

ORIGINAL RESEARCH ARTICLE

A greenhouse pot experiment assessing the zinc-accumulating behavior of *Lupinus uncinatus* Schldl

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Abstract: A greenhouse pot experiment was conducted to evaluate the zinc (Zn) accumulation potential of *Lupinus uncinatus* Schldl. The effects of varying Zn concentrations on plant dry matter yield, metal tolerance, and Zn accumulation and distribution in roots, stems, and leaves were investigated. Zn was applied as ZnCl₂ at rates of, 200, 400, and 600 mg/kg. One-way analysis of variance followed by Tukey's multiple comparison test ($p < 0.05$) revealed significant effects of Zn on root dry weight, Zn uptake in roots, stems, and leaves, and the shoot-to-root Zn ratio. Root dry weight was significantly reduced, with the highest Zn treatment (600 mg/kg) causing a 57% reduction compared to control plants. However, no significant differences were observed in overall plant dry matter yield. Metal tolerance declined with increasing Zn stress. Zn accumulation in leaves reached 9,632 mg/kg and 14,771 mg/kg at soil Zn application rates of 400 mg/kg and 600 mg/kg, respectively. The shoot-to-root Zn ratio exceeded one, and more than 64% of the total Zn absorbed by *L. uncinatus* was translocated to the shoots at 600 mg/kg. These results position *L. uncinatus* as a promising species for Zn phytoremediation, encouraging future studies under field conditions and with other toxic metals.

Keywords: Metal uptake; Zinc toxicity; Phytoremediation; Tolerance index; Lupin plants; Soil contamination

1. Introduction

Modern advancements in agriculture, industry, and urban development have led to significant growth, but they have also resulted in serious environmental issues, particularly the accumulation of heavy metals and toxic substances in soil.¹⁻⁴ Large regions worldwide are impacted by anthropogenic deposition of heavy metals.⁵ In recent years, concerns have grown regarding the health and ecological impacts of heavy metals in soil and their subsequent absorption by plants, prompting the scientific community to explore effective mitigation strategies.⁶ Human activities such as mining, smelting, waste disposal, manufacturing, battery disposal, and agricultural practices, including the use of inorganic fertilizers and pesticides, are recognized as major sources of environmental pollution.⁷ Soil contamination directly affects the health of living organisms because toxic substances can readily enter the food chain.⁸ As a result, the widespread distribution of toxic elements in soil, water, and air poses serious and unpredictable risks to human health.⁹

At low concentrations, however, heavy metals are crucial for biological functions. They serve as cofactors for many enzymes, including proteinases, dehydrogenases, and peptidases. Zinc (Zn), for example, is essential for the synthesis of carbohydrates, proteins, phosphates, auxins, RNA, and ribosomes,¹⁰ and it plays a vital role in the metabolic processes of both plants and animals. Nevertheless, Zn can accumulate to toxic levels in the environment, adversely affecting plants as well as soil-dwelling organisms.¹¹ Although Zn is an integral part of Earth's biogeochemical cycles and is required by plants in trace amounts, human activities such as excessive agrochemical use, industrial and municipal waste disposal, and mining have led to elevated Zn concentrations in soils, which may become detrimental to plants^{12,13} and frequently reach toxic levels for soil microbes.¹⁴ Phytoextraction, the process of removing heavy metals from soil using plants, is increasingly regarded as an eco-friendly and cost-effective approach to the remediation of soils contaminated with potentially toxic substances.¹⁵⁻¹⁸ More broadly, phytoremediation, the use of plants to restore polluted soils, is considered an innovative and promising strategy for soil decontamination, offering several advantages over traditional methods, such as being *in situ*, cost-effective, and environmentally friendly.^{19,20} Recent studies have demonstrated that certain plant species, due to their unique traits, possess the capability to absorb and metabolize xenobiotics contaminating the environment.²¹

Plants selected for the uptake of toxic elements from soil should be contamination-specific, meaning they must be capable of tolerating the specific pollutants present, thrive under local soil and climatic conditions, accumulate metals in harvestable tissues, and possess root systems that spatially align with the geographical distribution of contaminants. *Lupinus* species exhibit ecophysiological traits that enable them to solubilize and absorb toxic elements from soil due to their extensive and deep root systems. These plants also demonstrate adaptability to environmental stress factors, such as excess nitrates, low root temperatures, detopping,^{22,23} lime excess,²⁴ and salinity,²⁵ that would otherwise inhibit normal plant growth.²⁶

Reay and Waugh²⁷ have reported the uptake of manganese (Mn) and aluminum (Al) by *Lupinus* shoots and roots, while other studies have shown the absorption of cadmium (Cd) and mercury (Hg).^{28,29} *Lupinus albus* L. has shown the ability to detoxify water by absorbing Cd, plumbum (Pb), and chromium (Cr),³⁰ with Cd retention attributed to thiol groups in cell walls.³¹ These findings highlight the considerable potential of this genus for remediating contaminated soils.³² Furthermore, *Lupinus luteus* L. (yellow lupine) has been demonstrated to grow without toxicity when exposed to benzo(a)pyrene. Endophytes associated with this species not only enhanced plant growth and tolerance to benzo(a)pyrene but also improved resistance to other organic pollutants such as diesel and polychlorinated biphenyls. These endophytes were also capable of metabolizing some of the organic pollutants.³³

Unlike hyperaccumulator plants such as *Thalspi caerulescense*, *Lupinus* species act as metal excluders, restricting the movement of metals from roots to shoots across a broad range of soil metal concentrations. This exclusion mechanism may involve alterations in membrane permeability, changes in the metal-binding capacity of cell walls, or the exudation of chelating substances from roots, as has been proposed for *L. albus*.³⁴

In a recent study by Saladin *et al.*,³⁵ *L. albus* exposed for 3 weeks in a greenhouse to soils from former French mines (Pontgibaud and Vaulry) contaminated with metal(loid)s, including high concentrations of arsenic (As) and Pb (772–1,064 mg/kg and 121–12,340 mg/kg, respectively), showed less growth inhibition than two other species (common vetch and buckwheat). This resilience was attributed to lower metal(loid) concentrations in the roots and aerial tissues of lupins, suggesting that *L. albus* may be the most suitable species for phytostabilization among the crops

tested. However, much of the existing research on this genera has been conducted in non-soil media, such as hydroponic systems, with limited information available on its performance in contaminated soils. Therefore, it was important to evaluate the response of *Lupinus uncinatus*—an understudied species within the same genus as *L. albus*—to varying soil Zn treatments.

2. Materials and methods

A greenhouse pot experiment was conducted to evaluate the phytoremediation potential of *L. uncinatus*. Soil was collected from the top 25 cm layer of an agricultural field at San Pablo Ixayoc, State of Mexico, Mexico, and analyzed for Zn concentration along with other physical and chemical properties (Table 1). Seeds of *L. uncinatus* were also collected from the same site.

Plastic pots (15 cm diameter) were filled with 5 kg of soil. Seeds were sown directly in the pots and germinated within 5–7 days. One week after germination, seedlings were thinned to three plants per pot. After 4 weeks of growth, plants were treated with ZnCl₂ at concentrations of 200, 400, and 600 mg/kg, applied in 15 mL of deionized water.

The pots were arranged in a randomized design under normal greenhouse conditions. Average daytime and nighttime temperatures ranged between 25–29°C and 8–11°C, respectively. Pots were re-randomized 3 times during the experiment. Four replicates were established per treatment (200, 400, and 600 mg/kg) and for the control (no Zn added only having background soil Zn concentration of 65 mg/kg). Pots were irrigated with distilled water every 2–3 days to maintain 70% of field capacity. Plant growth was assessed by measuring dry weight accumulation under each Zn treatment, while Zn content and distribution in roots, stems, and leaves were determined at harvest, which marked the end of the experiment.

Table 1. Selected physical and chemical characteristics of pot soil

Parameter	Value
pH	6.29
Electrical conductivity	0.16 dS/m
Organic matter	1.19%
Soil texture	Sand (34%); silt (32%); clay (34%)
Bulk density	1.13 Mg/m ³
Total zinc content	65 mg/kg

Two weeks after Zn application, plants were harvested and separated into roots, stems, and leaves. Plant tissues were thoroughly washed with tap water, followed by repeated rinsing with deionized water. Samples were then oven-dried at 70°C for 72 h to record dry weight, ground to a fine powder, and subsampled (0.1 g) for digestion with nitric perchloric acid. Zn concentrations (mg/kg) in plant organs were quantified using an atomic absorption spectrophotometer (Model 3110, Perkin-Elmer, USA).

Data were analyzed using one-way analysis of variance, and Tukey's test was applied for multiple comparisons of means at a significance level of $p < 0.05$. Homogeneity of variance was verified using Levene's test ($p > 0.05$). All statistical analyses were performed using SAS software (version 9.1; SAS Institute, United States).³⁶

Metal tolerance was calculated using Equation I:

$$X = \frac{A}{B} \times 100 \quad (I)$$

Where A = mean dry weight of plants grown under Zn treatments, and B = mean dry weight of control plants (no Zn treatment).

Accordingly, a tolerance index of 50% indicates that plants produced 50% of the optimum biomass relative to the control, which is considered the minimum acceptable biomass under toxic soil conditions.³⁷

3. Results and discussion

3.1. Plant growth and dry matter yield

The dry matter yield of *L. uncinatus* plants after 2 weeks of exposure to varying Zn supply levels is presented in Table 2. The roots, being in direct contact with the heavy metal and serving as primary sites of toxic action, showed a significant decrease in dry matter

Table 2. Dry matter yield of *Lupinus uncinatus* after 2 weeks of exposure to different zinc supply levels

Zinc supply levels (mg/kg)	Dry matter yield (g/pot)		
	Shoot	Root	Plant
Control	1.90±0.18 ^b	0.56±0.04 ^a	2.46±0.17 ^b
200	3.29±0.27 ^a	0.31±0.02 ^b	3.60±0.26 ^a
400	2.99±0.21 ^a	0.30±0.03 ^{bc}	3.29±0.21 ^a
600	3.36±0.31 ^a	0.32±0.02 ^c	3.68±0.30 ^c

Note: Values in the same column followed by different letters differ significantly ($p < 0.05$) according to Tukey's test ($n=4$).

with increasing Zn supply levels, as expected. At the 600 mg/kg treatment, root dry matter was reduced by 57% compared to control plants.

The negative effects of toxic elements on root growth are well documented. For example, Luo *et al.*³⁸ reported that elevated Zn levels inhibited the growth of *Jatropha* seedlings by disrupting normal cellular metabolism and inducing visible injuries and physiological disorders. Moreover, root growth was identified as the first visible site of damage under excessive Zn, due to reduced cell division.³⁹ Additionally, cell walls have been reported to rupture under physical stress when their elasticity is significantly reduced by Pb and Cd exposure.⁴⁰ Significant decreases in plant biomass production have also been reported in *Lupinus ballianus*⁴¹ and *Lupinus termis* when grown either in 100% mine waste substrates containing 3,780 mg/kg Zn or in soil-filled pots supplemented with 100 mg/L Zn solutions, respectively.⁴²

Shoot dry matter yield per pot ranged from 1.90 g to 3.36 g. A significant increase in shoot dry matter was observed compared to the control, and total plant biomass followed the same trend. Similar increases in plant biomass in response to heavy metal treatments have been reported by other researchers for Mn and copper,⁴³ as well as Cd.^{44,45}

3.2. Metal tolerance

In this study, we employed the index of tolerance (IT) to evaluate the ability of *L. uncinatus* to withstand Zn treatments after 2 weeks of growth in contaminated soil. Figure 1 illustrates the tolerance of *Lupinus* plants under Zn exposure. An IT of 50% represents 50% of optimum plant growth and is considered the minimum acceptable biomass for plants grown in the presence of toxic soil elements.³⁷

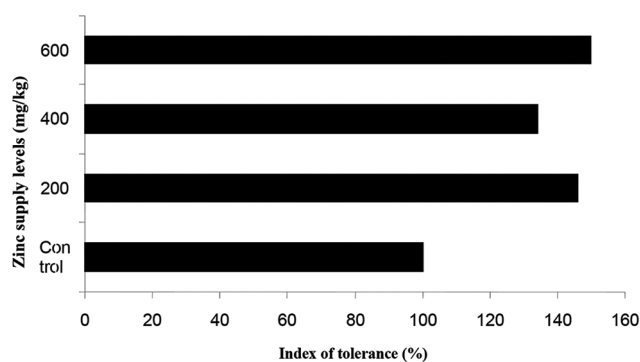


Figure 1. Index of tolerance exhibited by *Lupinus uncinatus* exposed to different zinc supply levels for 2 weeks ($n = 4$)

Zinc treatments appeared to have a stimulatory effect on plant dry matter yield, as IT values for Zn supply levels of 200, 400, and 600 mg/kg were 146%, 134% and 150% respectively. Two fundamental mechanisms have been reported for plant tolerance to toxic elements: Metal exclusion and metal detoxification.⁴⁶ In the exclusion pathway, plants restrict metal absorption into the roots, thereby preventing translocation and accumulation in aerial tissues.⁴⁷ By contrast, hyperaccumulator species actively take up toxic elements and tolerate them through internal detoxification mechanisms. Several hyperaccumulator species have been identified for Zn. To cope with excess Zn in the cytoplasm, plants may employ one or more of the following pathways: (i) Reduced accumulation across the plasma membrane, (ii) sequestration in subcellular organelles, (iii) precipitation as insoluble salts, (iv) binding of Zn to low-molecular-weight organic compounds, and (v) removal through the plasma membrane into the apoplast.⁴⁸

The IT values obtained in this study were compared with those reported in other investigations (Table 3). The results indicate that *Lupinus* species (both *L. uncinatus* in the present study and *L. albus*³²) exhibited a high capacity for Zn accumulation and efficient translocation to aerial tissues, particularly the stem, and consequently demonstrated higher tolerance indices compared to other hyperaccumulator plants. However, these comparisons should be interpreted cautiously, as the experimental conditions differed among studies in terms of exposure duration, soil pH, texture, and growth medium.

Further research is required to directly compare the effectiveness of different plant genera in phytoextraction of heavy metals. To date, only a few species have been reported in the literature as potential soil Zn hyperaccumulators, notably those belonging to the genera *Thlaspi* and *Brassica* spp.; however, confirmation under standard growth conditions remains necessary.

The high metal IT values observed in this study indicate that *L. uncinatus* has considerable potential for application in Zn phytoremediation. However, biochemical mechanisms conferring metal tolerance and uptake are both species-specific and metal-specific. A deeper understanding of the physiological and biochemical characteristics of *Lupinus* species that enable them to tolerate excessive concentrations of metals is essential to fully harness their phytoremediation potential.

3.3. Plant accumulation and transport of zinc

An unresolved issue in phytoremediation, apart from tolerance, is the uptake and internal distribution

Table 3. Comparison of different phytoremediation strategies

Plant	Zinc Concentration in medium (mg/kg)	Zinc concentration in plant (mg/kg)	Medium, time of exposure	Tolerance index (%)	References
<i>Lupinus uncinatus</i>	600	10,000 (root); 18,000 (shoot)	Soil in pot, 14 days	149	The present study
<i>Lupinus albus</i>	500	5,200 (root); 1,638 (shoot)	Soil in pots, 84 days	38	32
<i>Brassica juncea</i>	6.5 mg/L	10,000 (root); 1,500 (shoot)	Hydroponics, 12 days	122	49
<i>Thlaspi caerulescens</i>	210	1,200 (root); 8,700 (shoot)	Soil in pots (industrial contamination), 100 days	–	50
<i>Hordeum vulgare</i> (barley, non-tolerant)	100	1 (root); 4.5 (plant)	Soil in pots, 48 days	41	51

of metals within plants, particularly their transport from roots to aerial tissues. Despite the relative short exposure period of 2 weeks, considerably elevated Zn concentrations were detected in the roots, stems, and leaves of *L. uncinatus*.

As shown in Figure 2, Zn concentrations in all plant parts increased proportionally with rising Zn supply levels, indicating the substantial accumulation potential of this species under the experimental conditions. These findings are consistent with previous reports for *L. albus* by Ximénez-Embún *et al.*³⁷ and Pastor *et al.*³² In *L. uncinatus*, Zn concentrations in stems ranged from 3,720 mg Zn/kg to 14,771 mg Zn/kg, while leaves contained 3,090–4,062 mg Zn/kg. Roots accumulated between 5,818 and 10,568 mg Zn/kg. For comparison, *Noccaea caerulescens* (formerly known as *Thlaspi caerulescens*) can accumulate up to 40,000 mg Zn/kg in shoot dry biomass without toxic symptoms,^{52,53} whereas the normal Zn concentration in most plants is 30–100 mg/kg dry mass.⁵⁴

These results demonstrate that *L. uncinatus* is capable of substantial Zn uptake under slightly acidic conditions (as tested in the present study). Given that neutral soils are among the preferred environments of this species,⁵⁵ it is reasonable to infer that similar uptake potential may also occur under neutral conditions.

They also align with observations by Pastor *et al.*,³² who reported Zn uptake of 3,605 mg Zn/kg in *L. albus* grown in acidic soil contaminated with 300 mg Zn/kg. Furthermore, lupins have been reported to accumulate other heavy metals. For instance, *L. albus* accumulated 4,900, 2,300, 400, and 200 mg/kg of Cd, Hg, Pb, and Cr, respectively, when grown in contaminated sand.³⁷ In related experiments using soil and nutrient solution, Zn and Cd accumulation in different plant parts of *L. uncinatus* was also observed.^{56,57} Similarly, Vázquez *et al.*⁵⁸ reported that the growth of *L. albus* grown in perlite containing

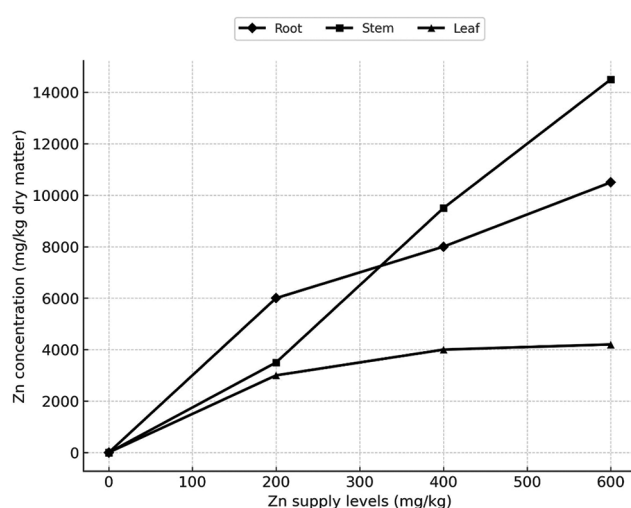


Figure 2. Zinc (Zn) uptake by roots and its accumulation and transport to aerial tissues (stems and leaves) of *Lupinus uncinatus* exposed to different Zn supply levels for 2 weeks

Notes: Vertical bars represent \pm SE ($n=4$). Zn 000 corresponds to the control (65 mg/kg soil Zn without additional Zn application); Zn 200, Zn 400, and Zn 600 represent Zn supply levels of 200, 400, and 600 mg/kg, respectively.

10–100 μ M As showed enhanced tolerance to arsenate, supporting its potential application in phytoremediation or revegetation of As-contaminated sites.

Additional studies further confirm the capacity of lupins to thrive under metal stress. For example, *L. albus* grown in vermiculite with elevated Zn concentrations at pH 6.7 for 3 weeks achieved Zn concentrations of 1,400 mg/kg of Zn in shoots and 4,100 mg/kg in roots.³² Among Andean high-elevation plants, *L. ballianus* exhibited the highest Cd accumulation in roots, with 287.3 mg/kg dry matter when grown in a substrate consisting entirely of mine waste.⁴¹

Martínez-Alcalá *et al.*⁵⁹ reported that Zn concentrations were slightly higher in shoots ($46 \pm 10 \mu\text{g/g}$) than in roots ($34 \pm 1 \mu\text{g/g}$ of *L. albus* cultivated for 74 days in calcareous pot soil containing high levels of Zn and Pb (2,058 and 2,947 $\mu\text{g/g}$, respectively). Similar shoot concentrations (40–70 $\mu\text{g/g}$) were reported in *L. albus* without apparent detrimental effects.⁶⁰ In contrast, other studies have demonstrated that Zn absorbed by this species can accumulate predominantly in roots, particularly when soil amendments are applied to stabilize metals in the soil.^{31,61} Taken together with the findings of the present study, *Lupinus* species hold considerable potential for phytoremediation of metal-contaminated soils.

A notable feature of Zn accumulation in *L. uncinatus* is the relatively high concentrations detected in stems and leaves compared with roots, as reflected by shoot-to-root ratios approaching 1.78 at the 600 mg/kg treatment (Table 4). Ratios >1 indicate efficient translocation of Zn from roots to shoots, with Zn mobility increasing with high Zn additions to the soil.

Lupin plants have also demonstrated strong root-to-shoot transport capacity in the case of Hg hyperaccumulation,³⁷ suggesting the presence of an underlying cellular mechanism for heavy metal transport. In addition to efficient uptake, it is possible that lupin roots may also enhance the bioavailability of heavy metals in the rhizosphere, thereby enlarging the pool of Zn available for uptake.⁵⁰ In general, Zn-efficient genotypes (e.g., oilseed rape) tend to contain lower Zn concentrations in roots and higher concentrations in young leaves, indicating a superior capacity for Zn translocation and utilization.⁶²

The mechanisms governing Zinc uptake and translocation in lupins, as well as the effect of soil factors on these processes, remain poorly characterized due to the limited literature available. Nonetheless, the observed accumulation and distribution patterns highlight the potential value of *Lupinus* species as phytoremediators in slight acidic or neutral soils.

Table 4. Shoot-to-root Zn concentration ratios in *Lupinus uncinatus* grown in Zn-contaminated soil ($n=4$)

Zn supply levels (mg/kg)	Shoot: Root Zn ratio
Control	1.06±0.10
200	1.17±0.14
400	1.63±0.22
600	1.78±0.19

Abbreviation: Zn: Zinc.

3.4. Metal distribution within *L. uncinatus* at various zinc concentrations

The distribution of Zn in leaves, stems, and roots varied across treatments. Zn concentration in the shoots (stems and leaves) increased, while root concentrations decreased, with increasing Zn supply levels from 200 to 600 mg Zn/kg (Figure 3).

At 200 mg Zn/kg, 54% of total Zn uptake was allocated to the shoots (stems + leaves) and 46% to the roots. By contrast, at 600 mg Zn/kg, shoot allocation increased to 64% while root allocation decreased to 36%. These results indicate that:

- At higher Zn concentrations, shoots tend to accumulate a greater proportion of the absorbed metal, as previously reported;³⁶
- The shift in Zn partitioning with increasing contamination may represent an adaptive response mechanism to mitigate the imposed metal stress.

The presented data indicate that *L. uncinatus* possesses a considerable capacity for Zn accumulation, reflected in high metal IT values, shoot-to-root ratios above 1, and increased dry biomass under Zn stress. Although root growth showed a significant reduction in dry weight after 2 weeks of exposure, overall plant biomass exhibited a stimulatory effect relative to the control.

The mobility of Zn within plants is generally considered intermediate, falling between highly mobile elements such as potassium and phosphorus and relatively immobile elements such as calcium.⁵⁴ Nevertheless, Zn has been found to be readily transported in the phloem of some species (e.g., wheat).^{63–65} Furthermore, Zn

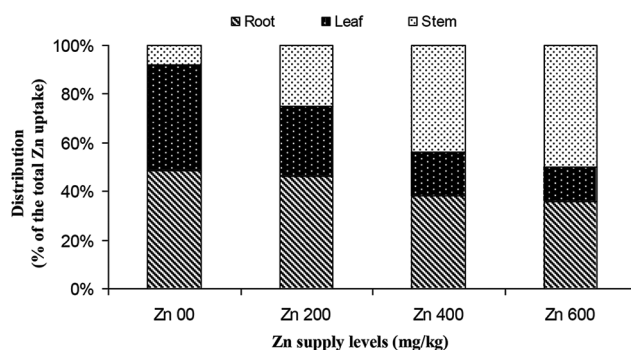


Figure 3. Zinc (Zn) distribution in different plant parts of *Lupinus uncinatus* grown at different Zn supply levels for 2 weeks ($n = 4$)

Notes: Zn 00 corresponds to the control (65 mg/kg soil Zn without additional Zn application); Zn 200, Zn 400, and Zn 600 represent Zn supply levels at 200, 400, and 600 mg/kg, respectively.

redistribution has been reported to depend on plant age and the Zn concentration in source organs.⁶⁶

4. Conclusion

The response of *L. uncinatus* to elevated soil Zn concentrations—evaluated in terms of plant survival, toxicity symptoms, and vegetative growth (increased dry matter)—demonstrated the ability of this species to withstand adverse conditions. The high Zn concentrations detected in different plant organs, coupled with efficient translocation to shoots and elevated IT values, confirm the considerable potential of *Lupinus* species for the phytoremediation of Zn-contaminated soils.

Future research should be conducted under open-field conditions to verify the accumulation potential of this species, not only for Zn but also for other toxic metals. The present experiment was designed to provide preliminary insights into the response of *Lupinus* plants to high Zn concentrations and their pattern of metal accumulation, forming a foundation for broader applications in phytoremediation.

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Conflict of interest

Muhammad Ehsan is an Editorial Board Member of this journal but was not involved in any way in the editorial and peer-review process conducted for this paper, either directly or indirectly. Separately, the other authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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Availability of data

Data are available from the corresponding author upon reasonable request.

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