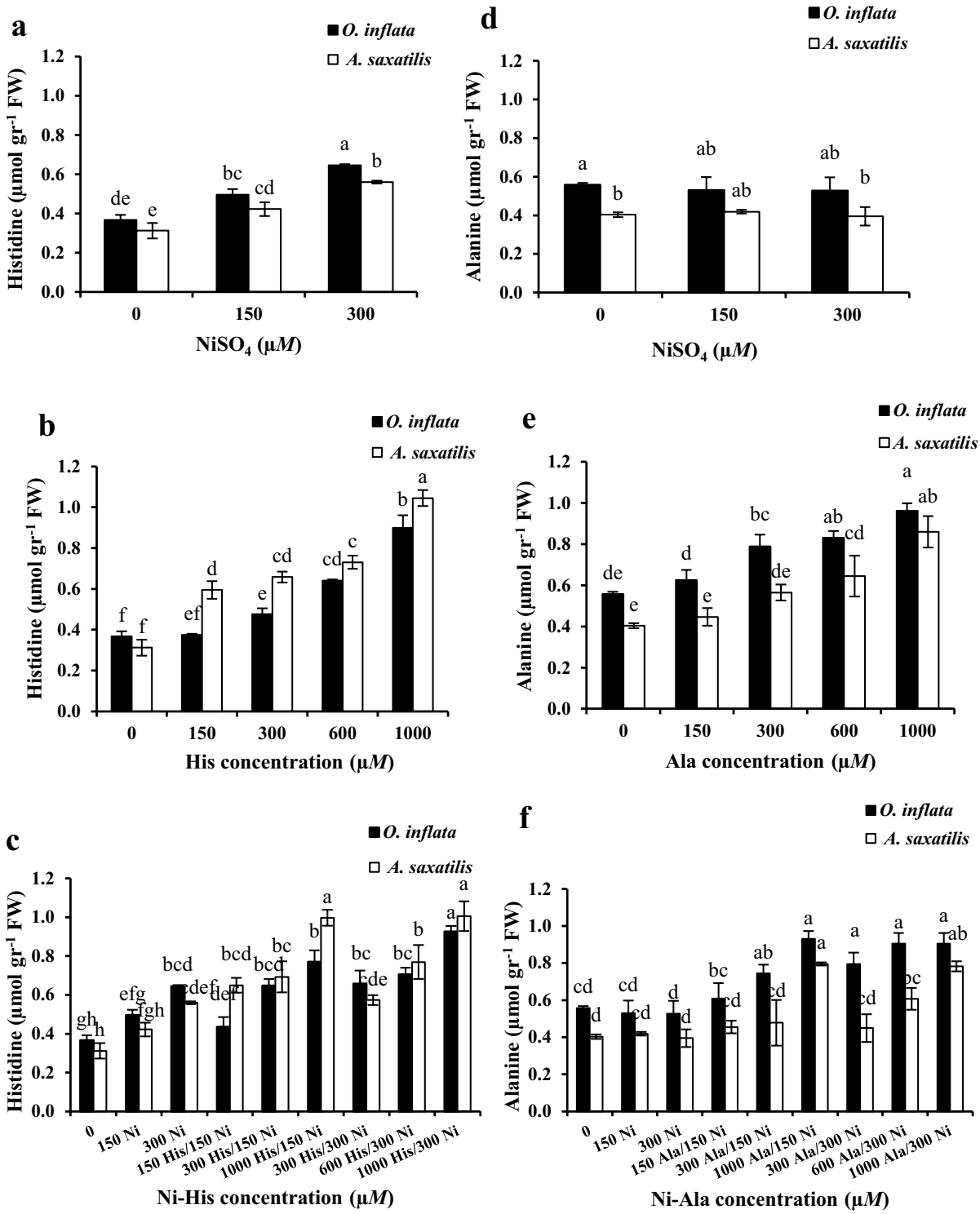
the shoot L-His or L-Ala concentrations in L-His or L-Ala pretreated plants, respectively (Fig. 7c, f, Fig. 7b, e).

Treatment with 300 µM Ni for 48 h, significantly decreased the shoot dry weight (SDW) of the non-accumulator *A. saxatilis*, by approximately 16%, compared to the control plants. Ni treatments had no significant effect on the SDW of the hyperaccumulator, *O. inflata* (Fig. 8a). The highest concentrations of L-His or L-Ala used for the pretreatments (1000 µM) significantly decreased SDW in *A. saxatilis*, by 13.03% and 16.40%, respectively, compared to the control plants. However, none of the L-His and L-Ala pretreatments showed a significant impact on *O. inflata* SDW, when no Ni was added to the medium (Fig. 8b, d). Pretreatment with 1000 µM L-His prior to the Ni treatments increased the *A. saxatilis* SDW, by approximately 6.5% and 7.6%, respectively, compared to non-pretreated plants exposed to 150 and 300 µM Ni (Fig. 8c). In both species, pretreatment with L-Ala before Ni exposure (150 and 300 µM Ni) had no significant effect on SWD in both species, compared with Ni-only treated plants (Fig. 8e).

Discussion

Exogenous L-His pretreatment stimulated the Ni translocation in both hyperaccumulator and non-accumulator plants

As shown by our results, pretreating plants with L-His, but not with L-Ala, significantly increased the shoot Ni concentration in both species, in agreement with previous observations in many other Brassicaceae (Kerkeb and Krämer 2003; Kozhevnikova et al. 2021a,b; Seregin et al. 2022). However, this was not associated with significant changes in the concentrations of Zn and Fe or other micro- and macronutrient concentrations in shoots (data not shown), although exogenous L-His supply has been shown to increase the translocation of, at least, Zn in a number of other hyperaccumulator and non-accumulator Brassicaceae (Kozhevnikova et al. 2021a,b; Seregin et al. 2022). For *O. inflata*, this effect of exogenous L-His supply, i.e., enhanced shoot Ni accumulation, is surprising, since Seregin et al. (2019) did not observe enhanced Ni concentrations upon exogenous L-His supply in the xylem sap of the majority of *Alyssum* (*Odontarrhena*) Ni hyperaccumulators tested. The huge increase of shoot Ni in *A. saxatilis*, on the other hand, is in agreement with the observation of a


a
O. inflata
A. saxatilis
Histidine (µmol gr-1 FW)
NiSO4 (µM)
d
Alanine (µmol gr-1 FW)
b
His concentration (µM)
e
Ala concentration (µM)
c
Ni-His concentration (µM)
0
150 Ni
300 Ni
150 His/150 Ni
300 His/150 Ni
1000 His/150 Ni
300 His/300 Ni
600 His/300 Ni
1000 His/300 Ni
f
Ni-Ala concentration (µM)
150 Ala/150 Ni
300 Ala/150 Ni
1000 Ala/150 Ni
300 Ala/300 Ni
600 Ala/300 Ni
1000 Ala/300 Ni

◂**Fig. 7** The effect of different concentrations of Ni (**a**), L-His (**b**), and L-His pretreatment and Ni (**c**) on shoot His concentration, and Ni (**d**), L-Ala (**e**), and L-Ala pretreatment and Ni (f) on shoot Ala concentration of *O. inflata* (closed bars) and *A. saxatilis* (open bars). The concentration of amino acids was determined in shoots of 8-week-old plants pre-exposed to L-His for 4 h and then supplemented with Ni (48 h). Values represent means ± SE of three replicates. Different letters show significant differences ($P < 0.05$), based on Duncan's test

significantly increased Ni concentration in the xylem of this species by the same authors. The lack of effect of exogenous L-His on Ni translocation in many *Alyssum*/*Odontarrhena* Ni hyperaccumulators might well be due to a strong induction of L-His biosynthesis upon Ni exposure (Kerkeb and Krämer 2003; Krämer et al. 1996). Concerning the mechanism of action L-His, it is relevant that the chelation of Ni (and/or Zn) by L-His strongly inhibited their uptake into right-side-out energized tonoplast vesicles isolated from *N. caerulescens* roots (Kozhevnikova et al. 2014b; Richau et al. 2009), suggesting that L-His may promote these metals' translocation to the shoot through inhibiting their vacuolar retention in peripheral root cells (Seregin and Kozhevnikova 2021). In this respect, it is interesting that exogenous L-His increased the shoot Ni concentration relatively less strongly in *O. inflata* (± 1.5-fold) than in *A. saxatilis* (± 4-fold), which might be attributable to a strong accumulation of plant-internal free L-His upon Ni exposure in the former species, which seems to be characteristic of *Alyssum*/*Odontarrhena* Ni hypeaccumulators (Kerkeb and Krämer 2003; Krämer et al. 1996), but lacking or being much less conspicuous in Noccaea Zn/Ni hyperaccumulator and non-accumulator Brassicaceae (AD Khozevnikova and IV Seregin, unpublished results). In any case, the shoot Ni concentration upon pretreatment with exogenous L-His remained significantly lower (about 2-fold) in *A. saxatilis* than in *O. inflata*, while the shoot L-His concentrations were not significantly different (Fig. 1), as previously observed in other Brassicaceae non-accumulators (Kozhevnikova et al. 2021a,b; AD Kozhevnikova and IV Seregin, unpublished). This may be taken to indicate that the Ni translocation in unpretreated plants of both species is, at first instance, limited by the vacuolar retention of Ni in the roots, but in L-His-pretreated plants by their Ni xylem loading capacity, and that the Ni xylem loading capacity is lower in *A. saxatilis* than in *O. inflata*. However, the precise mechanisms of Ni xylem loading in plants are poorly understood to date. Further detailed studies are urgently required. In any case, it is unlikely that Ni and L-His are loaded simultaneously, as a complex, into the xylem (see above). Moreover, when grown under Ni exposure from germination onward, Odontarrhena/Alyssum Ni hyperaccumulators didn't show exceptionally high L-His concentrations in their xylem fluid (Alves et al. 2011; Centofanti et al. 2013), such as observed shortly upon sudden Ni exposure in plants that were raised without Ni in the nutrient solution (Kerkeb and Krämer 2003; Krämer et al. 1996). Although this is strong evidence that L-His doesn't serve as a Ni chaperone during xylem transport indeed, it does not prove that L-His is not essential for Ni translocation in Ni hyperaccmulators. These results merely confirm that the role for L-His does not lie in the chelation of Ni within the xylem itself, but rather in the root cell cytoplasm, where it seems to counteract the vacuolar retention of Ni (or Zn) in root cortical cells, at least in the Ni/Zn hyperaccumulator, *Noccaea caerulescens* (Kozhevnikova et al. 2014a; Richau et al. 2009). Therefore, the increase of the L-His concentrations observed in the xylem exudates of Ni hyperaccumulators shortly upon sudden Ni exposure is probably a transient phenomenon, caused by a Ni-imposed stimulation of L-His biosynthesis in the roots. Of course, this does not preclude any essential role for L-His in Ni hyperaccumulation, since L-His most likely impacts upon the vacuolar retention of Ni in cortical root cells, but probably not upon the (the last step in) Ni xylem loading as such, and (almost) certainly not upon the Ni transport rate through the xylem vessels themselves. Concerning the latter, it is important to keep in mind that the His-Ni complex is highly stable at cytoplasmic pH (about 7.2), but expected to dissociate at xylem pH (< 7.0), owing to protonation of the imidazole nitrogen (Blindauer and Schmid 2010; Monsant et al. 2011; Salt et al. 1999). Moreover, although the Ni xylem loading mechanisms in Ni hyperaccumulators are poorly known, it seems unlikely that Ni can be loaded as undissociated Ni-His, in view of the abundance of His residues in the metal-binding regions of the vast majority of the candidate transporters known thus far (Krämer et al. 2007; Williams and Mills 2005). Thus, in summary, if L-His would play a role in Ni hyperaccumulation, one would expect enhanced L-His concentrations in Ni hyperaccumulators, compared

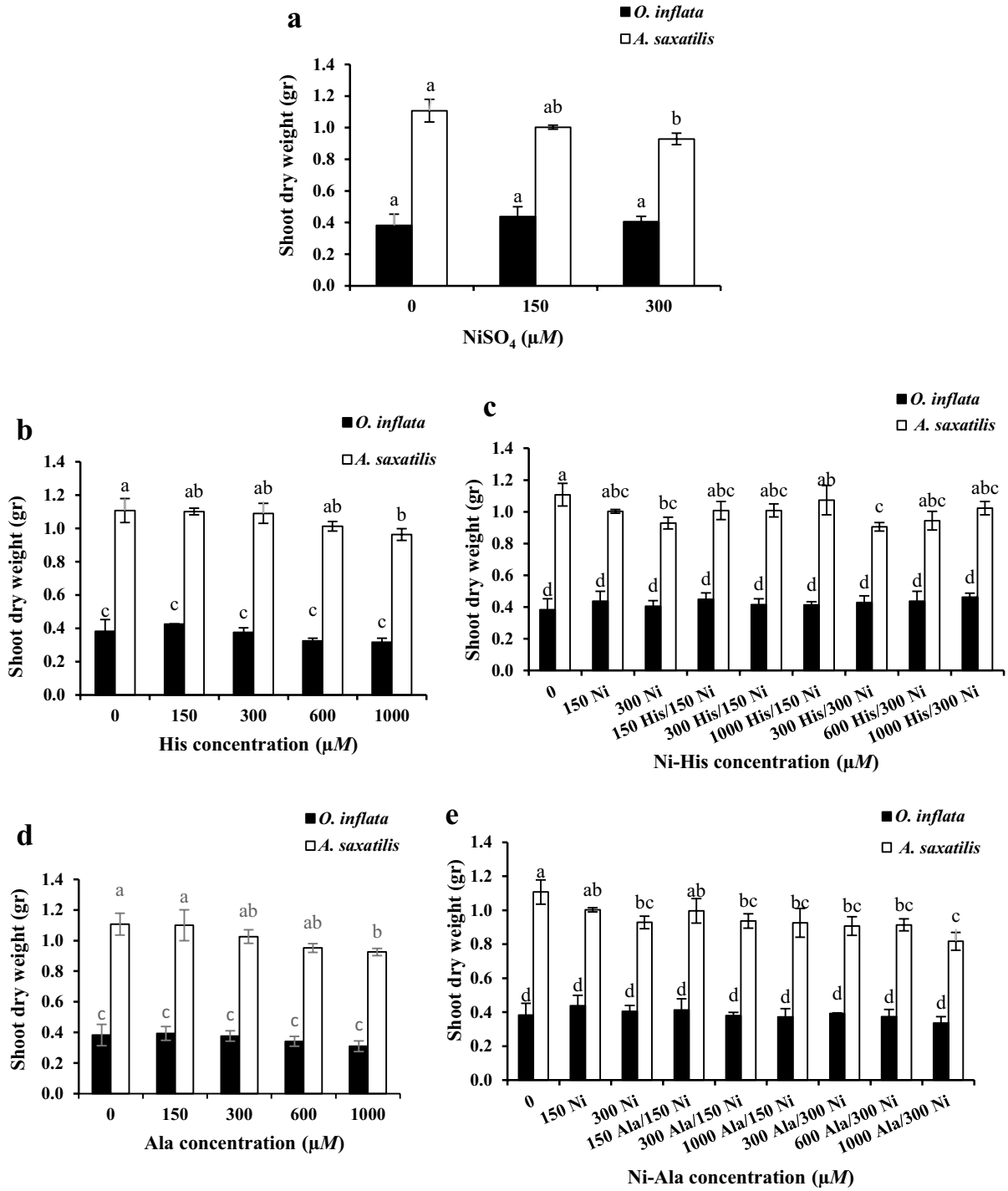


Fig. 8 The effect of different concentrations of Ni (**a**), L-His (**b**), L-His pretreatment and Ni (**c**), L-Ala (**d**), and L- Ala pretreatment and Ni (**e**) on shoot dry weight of *O. inflata* (closed bars) and *A. saxatilis* (open bars). The roots of 8-week-old plants were pretreated with different concentrations of L-His or L-Ala for 4 h, then supplemented with Ni (48 h). Values represent means ± SE of three replicates. Different letters show significant differences ($P < 0.05$), based on Duncan test

to non-accumulators, at least in the roots, either constitutively, or inducible by Ni exposure. The latter (Ni-induced increased L-His concentrations in roots) occurs almost certainly in *Odontarrhena* Ni hyperaccumulators (Kerkeb and Krämer 2003; Krämer et al. 1996), while *Noccaea* Ni hyperaccumulators are characterized by strongly enhanced constitutive L-His concentrations, though only in their roots, not in their shoots (AD Kozhevnikova and IV Seregin, unpublished results). In any case, the L-His concentrations in the xylem exudates or shoots are probably without direct relevance concerning the role of L-His in Ni translocation in Ni hyperaccumulators.

The possible mitigating effects of L-histidine on the toxicity of Ni in shoots

L-His has also been suggested to mitigate Ni toxicity in plants both in Ni hyperaccumulators and non-accumulators (Ingle et al. 2005; McNear et al. 2010). Our results are in line with this viewpoint, since pretreatment with L-His, but not with L-Ala completely abolished the decreased SDW upon Ni-exposure (300 μM) in *A. saxatilis*, which was not apparent in unpreatreated *O. inflata*, probably due to the presence of adaptive Ni hypertolerance in the latter (serpentine) species (Fig. 8). Ni treatments significantly increased free L-His but not L-Ala concentrations in shoots of both *O. inflata* and *A. saxatilis*. Moreover, despite some variations, by increasing the concentration of L-His pretreatments, a remarkable reduction of APX and CAT activities was observed in *O. inflata* and *A. saxatilis*, compared to the only Ni-treated plants (Figs. 4c and 5c). These observations might signify a particular role for L-His in Ni detoxification in both species. The precise mechanisms underlying L-His-mediated Ni tolerance in plant shoots are unknown yet, but may be assumed to depend mainly on the chelation of Ni by L-His at cytoplasmic pH, and/or the resulting alterations of the Ni distribution patterns over organs, tissues, cells, or cellular compartments. As reported by Ingle et al. (2005), an increased Ni tolerance and shoot-free His concentration was observed in transgenic *Arabidopsis thaliana* lines with the overexpression of *A. lesbiacum* (Ni hyperaccumulator) ATP phosphoribosyl transferase (*ATP-PRT*) cDNA encoding the first and regulatory enzyme of the His biosynthesis pathway.

It is important to note that the L-His pretreatment itself also seems to be toxic, at least in *A. saxatilis*, as judged from the significant decrease in SDW at 1000 μM L-His (Fig. 8b). Thus, although it reduces Ni toxicity, excessive concentrations of L-His as such might cause metabolic disturbances, at least in *A. saxatilis*, but possibly also in *O. inflata*, as suggested by the huge induction of POD by L-His (without Ni) (Fig. 4c), although there were no significant effects of the L-His treatment (without Ni) on SDW in the latter species (Fig. 8b). The same pattern was observed for the L-Ala pretreatments (Fig. 8d), the main difference being that L-His pretreatment protected *A. saxatilis* from Ni toxicity, but that the L-Ala pretreatment did not (Fig. 8c, e). The cellular processes and biomass production (Fig. 8b and d) of these plant species can be affected by the high concentration of amino acids (Fig. 7b) in various ways. The imidazole side chain of histidine can act as a weak base, and excess histidine concentrations might lead to cellular pH change, which can disrupt the pH optimum for the maximal catalytic activity of enzymes and affect ion channel function, and protein stability (Liao et al. 2013; Monsant et al. 2011; Nelson and Cox 2017; Pantoja 2021; Raven and Smith 1976; Zhang et al. 2015). In addition to Ni, histidine can bind various metal ions including Zn, Cu, and Co, therefore, excess levels of this amino acid can disturb the homeostasis of other metal ions in plant cells (Homer et al. 1991; Monsant et al. 2011; van der Ent et al. 2020). The higher concentrations of amino acids outside the cell than inside, or intracellular excess amino acids cause cell dehydration and cell swelling, respectively. As a result, these processes can cause an imbalance in cell water potential and disturb normal cell functions, ultimately affecting the production of plant biomass (Kowles 2010; Taiz et al 2015).

Our observation that Ni exposure (300 μM) caused H_2O_2 accumulation in the shoots of (unpretreated) *A. saxatilis*, but barely or not in *O. inflata* (Fig. 2a), probably also reflects the superior Ni tolerance of the latter species. It is noteworthy that this Ni-imposed H_2O_2 accumulation was not reversed, but even increased by the 1000-μ*M* L-His pretreatment (Fig. 2c), suggesting that increased L-His synthesis, as such, may not be sufficient to prevent Ni-imposed oxidative stress in the genetic background of *A. saxatilis*. On the other hand, also Ni-exposed *O. inflata* showed enhanced, rather than decreased Ni-imposed shoot H_2O_2 accumulation

after L-His pretreatment (Fig. 2c), but this was probably owing to excessive accumulation of L-His itself, in view of the absence of any significant H_2O_2 accumulation in Ni-exposed unpretreated plants (Fig. 2a), and the very similar effects of L-His in plants with and without Ni exposure (Fig. 2b, c).

It is remarkable that, upon Ni exposure, the antioxidant enzyme activities, at least those of APX and CAT, but possibly also that of POD, were more upregulated in *O. inflata* than in *A. saxatilis* (Figs. 3a, 4a and 5a), in spite of the superior Ni tolerance and the absence of any Ni-imposed H_2O_2 accumulation in the former species. In addition, also the L-His and L-Ala pretreatments more strongly increased these enzyme's capacities (Figs. 3b, d and 4b, d), except that of CAT (Fig. 5b, d). Except for POD in L-His-pretreated plants (Fig. 3c), the antioxidant enzyme activities were usually higher in L-His or L-Ala pretreated Ni-exposed (300 µM) *O. inflata* than in *A. saxatilis* (Figs. 4c, e and 5c, e). These results may be taken to indicate that the antioxidant capacity under Ni exposure is overall higher in *O. inflata* than in *A. saxatilis*, which could explain the absence of any detectable Ni-imposed H_2O_2 accumulation in the former species. It is even arguable that an enhanced enzymatic antioxidant capacity might represent an essential component of the Ni hypertolerance syndrome in *O. inflata*. On the other hand, this superior antioxidant capacity is only apparent after Ni exposure, but not at all in the absence of Ni (Fig. 3a, 4a and 5e), or in other words, Ni-inducible, whereas metal-hypertolerances are usually constitutive, rather than inducible by exposure to the metal in question (Ernst et al. 1992). However, the possibility that Ni-inducible antioxidant capacity contributes to Ni hypertolerance in *O. inflata* cannot be excluded with certainty.

Conclusions

Exogenous L-histidine promoted Ni translocation to the shoot in both Ni hyperaccumulator *O. inflata* and non-accumulator *A. saxatilis* plants. The shoots of *O. inflata* seemed to be equipped with a high enzymatic antioxidant capacity, though only after Ni exposure, which possibly prevented Ni-induced H_2O_2 accumulation. However, in both species the shoot H_2O_2 concentrations upon Ni exposure were unaffected, or slightly increased by pretreating the plants with exogenous L-His or L-Ala.

Next to enhancing the root-to-shoot Ni translocation in both species, exogenous L-His supply significantly improved Ni tolerance, as estimated from the effect on shoot dry weight, at least in *A. saxatilis* and on the activity of antioxidant enzymes, in both species.

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Data availability Data is available based on request.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

References

Aebi H, Lester P (1984). In: Packer L (ed) Methods in Enzymology 121–126. Academic Press, New York. https://doi.org/10.1016/S0076-6879(84)05016-3

Alves S, Nabais C, Gonçalves MDLS, Dos Santos MMC (2011) Nickel speciation in the xylem sap of the hyperaccumulator *Alyssum serpyllifolium* ssp. *lusitanicum* growing on serpentine soils of northeast Portugal. J Plant Physiol 168:1715–1722. https://doi.org/10.1016/j.jplph.2011.04.004

Baker AJ, Brooks R (1989) Terrestrial higher plants which hyperaccumulate metallic elements. A review of their distribution, ecology and phytochemistry. Biorecovery 1:81–126

Blindauer CA, Schmid R (2010) Cytosolic metal handling in plants: determinants for zinc specificity in metal transporters and metallothioneins. Metallomics 2:510–529. https://doi.org/10.1039/c004880a

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Broadhurst CL, Chaney RL (2016) Growth and metal accumulation of an *Alyssum murale* nickel hyperaccumulator ecotype co-cropped with *Alyssum montanum* and perennial ryegrass in serpentine soil. Front Plant Sci 7:45. https://doi.org/10.3389/fpls.2016.00451

Brooks RR, Lee J, Reeves RD, Jaffré T (1977) Detection of nickeliferous rocks by analysis of herbarium specimens

of indicator plants. J Geochem Explor 7:49–57. https://doi.org/10.1016/0375-6742(77)90074-7
Callahan DL, Baker AJ, Kolev SD, Wedd AG (2006) Metal ion ligands in hyperaccumulating plants. J Biol Inorg Chem 11:2–12. https://doi.org/10.1007/s00775-005-0056-7
Centofanti T, Sayers Z, Cabello-Conejo MI, Kidd P, Nishizawa NK, Kakei Y, Davis AP, Sicher RC, Chaney RL (2013) Xylem exudate composition and root-to-shoot nickel translocation in Alyssum species. Plant Soil 373:59–75. https://doi.org/10.1007/s11104-013-1782-1
Clemens S (2019) Metal ligands in micronutrient acquisition and homeostasis. Plant Cell Environ 42:2902–2912. https://doi.org/10.1111/pce.13627
Corso M, de la Torre VSG (2020) Biomolecular approaches to understanding metal tolerance and hyperaccumulation in plants. Metallomics 12:840–859. https://doi.org/10.1039/D0MT00043D
Ernst WHO, Verkleij JAC, Schat H (1992) Metal tolerance in plants. Acta Bot Neerl 41:229–248. https://doi.org/10.1111/j.1438-8677.1992.tb01332.x
Gajewska E, Skłodowska M (2007) Effect of nickel on ROS concentration and antioxidative enzyme activities in wheat leaves. Biometals 20:27–36. https://doi.org/10.1007/s10534-006-9011-5
Ghaderian SM, Mohtadi A, Rahiminejad MR, Baker AJM (2007) Nickel and other metal uptake and accumulation by species of *Alyssum* (Brassicaceae) from the ultramafics of Iran. Environ Pollut 145:293–298. https://doi.org/10.1016/j.envpol.2006.03.016
Ghasemi R, Ghaderian SM, Krämer U (2009) Interference of nickel with copper and iron homeostasis contributes to metal toxicity symptoms in the nickel hyperaccumulator plant *Alyssum inflata*. New Phytol 184:566–580. https://doi.org/10.1111/j.1469-8137.2009.02993.x
Hassan MU, Chattha MU, Khan I, Chattha MB, Aamer M, Nawaz M, Ali A, Khan MAU, Khan TA (2019) Nickel toxicity in plants: reasons, toxic effects, tolerance mechanisms, and remediation possibilities—a review. Environ Sci Pollut Res 26:12673–12688. https://doi.org/10.1007/s11356-019-04892-x
Hendriks KP, Kiefer C, Al-Shehbaz IA, Bailey CD, van Huysduynen AH, Nikolov LA, Nauheimer L, Zuntini AR, German DA, Franzke A, Koch MA (2023) Global Brassicaceae phylogeny based on filtering of 1,000-gene dataset. Curr Biol. https://doi.org/10.1016/j.cub.2023.08.026
Homer FA, Morrison RS, Brooks RR, Clemens J, Reeves RD (1991) Comparative studies of nickel, cobalt, and copper uptake by some nickel hyperaccumulators of the genus Alyssum. Plant Soil 138:195–205. https://doi.org/10.1007/BF00012246
Ingle RA, Mugford ST, Rees JD, Campbell MM, Smith JAC (2005) Constitutively high expression of the histidine biosynthetic pathway contributes to nickel tolerance in hyperaccumulator plants. Plant Cell 17:2089–2106. https://doi.org/10.1105/tpc.104.030577
Kachenko AG, Singh B, Bhatia N (2010) The role of low molecular weight ligands in nickel hyperaccumulation in *Hybanthus floribundus* subspecies *floribundus*. Funct Plant Biol 37:1143–1150. https://doi.org/10.1071/FP10080
Kerkeb L, Krämer U (2003) The role of free histidine in xylem loading of nickel in *Alyssum lesbiacum* and *Brassica juncea*. Plant Physiol 131:716–724. https://doi.org/10.1104/pp102.010686
Koch M, Al-Shehbaz IA (2009) Phylogeny of *Brassica* and wild relatives. In: Gupta SK (ed) Biology and breeding of Crucifers. Florida, Boca Raton, pp 1–19
Kowles RV (2010) Regulation of Water in Plant Cells. Bioscene: J College Biol Teach 36:34–42
Kozhevnikova AD, Seregin IV, Erlikh NT, Shevyreva TA, Andreev IM, Verweij R, Schat H (2014a) Histidine-mediated xylem loading of zinc is a species-wide character in *Noccaea caerulescens*. New Phytol 203:508–519. https://doi.org/10.1111/nph.12816
Kozhevnikova AD, Seregin IV, Verweij R, Schat H (2014b) Histidine promotes the loading of nickel and zinc, but not of cadmium, into the xylem in *Noccaea caerulescens*. Plant Signal Behav 9:29580. https://doi.org/10.4161/psb.29580
Kozhevnikova AD, Seregin IV, Zhukovskaya NV, Kartashov AV, Schat H (2021a) Histidine-mediated nickel and zinc translocation in intact plants of the hyperaccumulator *Noccaea caerulescens*. Russ J Plant Physiol 68:37–50. https://doi.org/10.1134/S1021443721070074
Kozhevnikova AD, Seregin IV, Schat H (2021b) Translocation of Ni and Zn in *Odontarrhena corsica* and *Noccaea caerulescens*: the effects of exogenous histidine and Ni/Zn interactions. Plant Soil 468:295–318. https://doi.org/10.1007/s11104-021-05080-y
Krämer U, Cotter-Howells JD, Charnock JM, Baker AJ, Smith JAC (1996) Free histidine as a metal chelator in plants that accumulate nickel. Nature 379:635–638. https://doi.org/10.1038/379635a0
Krämer U, Smith RD, Wenzel WW, Raskin I, Salt DE (1997) The role of metal transport and tolerance in nickel hyperaccumulation by *Thlaspi goesingense Halacsy*. Plant Physiol 115:1641–1650. https://doi.org/10.1104/pp.115.4.1641
Krämer U, Talke IN, Hanikenne M (2007) Transition Metal Transport. FEBS Let 581:2263–2272. https://doi.org/10.1016/j.febslet.2007.04.010
Lasat MM, Pence NS, Garvin DF, Ebbs SD, Kochian LV (2000) Molecular physiology of zinc transport in the Zn hyperaccumulator *Thlaspi caerulescens*. J Exp Bot 51(342):71–79
Li Y, Feng Y, Lv G, Liu B, Qi A (2015) The phylogeny of Alyssum (Brassicaceae) inferred from molecular data. Nord J Bot 33:715–721. https://doi.org/10.1111/njb.00588
Liao SM, Du QS, Meng JZ, Pang ZW, Huang RB (2013) The multiple roles of histidine in protein interactions. Chem Cent J 7:1–2. https://doi.org/10.1186/1752-153X-7-44
McNear DH Jr, Chaney RL, Sparks DL (2010) The hyperaccumulator *Alyssum murale* uses complexation with nitrogen and oxygen donor ligands for Ni transport and storage. Phytochem 71:188–200. https://doi.org/10.1016/j.phytochem.2009.10.023
Merlot S, Garcia de la Torre VS, Hanikenne M (2018) Physiology and molecular biology of trace element hyperaccumulation. In: Van der Ent A, Echevarria G, Baker A, Morel J (eds) Agromining: farming for metals pp 93–116. Mineral Resource Reviews Springer, Berli. https://doi.org/10.1007/978-3-319-61899-9_6
Monsant AC, Kappen P, Wang Y, Pigram PJ, Baker AJ, Tang C (2011) In vivo speciation of zinc in *Noccaea caerulescens* in response to nitrogen form and zinc exposure. Plant Soil 348:167–183. https://doi.org/10.1007/s11104-011-0887-7
Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol 22:867–880. https://doi.org/10.1093/oxfordjournals.pcp.a076232
Nelson DL, Cox MM (2017) Lehninger Principles of Biochemistry, 7th edn. Freeman, New York, W.H

Ouerdane L, Mari S, Czernic P, Lebrun M, Łobiński R (2006) Speciation of non-covalent nickel species in plant tissue extracts by electrospray Q-TOFMS/MS after their isolation by 2D size exclusion-hydrophilic interaction LC (SEC-HILIC) monitored by ICP-MS. J Anal at Spectrom 21:676–683. https://doi.org/10.1039/B602689C

Pantoja O (2021) Recent advances in the physiology of ion channels in plants. Annu Rev Plant Biol 72:463–495. https://doi.org/10.1146/annurev-arplant-081519-035925

Persans MW, Yan X, Patnoe JMM, Krämer U, Salt DE (1999) Molecular dissection of the role of histidine in nickel hyperaccumulation in *Thlaspi goesingense* (Halacsy). Plant Physiol 121:1117–1126. https://doi.org/10.1104/pp.121.4.1117

Plewa MJ, Smith SR, Wagner ED (1991) Diethyldithiocarbamate suppresses the plant activation of aromatic amines into mutagens by inhibiting tobacco cell peroxidase. Mutat Res-Fund Mol M 247:57–64. https://doi.org/10.1016/0027-5107(91)90033-K

Rao KM, Sresty TVS (2000) Antioxidative parameters in the seedlings of pigeonpea (*Cajanus cajan* (L.) Millspaugh) in response to Zn and Ni stresses. Plant Sci 157:113–128. https://doi.org/10.1016/S0168-9452(00)00273-9

Raven JA, Smith FA (1976) Nitrogen assimilation and transport in vascular land plants in relation to intracellular pH regulation. New Phytol 76:415–431. https://doi.org/10.1111/j.1469-8137.1976.tb01477.x

Reeves RD, Baker AJ, Jaffré T, Erskine PD, Echevarria G, van der Ent A (2018) A global database for plants that hyperaccumulate metal and metalloid trace elements. New Phytol 218:407–411. https://doi.org/10.1111/nph.14907

Richau KH, Kozhevnikova AD, Seregin IV, Vooijs R, Koevoets PL, Smith JAC, Ivanov VB, Schat H (2009) Chelation by histidine inhibits the vacuolar sequestration of nickel in roots of the hyperaccumulator *Thlaspi caerulescens*. New Phytol 183:106–116. https://doi.org/10.1111/j.1469-8137.2009.02826.x

Salt DE, Prince RC, Baker AJ, Raskin I, Pickering IJ (1999) Zinc ligands in the metal hyperaccumulator *Thlaspi caerulescens* as determined using X-ray absorption spectroscopy. Environ Sci Technol 33:713–717. https://doi.org/10.1021/es980825x

Scheible WR, González-Fontes A, Morcuende R, Lauerer M, Geiger M, Glaab J, Gojon A, Schulze ED, Stitt M (1997) Tobacco mutants with a decreased number of functional nia genes compensate by modifying the diurnal regulation of transcription, post-translational modification and turnover of nitrate reductase. Planta 203:304–319. https://doi.org/10.1007/s004250050196

Seregin IV, Kozhevnikova AD (2006) Physiological role of nickel and its toxic effects on higher plants. Russ J Plant Physiol 53:257–277. https://doi.org/10.1134/S1021443706020178

Seregin IV, Kozhevnikova AD (2021) Low-molecular-weight ligands in plants: role in metal homeostasis and hyperaccumulation. Photosynth Res 150:51–96. https://doi.org/10.1007/s11120-020-00768-1

Seregin IV, Kozhevnikova AD (2023) Nicotianamine: a key player in metal homeostasis and hyperaccumulation in plants. Int J Mol Sci 24:13. https://doi.org/10.3390/ijms241310822

Seregin IV, Kozhevnikova AD, Schat H (2019) Comparison of L-histidine effects on nickel translocation into the shoots of different species of the genus Alyssum. Russ J Plant Physiol 66:340–344. https://doi.org/10.1134/S1021443719020122

Seregin IV, Kozhevnikova AD, Schat H (2022) Histidine-mediated nickel and zinc translocation in *Arabidopsis thaliana* and *Lepidium ruderale*. Russ J Plant Physiol 69:13. https://doi.org/10.1134/S1021443722010186

Singh K, Pandey SN (2011) Effect of nickel-stresses on uptake, pigments and antioxidative responses of water lettuce. Pistia Stratiotes l J Environ Biol 32:391

Španiel S, Kempa M, Salmerón-Sánchez E, Fuertes-Aguilar J, Mota JF, Al-Shehbaz IA, German DA, Olšavská K, Šingliarová B, Zozomová-Lihová J, Marhold K (2015) AlyBase: database of names, chromosome numbers, and ploidy levels of Alysseae (Brassicaceae), with a new generic concept of the tribe. Plant Syst Evol 301:2463–2491. https://doi.org/10.1007/s00606-015-1257-3

Taiz L, Zeiger E, Moller IM, Murphy A (2015) Plant Physiology and Development. Sinauer Associates, Sunderland, CT

Tappero R, Peltier E, Gräfe M, Heidel K, Ginder-Vogel M, Livi KJT, Rivers ML, Marcus MA, Chaney RL, Sparks DL (2007) Hyperaccumulator *Alyssum murale* relies on a different metal storage mechanism for cobalt than for nickel. New Phytol 175:641–654. https://doi.org/10.1111/j.1469-8137.2007.02134.x

Valenti LE, De Pauli CP, Giacomelli CE (2006) The binding of Ni (II) ions to hexahistidine as a model system of the interaction between nickel and His-tagged proteins. J Inorg Biochem 100:192–200. https://doi.org/10.1016/j.jinorgbio.2005.11.003

Van Der Ent A, Vinya R, Erskine PD, Malaisse F, Przybyłowicz WJ, Barnabas AD, Harris HH, Mesjasz-Przybyłowicz J (2020) Elemental distribution and chemical speciation of copper and cobalt in three metallophytes from the copper–cobalt belt in Northern Zambia. Metallomics 12:682–701. https://doi.org/10.1039/C9MT00263D

Velikova V, Yordanov I, Edreva A (2000) Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. Plant Sci 151:59–66. https://doi.org/10.1016/S0168-9452(99)00197-1

Williams LE, Mills RF (2005) P1B-ATPases–an ancient family of transition metal pumps with diverse functions in plants. Trends Plant Sci 10:491–502. https://doi.org/10.1016/j.tplants.2005.08.008

Zhang YK, Zhu DF, Zhang YP, Chen HZ, Xiang J, Lin XQ (2015) Low pH-induced changes of antioxidant enzyme and ATPase activities in the roots of rice (*Oryza sativa* L.) seedlings. PloS one 10:e0116971. https://doi.org/10.1371/journal.pone.0116971