The Role of L-histidine on Nickel Translocation and Antioxidant enzymes activity in Hyperaccumulator (Odontarrhena inflata) and Non-accumulator (Aurinia saxatilis) plants

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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 toxicity in shoots of *Odontarrhena inflata* and *Aurinia saxatilis*.

Methods

In the preliminary experiments, the Ni content of plants was quantified in pretreated plants using L-histidine and L-alanine (L-Ala) for 4 hours and then exposed to Ni for 8 hours. Hydrogen peroxide (H_2O_2) and activity of some antioxidant enzymes were determined after 4 hours of pretreatment using L-His and L-Ala before 48 hours 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 higher activity of APX and CAT in *O. inflata*. Ni revealed a concentration-dependent increase in H_2O_2 content in *A. saxatilis*. L-His pretreatment increased H_2O_2 concentration in Ni-treated plants. Pretreatment with L-His decreased the activity of POD, APX, and CAT only at 300 µM Ni in *O. inflata* while decreasing the activity of CAT, but increased POD activity at 150 and 300 µM Ni in *A. saxatilis*. Pretreatment with L-Ala decreased POD and APX activity but had no significant impact on H_2O_2 content and CAT activity.

Conclusion

L-Histidine promoted root-to-shoot Ni translocation and alleviated Ni toxicity by inducing of antioxidant enzymes in hyperaccumulator and non-accumulator plants. The role of histidine in Ni hyperaccumulation may not be limited to Ni transport, but linked to detoxification of Ni.

Introduction

The main aim of the present study is to understand the response of plant exposure to heavy metals and characterize the biochemical mechanisms of selective accumulation of metals in hyperaccumulating plants. A minority of plant species (about 0.2% of the total angiosperms) are capable to grow and reproduce on metal-enriched soils (Reeves et al. 2018; van der Ent et al. 2020). Hyperaccumulator plants have higher foliar metal accumulation than those found in non-metallophytes. Approximately 500 species of 720 hyperaccumulator plant species are nickel accumulators which are endemic to serpentine

soils. They are able to absorb and accumulate Ni more than 1000 µg/g dry weight in their leaves (Broadhurst and Chaney 2016; Reeves et al. 2018). About 50 species of the genus *Odontarrhena* (syn. *Alyssum*) from the Brassicaceae family are Ni hyperaccumulators and the best candidate for investigating Ni accumulation responses in plants (Baker and Brooks 1989; Koch and Al-Shehbaz 2009).

It appears that non-accumulating plants keep the heavy metal taken up by roots through their root vacuolar sequestration, thereby, decreasing the availability of heavy metal ions in roots and cell-radial transport toward the stele. In contrast, in hyperaccumulator plants, the heavy metal is significantly loaded into the xylem and efficiently transported to the shoot and accumulates in an innocuous form (Brooks et al. 1977; Corso and de la Torre 2020; Lasat et al. 2000; Richau et al. 2009). Excess Ni concentrations disrupt iron (Fe) homeostasis and subsequently cause Fe-mediated chlorosis (Ghasemi et al. 2009), and inhibit photosynthesis through the disorder of electron transport, chloroplast structure, chlorophyll biosynthesis, and the replacement of cofactors in the active site of metalloenzymes (Hassan et al. 2019; Seregin and Kozhevnikova 2006; Singh and Pandey 2011).The induction of oxidative stress and the 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 due to the high toxicity of Ni accumulation (Corso and de la Torre 2020; Merlot et al. 2018), which involves employing high-affinity ligand molecules for the facilitation of metal chelation, transport, and sequestration (Clemens 2019).

The Ni accumulation may not be achieved by the complex forming with high-molecular-weight molecules including phytochelatins and metallothioneins for instance, Ni is mainly chelated with low-molecular-weight polar ligands such as organic acids and amino acids (Kachenko et al. 2010). Histidine has been frequently reported as the most important chelating agent involved in Ni accumulation (Kerkeb and Krämer 2003; Krämer et al. 1996; Richau et al. 2009). This amino acid serves as a tridentate ligand to form a Ni complex [$Ni(His)_2$] with high stability constant at physiological pH values (Callahan et al. 2006; Krämer et al. 1996; Valenti et al. 2006). Stable Ni–histidine complexes have also been identified in roots, xylem sap, and shoots of *Thlaspi caerulescens* (currently syn. *Noccaea caerulescens*) and in plant sap and vasculature tissue of *Alyssum murale* (McNear Jr et al. 2010; Ouerdane et al. 2006). It seems that a constitutively high free His content in root is a common property among Ni accumulators in Brassicaceae 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).

Despite of the important role of His in Ni binding and its transport, the mitigating role of this chelating compound on Ni toxicity has not been well understood in accumulating and non-accumulating plants. Therefore, in the present study to understand the role of His in Ni translocation, accumulation, and detoxification, we investigated the effect of exogenous L- His on Ni translocation into the shoots, H_2O_2 content, and the activity of antioxidant enzymes in Ni hyperaccumulator (*Odontarrhena inflata*) located in the west of Iran in serpentine soils and non-accumulator (*Aurinia saxatilis*) plants.

Material and method

Seedling growth

Seeds of *Odontarrhena inflata* (formerly syn. *Alyssum inflata* Nyár.) were harvested from the Iranian ultramafic region, Baneh (Ghaderian et al. 2007). *Aurinia saxatilis* seeds were supplied from Horti Tops-Tuinplus, Heerenveen, Netherlands. Seeds were cultured on Perlit and watered using ultrapure water for the first week then germinated seeds were grown by one-quarter-strength Hoagland 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) for two weeks. Afterward, 3-week-old seedlings were transferred into the 400 mL hydroponic culture vessels using the same Hoagland solution. The solutions were continuously aerated and renewed every 7 days with fresh nutrient solution. All cultures were grown in the growth chamber 16-h illumination/8-h dark day/night cycle (150 µmol m^{-2} s^{-1} light intensity) and 24/18 °C (day/night).

Experiment 1 To characterize the effect of exogenously supplied L-histidine, on root-to-shoot Ni translocation, the roots of 8 weeks old plants were transferred in 2-mM Mes/KOH-buffer containing 1 mM L-histidine or L-alanine (pH 5.5) and un-pretreated plants with amino acids were considered as control. After 4 h of pretreatment, the roots of plants were rinsed with ultrapure water properly, then transferred to a fresh nutrient solution supplemented with 300 µM $NiSO_4$. Finally, after 8 h post-Ni treatment, the shoots of plants were harvested, rinsed with ultrapure water, dried with paper towels then frozen in the liquid nitrogen, and stored at −80°C.

Experiment 2 Preliminary experiments showed that 48 hours 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 the MES/KOH buffered medium containing L-His and L-Ala (0,150, 300, 600, and 1000 µM, pH 5.5) for 4 hours. Un-pretreated plants with amino acids were used as control. Then the roots of pretreated plant were rinsed in ultrapure water and transferred to the one-quarter-strength Hoagland solution containing $NiSO_4$ (0,150, and 300 µM) for 48 h. Roots from four hours-treated plants only with amino acids were rinsed with ultrapure water and were transferred to a nutrient solution without Ni for 48 hours. In the end, shoots of plants were harvested, rinsed twice in ultrapure water, and dried carefully between paper towels. Shoot samples were weighted and immediately frozen in liquid nitrogen and stored at -80 ° C.

Activities of catalase, ascorbate peroxidase and peroxidase

Aliquots of 100 mg of frozen leaf tissues were homogenized by cooled mortar and pestle using 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 for 20 min at 15000 ***g*** at 4 °C. Various supernatant aliquots of approximately 100 µl were collected and used to determine the activities of different enzymes. The protein concentration was measured based on Bradford (1976). All enzymatic assays were conducted using a spectrophotometer (BioTek Synergy HTX).

Catalase activity was determined using Aebi (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 was determined 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 extension coefficient of 0.036 $mM^{-1}cm^{-1}$.

For the ascorbate peroxidase (APX) activity, the rate of absorbance reduction at 290 nm as a consequence of ascorbate oxidation in 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 extension coefficient of 2.8 mM^{-1} cm^{-1} for ascorbate. One unit of APX enzyme activity is expressed as the oxidation of 1 µmol /min ascorbic acid at 25°C. The activity of peroxidase (POD) was quantified by measuring the rate of absorbance increase at 470 nm. It was due to the tetraguaiacol formation resulting from guaiacol oxidation catalyzed by POD. 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).

Hydrogen peroxide (H_2O_2)

Hydrogen peroxide content was determined according to Velikova et al. (2000) method. Almost, 100 mg of fresh leaf biomass was homogenized with 1 ml of 0.1% (w/v) trichloroacetic acid (TCA) on ice. The resulting extract 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 subsequent supernatant. The content of H_2O_2 was calculated using the absorbance at 390 nm and the extinction coefficient of 0.28 μM^{-1} cm^{-1}, and reported as µmol H_2O_2 g^{-1} FW.

Quantification of L-histidine amino acid

Approximately, 200 mg of frozen homogenized leaf tissue powder Per sample 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 achieved through vortexing and incubation of 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. The extraction of pellets was repeated as described above, in two more steps, respectively by adding 400 µl of 50% (v/v) ethanol in 2.5 mM HEPES buffer (pH 7.5) and then 200 µl of 80% (v/v) ethanol. All three supernatants were collected and stored at -20 °C for further analysis. The concentrations of L-His in plant extracts were measured through LC-coupled MSE-based quantification using high-performance liquid chromatography (HPLC).

Element 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. Afterward, sample-containing tubes were incubated in a water bath at 90 °C for 4 hours. Followed by cooling, 1 ml of 30% (v/v) H_2O_2 was added per sample, and tubes were again placed at 90 °C for 1 hour. Eventually, the final volumes of solutions were made up 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 mean differences were calculated using Duncan's test $P < 0.05$ (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)

Roots pretreated with L-His significantly increased Ni concentration in the shoots of both *O. inflata* and *A. saxatilis* by 1.4 and 3.9 fold respectively compared to un-pretreated control plants (Fig. 1a).

In either species, the concentration of Ni in the shoot was slightly decreased approximately 10% and 4.5% for *O. inflata* and *A. saxatilis* respectively by exogenous L-Ala pretreatment compared with un-pretreated control plants (Fig. 1b). In both plant species, exogenous L-His pretreatment increased shoot L-His concentration significantly (Fig. 1b). Pretreatment with amino acids had a little effect on the concentration 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 (48h) on antioxidant enzymes activity in shoots

Increasing the concentration of Ni in the nutrient solution increased H_2O_2 content in shoots of *A. saxatilis* significantly however; it remained unchanged in *O. inflata* (Fig. 2a). L-His and L-Ala in the root nutrient medium (no Ni) had no significant effects on the shoot H_2O_2 contents in both species (Fig. 2b and d). In either species, L-His pretreatment (in particular 1000 µM) in plants treated with 150 and 300 µM Ni, the concentration of H_2O_2 in shoots was increased compared to the plants exposed to Ni without amino acid pretreatment (Fig. 2c).

Compared to exclusively Ni-exposed plants, no significant effect of L-Ala pretreatments was observed on shoot H_2O_2 concentrations of both species at 150 and 300 µM Ni (Fig. 2e).

In *O. inflata* and *A.* saxatilis, the presence of Ni in the root medium caused a significant increase in peroxidase (POD) activity (Fig. 3a). Interestingly, in hyperaccumulator plant (*O. inflata*) increasing His concentration (no Ni treatment) caused a remarkable POD activity in the shoot (more than 29-fold at 1000 µM L-His), while, the activity of POD in *A. saxatilis* was increased slightly (Fig. 3b).

Corresponding to Fig. 3c, in non-accumulator *A. saxatilis*, in pretreated plants with 1000 µM L-His compared to plants exposed to 150 and 300 µM Ni (no L-His) POD activity was increased significantly.

Interestingly, in the hyperaccumulator *O. inflata*, L-His pretreatments with 300 µM Ni, conversely and significantly decreased the enzyme activity compared to the plants treated only with Ni (no L-His) (Fig. 3c). Treatment with L-Ala (no Ni) increased the activity of POD in *O. inflata* and *A. saxatilis* (Fig. 4d). Pretreatments with L-Ala prior to 300 µM Ni caused a decrease in POD activity, with a similar trend in both species (Fig. 3e).

The activity of APX in both species was enhanced by increasing of Ni in the medium and in hyperaccumulator plant this increase was significantly higher than in non-accumulator e.g. up to 1.8-fold at 300 µM Ni (Fig. 4a). Different His concentrations (no Ni) significantly increased APX activity in *O. inflata* but not in *A. saxatilis* (Fig. 4b). A similar effect for L-Ala was observed on APX activity in *O. inflata*, but the activity of APX in *A. saxatilis* diminished by L-Ala treatment *(*Fig. 4d). As Figure 4c shows, in *O. inflata*, L-His pretreatments (300,600 and 1000 µM) prior to 300 µM Ni, decreased the enzyme activity compared to the un-pretreated plants. In contrast with L-His pretreatment, both plant species pretreated with L-Ala, drastically decreased APX activity at 300 µM Ni, compared to the un-pretreated plants (Fig. 4e).

Activity of CAT was increased by Ni exposure approximatly 45% and 38% fold at 300 µM Ni, for *O. inflata* and *A. saxatilis* compare to the control plants respectively (Fig. 5a). None of the L-His and L-Ala treatments showed a significant impact on CAT activity in both species when no Ni was in the medium (Fig. 5b, d). The CAT activity in *O. inflata* was decreased by L-His pretreatments significantly (52% at 1000 µM L-His pretreatment prior to 300 µM Ni). In *A. saxatilis* exogenous L-His dramatically decreased the activity of CAT, at both 150 and 300 µM Ni, compared with only Ni-treated plants (Fig. 5c). Unlike POD and APX, the activity of CAT in either species was not affected by the exogenous L-Ala when compared to the plants exposed to Ni only (Fig. 5e).

As expected, increasing Ni concentration in the hydroponic medium resulted in an elevating Ni content in shoots with remarkably higher Ni content in *O. inflata* than in *A. saxatilis* (more than 5-fold at 300 µM Ni, Fig. 6a). At both Ni concentrations (150 and 300 µM Ni), L-His pretreatments significantly increased Ni concentration in shoots of both species, compared with un-pretreated plants. In *O. inflata*, pretreatment with 1000 µM L-His increased shoot Ni concentrations by approximately 40% and 20% at 150 and 300 µM Ni respectively while in *A. saxatilis* for more than 72% and 73% for 150 and 300 µM Ni respectively (Fig. 6b). As previously reported, pretreatment of plants with L-Ala, resulted in a slight decrease of shoot Ni concentrations in both hyperaccumulator and non-accumulator, compared with Ni-only treated plants (Fig. 6c).

The 48 hours of Ni treatments (150 and 300 µM), increased His concentrations in the shoot of both *O. inflata* and *A. saxatilis* significantly compared to the control plants (Fig. 7a). In both species, L-histidine treatments (- Ni, Fig. 7b) and pretreated plants (+ Ni, Fig. 7c) increased shoot L-His concentrations in an ascending manner.

Discussion

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

Pretreatment of plants with exogenous L-His increased Ni accumulation in the shoots of both species, meanwhile, this was not associated with significant changes in Zn and Fe and other micro- and macronutrients content in shoots (data are not shown). As suggested before by Krämer (1996) and Kozhevnikova et al. (2021b) in Ni hyperaccumulators of genus *Alyssum*, histidine seemed to have a role in chelating and transport of Ni ions but probably no other metal elements. Similar reports revealed that exogenous L-His supply in Zn/Ni hyperaccumulator *N. caerulescens* and Ni hyperaccumulator *Odontarrhena corsica* (Kozhevnikova et al. 2014, 2021a,b) and non-accumulators *Alyssum montanum* and *B. juncea* (Kerkeb and Krämer 2003; Krämer et al. 1996) caused a significant increase in Ni translocation or loading into the xylem. This suggests that the Ni hyperaccumulation trait is probably primarily dependent on the constitutive free His concentration in the root. It should be noticed that considerable variation of histidine impact on Ni accumulation has been reported even in Ni hyperaccumulator plants (*Alyssum* genus). For instance, exogenous free His increased Ni loading in xylem sap in *Alyssum pintodasilvae* and *A. obovatum* but not in *Alyssum murale*, *Alyssum fallacinum*, *Alyssum corsicum*, *Alyssum tenium* and *Alyssum bertolini* (Seregin et al. 2019). These variations observations in *Alyssum* as Ni hyperaccumulators might be due to a difference in root endogenous L-His content and possible variation in metal transporter ability among these species (Ingle et al. 2005; Seregin et al. 2019). Moreover, frequently reported a positive correlation between L-His concentration and Ni accumulation capability and Ni-histidine complex formation in Ni hyperaccumulator plants (Ingle et al. 2005;Kerkeb and Krämer 2003; Mc Near Jr et al. 2010; Ouerdane et al. 2006; Richau et al. 2009). It indicates the integral role of histidine in the Ni hyperaccumulation process in plants.

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

Understanding the hyperaccumulation mechanisms is one of the main goals for studying heavy metal accumulator species. An important strategy of heavy metal detoxification is the participation of high-affinity of ligand agents in heavy metal complex formation and cellular compartmentation (Seregin and Kozhevnikova 2021). To investigate the possible mitigating effects of L-His as a low-molecular-weight ligand on Ni toxicity in shoots and limiting its interference with metabolic processes, the effects of L-His and L-Ala (as a control) pretreatments and subsequent Ni exposure were evaluated on H_2O_2 content and the activity of antioxidant enzymes in hyperaccumulator and non-accumulator species. Since the

production of reactive oxygen species (ROS) and oxidative stress are known as the primary consequences of heavy metal toxicity in plant cells (Mithöfer et al. 2004), Ni did not increase H_2O_2 in the shoots of hyperaccumulator *O. inflata* significantly, but strongly in non-accumulator *A. saxatilis.* It can be speculated that *O. inflata* as a hyperaccumulator species apparently contains an enhanced capacity for H_2O_2 detoxification compared to *A. saxatilis*. However, the H_2O_2 accumulation in the shoots of both species following pretreatment of plants with L-His might be due to the Ni translocation and accumulation in the shoots of either species by Ni + L-His complex formation, following an increase in H_2O_2 as a stress indicator reported by Gratão et al. (2005).

Nickel is considered as a redox-inactive metal, and it stimulates the activity of antioxidant enzymes in non- accumulators such as wheat and *Luffa cylindrica* (Gajewska and Skłodowska 2007; Wang et al. 2010) as well as Ni-accumulator including *Alyssum bertolonii* (Boominathan and Doran 2002). POD as an antioxidant enzyme is capable of scavenging harmful concentrations of H_2O_2, whereas APX uses ascorbate as an electron donor to reduce H_2O_2 and CAT consumes H_2O_2 as substrate (Gaspar et al. 1991). An increase of Ni-induced POD, APX, and CAT activities was observed in both plants at 300 µM Ni, but higher APX and POD activities were noticed in *O. inflata* compared to *A. saxatilis* (85% vs. 43% for APX and 45% vs. 38% for CAT respectively). Consistent with our results, when other Ni hyperaccumulator plants such as *Alyssum argenteum* were exposed to Ni, a significant increase of APX and glutathione reductase activities was reported. Similar patterns were observed in CAT and superoxide dismutase activities in *A. murale*, as Ni hyperaccumulator (Babaoğlu et al. 2009; Schickler and Caspi 1999). It seemed that the capability of H_2O_2 destruction by *O. inflata* shoot might have resulted from the higher activities of APX and CAT enzymes as a mechanism for high Ni accumulation (300 µM) in *O. inflata* compared to *A. saxatilis*.

Since *O. inflata* is endemic to serpentine soils and completes its entire life cycle in these Ni-enrich environments, it is expected to be genetically adapted to Ni (Konečná et al. 2020). Pre-adapted Ni hyperaccumulators, such as *O. inflata*, responded more quickly to Ni exposure than non-Ni-adapted species (*A. saxatilis*). Therefore, the higher antioxidant enzymes responses to Ni in *O. inflata* might be explained by its constitutively increased antioxidative defenses to quickly and strongly deal with Ni-derived ROS relative to non-accumulator *A. saxatilis*. This might be a logical strategy to protect the vital photosynthetic apparatus and membrane stability versus toxic ions entering to shoot cytoplasm (Asemaneh et al. 2006). As a support for our data, the exposure of *Nicotiana tabacum* (non-hyperaccumulator) hairy roots to cadmium (178 µM) increased the concentration of H_2O_2 up to five times compared to the control plants, while in the Cd- hyperaccumulator *Thalaspi caerulescens* no significant difference was observed in H_2O_2 content by Cd exposure. Interestingly, since CAT enzyme has a high affinity for substrate binding, similar to the pattern of our data, in *T. caerulescens*, H_2O_2 was maintained at a low quantity due to high CAT activity in this plant compared to *N. tabacum* (Boominathan and Doran 2003). Therefore as Krämer et al. (1997) concluded, a relationship between the ability of Ni toxicity tolerance and Ni-hyperaccumulation exists in *Thlaspi goesingense* compared to *T. arvense* as a non-accumulator plant.

It was noticeable that Ni treatments significantly increased free L-His content in shoots of both *O. inflata* and *A. saxatilis*. This might represent a role of His in the Ni detoxifying in both species. Moreover, despite of some variations, a remarkable reduction of APX and CAT activity was observed in *O. inflata* and *A. saxatilis* followed by increasing of L-His in the medium, compared to the only Ni treated plants, however, the increased POD activity in *A. saxatilis* was remarkable. Regarding the observed Ni-accumulation increase in the shoot of L-His pretreated plants, the reduced antioxidant enzyme activities (POD, APX, and CAT) particularly in *O. inflata* and at higher Ni concentration (300 µM), can be attributed to L-His role in Ni detoxification. This might be mediated through Ni-His complex formation and reducing the activity of free Ni ions in the metabolically active cytoplasm of shoot cells. This facilitates Ni sequestration in inactive compartments such as vacuoles of the epidermis cells probably through IREG/FPN transporters (Bidwell et al. 2004; Kachenko et al. 2008; Merlot et al. 2014; Tappero et al. 2007). We simulated this process in non-accumulator *A. saxatilis* by increasing His quantity in the shoot through L-His pretreatments for CAT enzyme. In agreement with this suggestion, an increased Ni tolerance and shoot-free His content was observed in transgenic *Arabidopsis thaliana* lines with the overexpression of *Alyssum lesbiacum* ATP phosphoribosyl transferase (ATP-PRT) cDNA, which encodes the first and regulatory enzyme of the His biosynthesis pathway (Ingle et al. 2005).

In the non-accumulator plant *A. saxatilis*, the pretreatment with the highest amount of L-His caused a slight increase in POD activity. One reason could be remarkably increased Ni root–to-shoot translocation with L-His and consequently Ni-induced activity of enzymes. However, we should remember that increased activity of POD and APX enzymes was also observed when L-His and L-Ala without Ni exposure were used in the medium. The question is "why amino acid treatment alone increased the activity of enzymes? The answer is not known but it might be due to the possible toxicity of high amounts of amino acids transferred to the shoots, which probably disrupted the homeostasis of metal ions or the osmotic potential in shoot cells and eventually induced the enzyme activities. The responses of *O. inflata* were more noticeable compared to *A. saxatilis*. As previously reported that high concentrations of certain amino acids such as proline, glutamate, and glycine have negative effects on plant growth and development (Biancucci et al 2015, Kan et al 2017). The decrease of POD and APX activities in both species caused by L-Ala pretreatments before exposure to Ni, should not be interpreted as the mitigating effects of L-Ala on Ni toxicity in shoots, because some evidence indicates that the ability of Ni-binding to His cannot be considered for Ala in Ni hyperaccumulators: since the Ni-Ala complex stability constant is 5-fold less than those with histidine (Dawson et al. 2002). Krämer et al. (1996) reported in *A. lesbiacum* as a model Ni hyperaccumulator plant, Ni exposure did not change the concentration of any other amino acids except His in the xylem sap (increased 36-fold). Additionally, analogs to our results, the absence of an increase of Ni in xylem sap followed by L-Ala pretreatments has also been reported for Ni hyperaccumulators of *Noccaea* genus (*N. caerulescens*) (Richau et al. 2009) and several *Alyssum* species, *A. murale*, *A. fallacinum*, *A. lesbiacum*, *A. bertolonii*, *A. pintodasilvae*, and *A. obovatum* (Seregin et al. 2019).

Conclusions

In the current study, the exogenous L-histidine promoted Ni translocation into the shoot of both accumulator *O. inflata* and non-accumulator *A. saxatilis* plants. The shoots of *O. inflata* seemed to be equipped with a robust capability for controlling Ni-inducted H_2O_2 production through employing pre-adapted antioxidant enzyme-boosted activities. L-His pretreatments increased H_2O_2 content but decreased the activity of antioxidant enzymes due to the facilitating of translocation and accumulation of Ni within the plants. The increased histidine concentrations in the shoots as a chelating agent reduced the Ni toxicity in plants and consequently, decreased the required activities of antioxidant enzymes. However, a high amount of L-His within the shoots in a particular concentration might be either toxic/detoxify compounds or disrupts the homeostasis of other nutrition elements in the plant cells. Despite of crucial roles of L-His in translocation, accumulation, and heavy metal tolerance in plants, understanding the mechanisms of L-His function in plant cells needs to be investigated in the future.

Declarations

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Competing Interests

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

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by [Soraya Soleymanifar], [Ali Akbar Ehsanpour], and [Henk Schat]. The first draft of the manuscript was written by [Soraya Soleymanifar] and all authors including [Rasoul Ghasemi] reviewed and corrected the manuscript.

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Figures

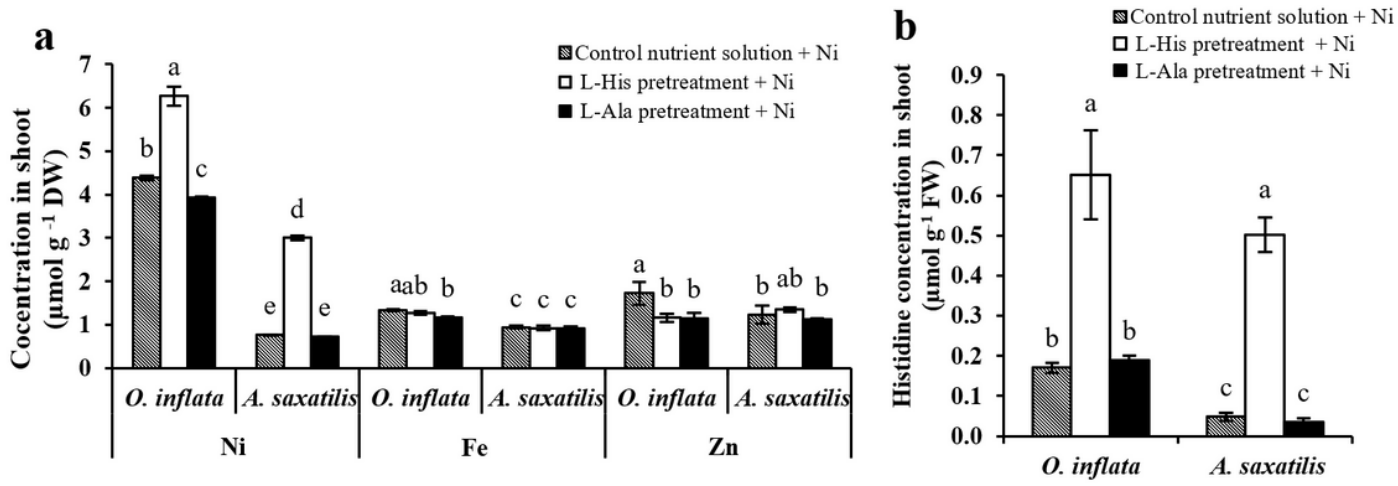

Figure 1

The effects of exogenously supplied L-His and L-Ala on the Ni, Fe, and Zn (a) and His (b) content in the shoots of *O.inflata*and *A. saxatilis* under short-term Ni exposure (8h). The root of 8-week-old plants was pretreated with 1 mM L-His (open bar), 1 mM L-Ala (closed bar), or control nutrient solution (dashed bar) 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 test

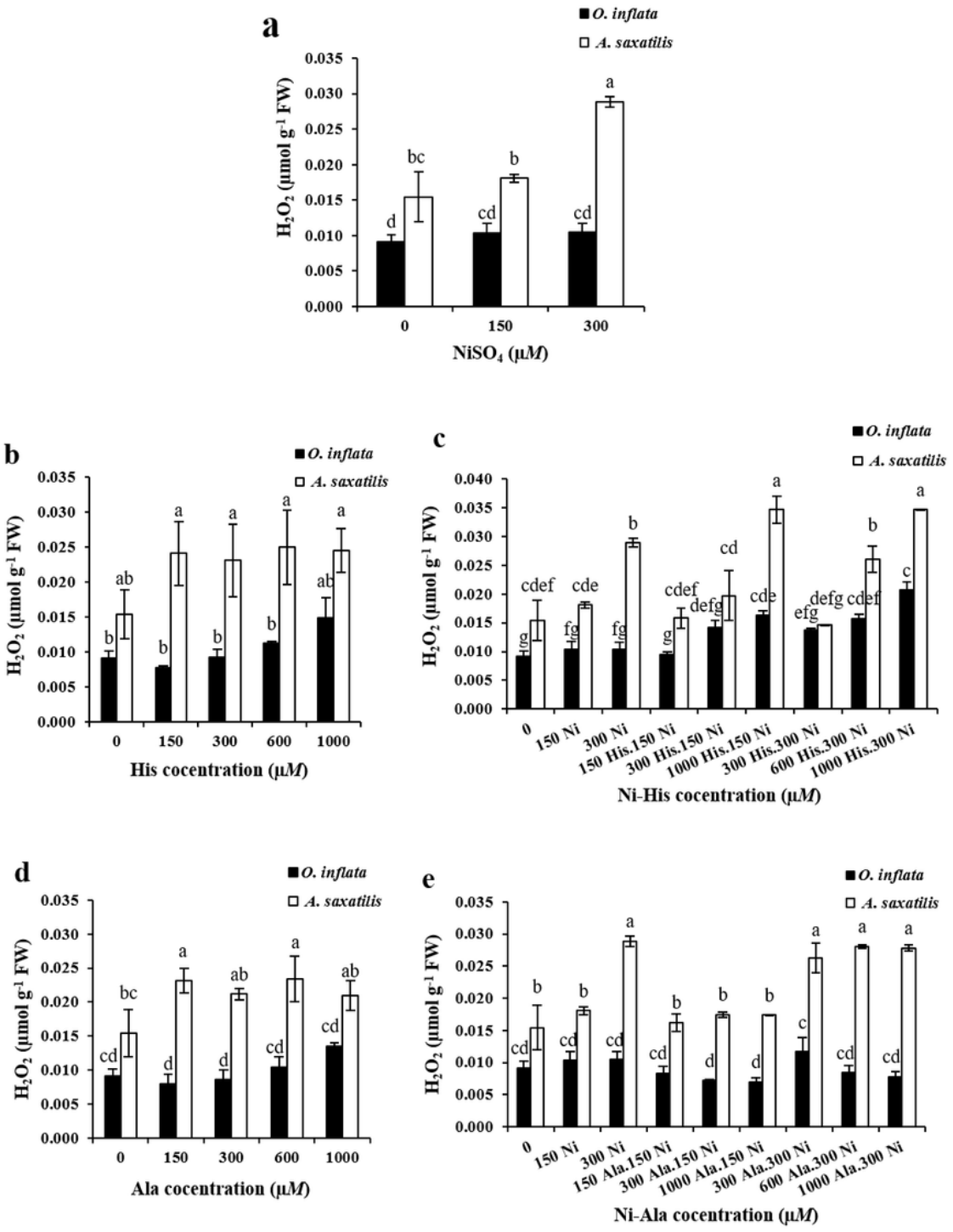

Figure 2

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 bar) and *A. saxatilis* (open bar). The root of 8-week-old plants was 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

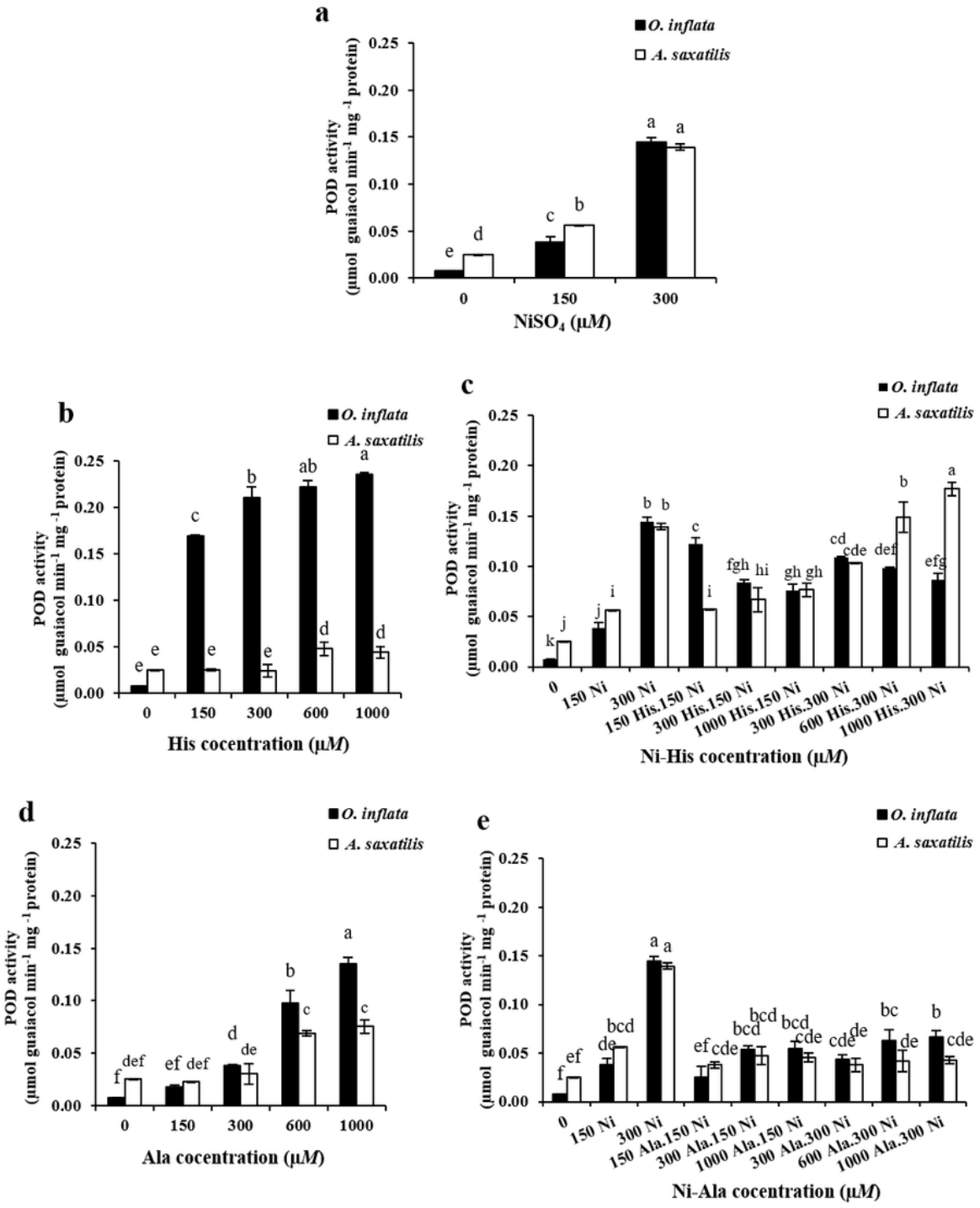

Figure 3

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 bar) and *A. saxatilis* (open bar). 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 test

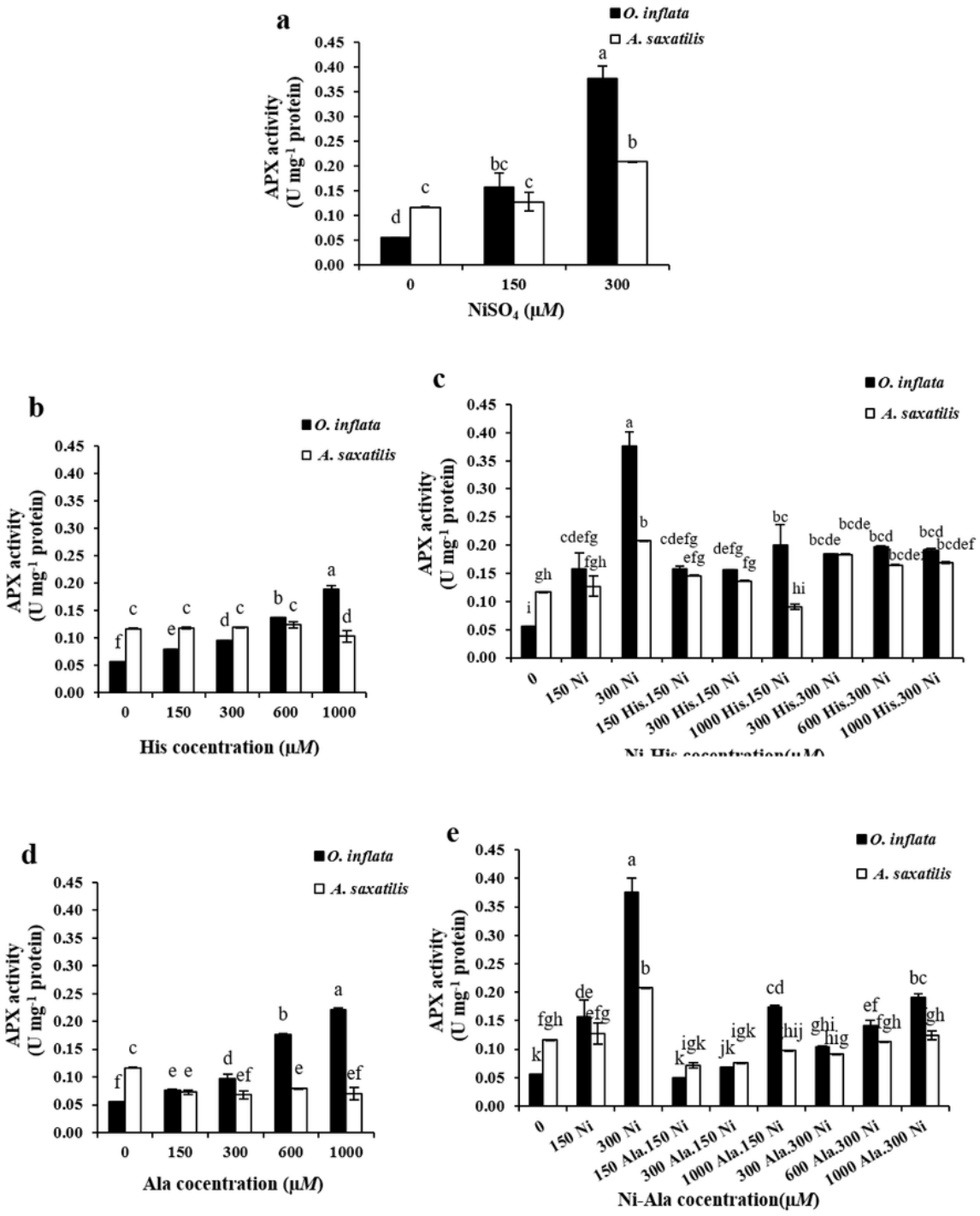

Figure 4

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 bar) and *A. saxatilis* (open bar). 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 test, 1 U (μmol/min)

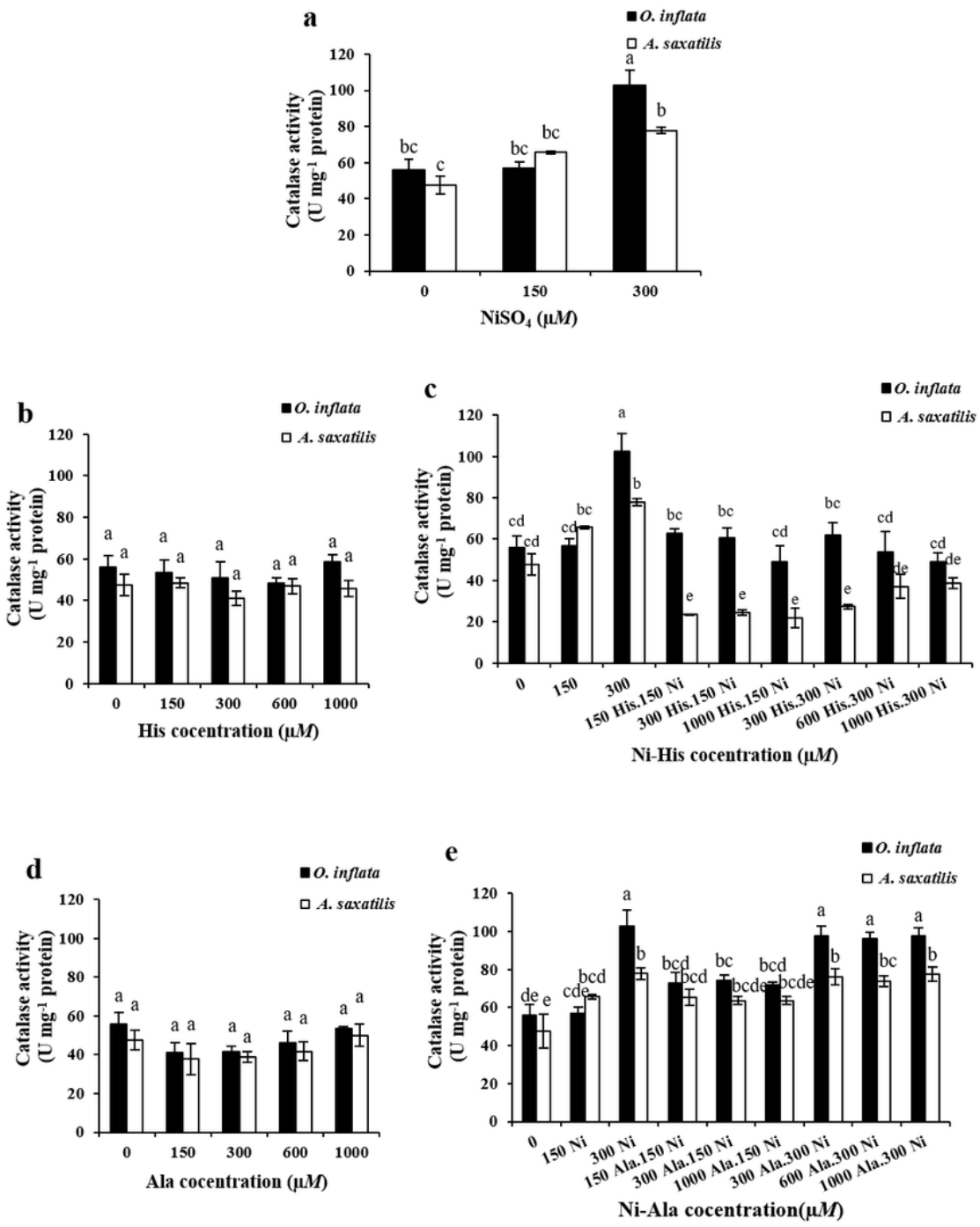

Figure 5

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 bar) and *A. saxatilis* (open bar).

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 test, 1 U (µmol/min)

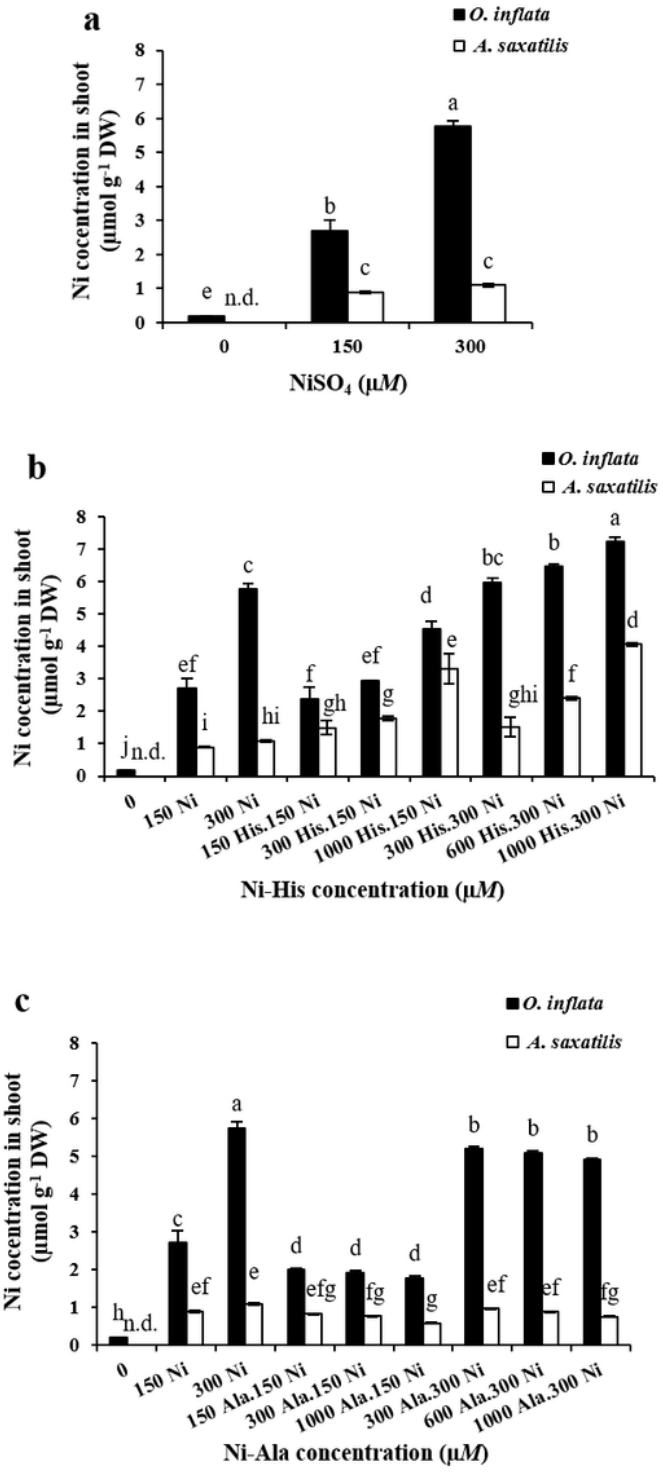

Figure 6

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 bar). 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 test, n. d., not detectable

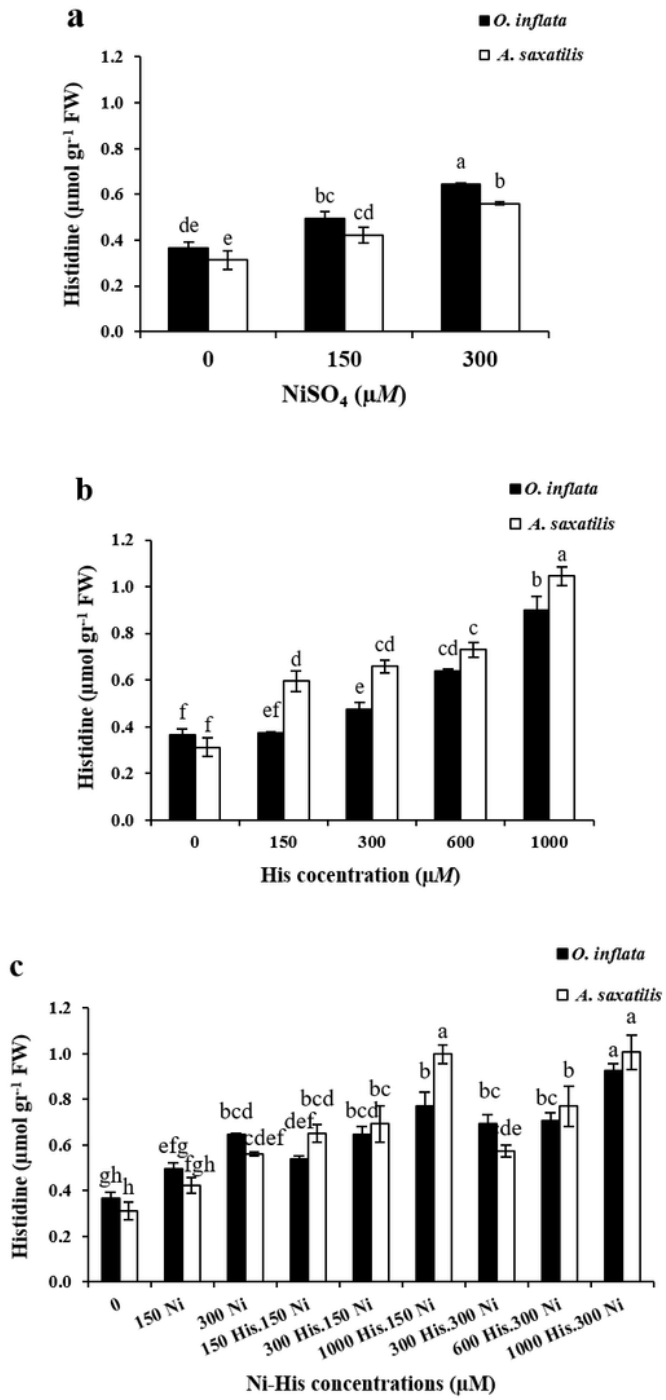

Figure 7

The effect of different concentrations of Ni (a), L-His (b), and L-His pretreatment and Ni (c) on shoot His concentration in *O. inflata* (closed bar) and *A. saxatilis* (open bar). His concentration 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 test