

## Roots of *Lupinus angustifolius* L. and enzyme activities in soil contaminated by toxic elements

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**Abstract:** The impact of toxic elements (TEs) contaminating the root zone of *Lupinus angustifolius* L. on enzymatic activities, nitrification rate, and changes in the root system was evaluated. Lupine was cultivated in a pot experiment using two types of soil – control and contamination (with a high degree of arsenic (As), cadmium (Cd), lead (Pb), and zinc (Zn) contamination). After harvesting lupine biomass, enzyme activities ( $\beta$ -glucosidase, acid phosphatase, arylsulphatase, lipase, chitinase, cellobiohydrolase, alanine aminopeptidase, and leucine aminopeptidase) in soils were analysed. Enzyme activities decreased with TE soil contamination. According to our results, arylsulphatase was found to be the most sensitive soil enzyme to TEs. The nitrification rate is closely related to soil contamination and plant activity, as it stimulates microbial growth and multiplication through root exudates. The close correlations confirmed this relationship ( $r = 0.73$ – $0.99$ ). An increasing trend in TE contents in the roots was observed with soil contamination. Plant hormones are crucial in regulating root growth and development under stress conditions. The levels of determined phytohormones in our experiment (auxins, abscisic acid (ABA), salicylic acid (SA), and bioactive cytokinins (bCKs)) were lower in the contamination compared to the control. Correlations confirmed a significant negative relationship between the TE content in the roots and the contents of phytohormones (auxins:  $r = -0.96$  to  $-0.97$ ; ABA:  $r = -0.83$  to  $-0.86$ ; SA:  $r = -0.95$  to  $-0.99$ , bCKs:  $r = -0.87$  to  $-0.93$ ). The ratios of these hormones (not their absolute values) appear to be the determining factor for regulating root development and protecting plants from oxidative stress.

**Keywords:** narrow-leaved lupine; nitrification; arylsulphatase; soil multi-contamination

Toxic elements (TEs) can adversely affect soil ecology, agricultural production, and groundwater quality and ultimately enter the food chain, which can be a risk to human health (Kwiatkowska-Malina 2018). The serious sources of TEs are anthropogenic activities, mainly mining and ore smelting, which can lead to increased concentrations of TEs in soil (Cui et al. 2021). The availability of TEs to plants from the soil depends on the total content of TEs in soils and the forms of TEs that occur in the soil. Moreover, the pH of soil solution, soil colloids, the degree of saturation with TE ions in the complex,

the sorption of the TEs on the mineral matrix, and the biodegradation of the organic fraction of the complex can influence TE mobility (Kwiatkowska-Malina 2018).

In response to soil contamination, the different root system architecture can affect root system plasticity. It can explain the different fitness and behaviour of the different plant species in polluted environments depending on the root type involved in the response (Vives-Peris et al. 2020). The toxic effects of the TEs are primarily associated with reduced primary root length, decreased lateral root proliferation, and

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reduced leaf area (Mathur et al. 2022). Additionally, TE stress can be alleviated by auxin regulation of root development and elongation processes, but their accumulation impairs auxin metabolism, which subsequently affects the developmental processes in plants.

Roots' uptake of TEs and nutrients is closely related to the root zone. It is an area at the root-soil interface with many physical, chemical, and biological processes due to the interaction between plant roots, microorganisms, and soil components (Yang et al. 2017). Plant roots respond to TE toxicity by creating barriers to TEs or sequestering elements in vacuoles, accompanied by changes in root growth and branching. In relation to this response, the roots secrete so-called exudates into the root zone, which have varied effects on the mobilisation of TEs. Exudates can form chelating compounds with TE ions at the interface in the root zone, thereby reducing their mobility and thus enabling plants to tolerate and resist TEs-induced toxicity in the soil (Vives-Peris et al. 2020). A decrease in soil pH during root exudation increases the dissolution of TE complexes in the soil solution, thereby creating more available TEs (Antoniadis et al. 2017). Also, root secretion is an important source of soil enzymes (Li et al. 2010).

Soil enzymes play a crucial role in nutrient cycling, as they are constantly synthesised, accumulated, inactivated, and degraded in the soil (Adetunji et al. 2017, Yang et al. 2017). They can be used as important indicators of soil quality or contamination due to their sensitivity to TE toxicity (Vaidya et al. 2020, Cui et al. 2021). Many papers confirmed that low contents of TEs in soil may stimulate enzyme activities, whereas higher ones inhibited them (Wahsha et al. 2017, Vaidya et al. 2020). Nikolova et al. (2023) published mainly negative alterations in enzyme activities, especially the activity of dehydrogenase and alkaline phosphatase, but there were also some stimulatory effects ( $\beta$ -glucosidase). According to Vig et al. (2003), TEs could inhibit soil enzyme activities through interacting with the enzyme active sites and substrate complexes and denaturing the enzyme protein.

This study aimed to assess the effect of TE pollution on the activities of soil enzymes in the root zone, which play crucial roles in soil nutrient cycling, and on changes in the root system in soil from an area affected by long-term pollution originating from the historic mining and smelting activities. Our research was based on the hypothesis that mining

and smelting activities influence soil TE content as a critical parameter inhibiting soil enzyme activities and affecting root growth and development. To better understand the relationship between these parameters, we analysed the contents of TEs, selected phytohormones in roots, and the activity of soil enzymes in the root zone. A crucial role of plant hormones was verified in regulating the root growth of lupine under TE stress.

## MATERIAL AND METHODS

**Design of the pot experiment and collection of samples.** A pot experiment was conducted in a greenhouse. Experimental soil (contamination) was collected from the Podleší locality in the Příbram district (49°42'24"N, 13°58'32"E) of the Czech Republic. The soil exhibited a high degree of contamination, especially with arsenic (As), cadmium (Cd), lead (Pb), and zinc (Zn), due to historic mining and smelting activities (Sucharová and Suchara 2004). As the control variant (control), soil with a low degree of contamination (according to Czech legislation, Czech Ministry of the Environment 2016) was collected from the Suchdol locality in the Prague district (50°8'8"N, 14°22'43"E) of the Czech Republic. The soil was collected from the top 30 cm at each location, air-dried, and homogenised. Basic soil characteristics and TEs pseudo-total content were determined after sieving through a 2 mm sieve (Table 1).

The soils were fertilised with N, P, and K (0.5 g N/pot in the form of  $\text{NH}_4\text{NO}_3$ , 0.16 g P and 0.4 g K/pot in  $\text{K}_2\text{HPO}_4$ ) during the preparation of the pot experiment and homogenised. 2.5 kg of soil was added to each pot. The eight pots were prepared for each type of soil. Four pots were sown with narrow-leaved lupine (*Lupinus angustifolius* L., cv. Boregine – 7 seeds per pot, AROS-osiva, Prague, Czech Republic) and the remaining four pots were left without plants. Pots were placed under natural light conditions with temperatures of 20–23 °C during the day (14 h) and 15–18 °C at night (10 h), along with a relative humidity of ~60% in a greenhouse for 30 days.

At the end of the experiment, plant samples were collected and weighed. Afterwards, some plant biomass was frozen in liquid nitrogen and stored at –80 °C. The rest was dried in an oven at 40 °C until reaching a constant weight and then homogenised. Soil samples were also collected, sieved through a 2 mm sieve, and stored at –80 °C for subsequent experiments.

Table 1. Basic characteristics and toxic element content of experimental soils

	Control – Suchdol	Contamination – Podlesí
Soil type	Haplic Chernozem	Haplic Cambisol
pH <sub>H<sub>2</sub>O</sub>	7.1 ± 0.1	6.0 ± 0.04
CEC (mmol <sub>+</sub> /kg)	230.1 ± 5.0	165.8 ± 15.1
C <sub>org</sub> (%)	1.8 ± 0.3	4.1 ± 0.1
As (mg/kg)	18.4 ± 0.8	68.2 ± 13.1
Cd (mg/kg)	0.4 ± 0.02	3.9 ± 0.5
Pb (mg/kg)	41.1 ± 3.8	1 009.1 ± 89.2
Zn (mg/kg)	92.9 ± 3.1	141.9 ± 14.0

CEC – cation exchange capacity; C<sub>org</sub> – organic carbon

**Determination of soil enzymes.** The enzymes evaluated in this publication were acid phosphatase (EC 3.1.3.2), alanine aminopeptidase (EC 3.4.11.2), arylsulphatase (EC 3.1.6.1),  $\beta$ -glucosidase (EC 3.2.1.21), cellobiohydrolase (EC 3.2.1.91), chitinase (EC 3.2.1.14) and leucine aminopeptidase (EC 3.4.11.1).

For soil enzyme determination, a suspension was prepared by homogenising 0.2 ± 0.002 g of soil sample and 20 mL of phosphate buffer (KH<sub>2</sub>PO<sub>4</sub>; c = 50 mmol/L; pH = 7.0) using an Ultra-Turrax (IKA Labortechnik, Staufen im Breisgau, Germany) for 30 s at 8 000 rpm. A solution of 10 mL of dimethyl sulfoxide (Thermo Scientific, Kandel, Germany) and the appropriate substrate was also prepared according to Hanč et al. (2018).

The 200  $\mu$ L of homogenised suspension and then 40  $\mu$ L of the substrate solution for the given enzyme were pipetted into the respective wells of the microtitre plate. For  $\beta$ -glucosidase and acid phosphatase, an additional 20  $\mu$ L of 99.9% dimethyl sulfoxide was added to the wells. Subsequently, the fluorescence of the substrates was measured in the microtiter plate using a Tecan Infinite® M200 device (Tecan Austria GmbH, Grödig, Austria) after 5 and 120 min in a Robbins 39 Scientific® 2000 microhybridisation incubator (SciGene, Sunnyvale, USA) at a temperature of 40 °C. Enzymatic activity was calculated from the difference between the initial and final values ( $\mu$ mol/g/h).

**Incubation test and determination of ammonia content.** The TE's effect on N-nitrification using incubation test was determined according to EN ISO 14238 (International Organisation for Standardisation 1997). The soil samples were incubated at 20 ± 2 °C for 14 days. Ammonia nitrogen (N-NH<sub>4</sub><sup>+</sup>) content in soil were determined after extraction by 1 mol/L

KCl solution using segmental flow-analysis using a colourimetric method on a Skalar<sup>plus</sup>System (Skalar Analytical B.V., Breda, The Netherlands).

**Determination of toxic elements content in root biomass.** Homogenised dry biomass (0.5 ± 0.05 g) was dissolved in 10 mL of 65% HNO<sub>3</sub> and 30% H<sub>2</sub>O<sub>2</sub> solution (4:1, v/v, Analytika®, Prague, Czech Republic). Low-pressure microwave digestion was performed using an Ethos 1 device (MLS GmbH, Leutkirch im Allgäu, Germany). The decomposed sample was diluted to 25 mL with demineralised water. The content of elements was analysed using an Agilent 720 inductively coupled plasma optical emission spectrometer (ICP-OES; Agilent Technologies Inc., Santa Clara, USA). Certified reference material (CRM NIST 1573a Tomato leaves, Analytika®, Prague, Czech Republic) was mineralised under the same conditions for quality assurance.

**Determination of phytohormones.** The extraction of phytohormone content in biomass was determined according to Přerostová et al. (2021). Analysis of phytohormones was performed with an LC/MS system consisting of a UHPLC 1290 Infinity II (Agilent Technologies Inc., Santa Clara, USA) coupled with a 6495 Triple Quadrupole Mass Spectrometer (Agilent Technologies Inc., Santa Clara, USA), operating in MRM mode, with quantification determined by the isotope dilution method. Data acquisition and processing were performed with Mass Hunter software B.08 (Agilent Technologies Inc., Santa Clara, USA).

**Statistical analyses.** Statistical results were processed using the Statistica 12.0 program (StatSoft, Tulsa, USA). One-way analysis of variance (ANOVA) with Fisher's *LSD* (least significant difference) post hoc test (*P* < 0.05) and Pearson's linear correlation (*r*; *P* < 0.05) were conducted to identify significant differences among the variants.

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## RESULTS AND DISCUSSION

Soil enzymes, derived from root exudates and microbial activity, are active in various biochemical processes and nutrient cycles and thus have vital roles in the recycling of soil nutrients and maintaining soil fertility (Bi et al. 2024). The enzymes evaluated in this publication play fundamental roles in the cycling of soil carbon (C), nitrogen (N), sulphur, and phosphorus. Toxic elements in soil mostly result in lower enzyme activities by causing protein denaturation, forming a complex with the substrate, interacting with the residues involved in enzyme function, or reacting with the enzyme-substrate complex (Hagmann et al. 2015). The changes in enzyme activities provide early warnings of soil biological changes, and therefore, as indicators of anthropogenic soil contamination could be useful for monitoring changes in ecosystems (Aponte et al. 2020). Our results showed a significant effect of TE contamination on enzyme activities and confirmed that changes in enzyme activities varied under the same TE contamination levels (Figure 1). Significant reductions in enzyme activities were measured in the contamination without plants compared to the control. The reductions were as follows: chitinase by 9%, leucine aminopeptidase by 44%,  $\beta$ -glucosidase by 46%, alanine aminopeptidase by 52%, acid phosphatase by 63%, cellobiohydrolase by 72%, arylsulphatase by 95%. The negative relationship between enzymes and TEs was confirmed by correlations (Table 2).

This finding is in line with several studies showing that enzyme activities decreased as the TE concentration increased (Vig et al. 2003, Narendrula-Kotha and Nkongolo 2017). Among the determined soil enzymes, arylsulphatase was the most sensitive to TEs, which is in accordance with the results of a meta-analysis conducted by Aponte et al. (2020). Wyszowska et al. (2016) evaluated the resistance of arylsulphatase in contaminated soils. They found that the toxic effect of TEs followed this order: Zn > Cu > Ni > Pb > Cd. According to the authors, arylsulphatase exhibited a higher resistance to the influence of TEs in neutral soils compared to acidic soils. In the case of acid phosphatase, a significant sensitivity to TEs was confirmed, making it a useful indicator for assessing soil contamination (Cui et al. 2021). Chowdhury and Rasid (2021) observed a strong negative correlation between acid phosphatase and TEs in soils contaminated mainly with Cd, Cr, Cu, Ni, Pb, and Zn. They found a highly significant negative

correlation for Zn and Cr. Similarly,  $\beta$ -glucosidase – a crucial enzyme for the degradation of soil organic matter and a reliable indicator of changes in soil organic compounds was strongly inhibited by TE contamination in our experiment. This finding is in accordance with the results reported by Hinojosa et al. (2004). Opposite results were observed by Nikolova et al. (2023), showing resistance of this enzyme to TEs, even stimulatory effects at slight to moderate levels of pollution.

A similar trend was observed in the variant without plants, showing a slight increase in lipase (by 14%) for contamination, indicating a rise in overall soil biogeochemical activity. Increased lipase activity was also confirmed by Agnello et al. (2016) in the presence of alfalfa with rhizosphere effects after 90 days in soil contaminated mainly with Cu, Pb, and Zn. On the other hand, Li et al. (2021) observed a significant decrease in lipase activity in the rhizosphere of *Salvia tiliifolia* Vahl at the highest applied dose of Cd (50 mg/kg). Both studies indicate variability in the response of soil enzyme activity to TEs and its relation to the plant effect.

Soil enzyme activity increases due to the plant effect and is a primary factor affecting soil C and N transformations (Bi et al. 2024). The effect of plants on enzyme activities was confirmed in our experiment (Figure 1). The results showed higher enzyme activities in the variant with lupine compared to the variant without plants, mainly in the contamination. The higher enzyme activity in the root zone can be explained by the stimulation of microbial activity mediated by the rhizodeposition of organic C by plants. This process creates an environment rich in organic substrates for microorganisms. Therefore, higher acid phosphatase activity was observed in pots where lupine grew in contaminated soil. Acid phosphatase is a typical root exudate (Kan et al. 2022), so the increased exudation of acid phosphatase by lupine may have occurred in response to a contaminated environment.

Additionally, a significant influence of roots on leucine aminopeptidase releasing leucine and other hydrophobic amino acids from the N-terminus of a polypeptide in soil was observed by Loeppmann et al. (2016) in a field study with hybrid maize where microbial biomass was also clearly increased by enhanced rhizodeposition. In our previous study (Novák et al. 2023), a similar trend was observed for  $\beta$ -glucosidase in the root zone of the As hyperaccumulator *Pteris cretica* cv. Albo-lineata. Controversially, the opposite

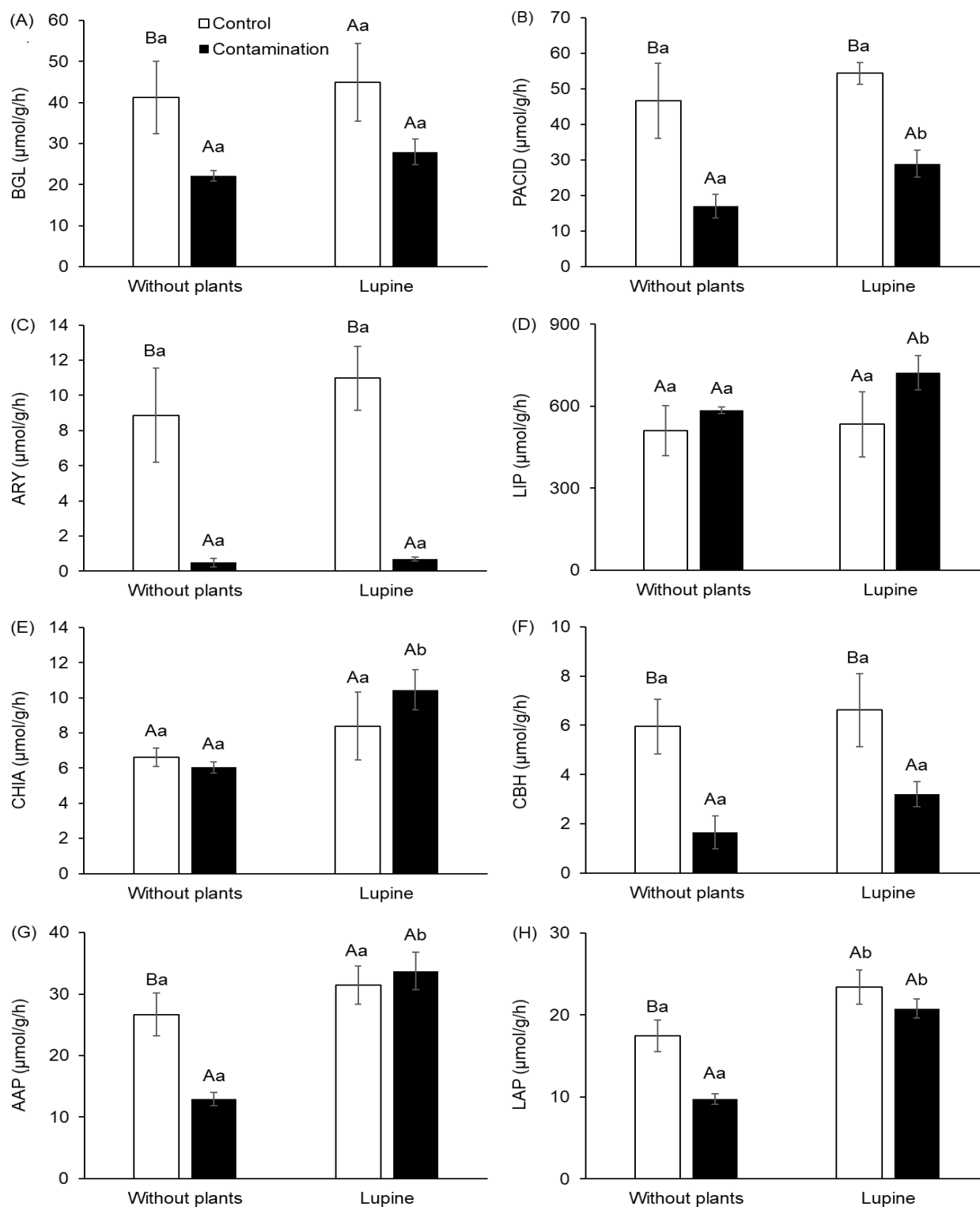


Figure 1. Enzymatic activity of (A)  $\beta$ -glucosidase (BGL); (B) acid phosphatase (PACID); (C) arylsulphatase (ARY); (D) lipase (LIP); (E) chitinase (CHIA); (F) cellobiohydrolase (CBH); (G) alanine aminopeptidase (AAP), and (H) leucine aminopeptidase (LAP) in the soil without plants and in the soil with sown *Lupinus angustifolius* (lupine). The data represent the mean  $\pm$  standard deviation ( $n = 3$ ). Different letters indicate significant differences ( $P < 0.05$ ) among soils (control  $\times$  contamination, uppercase letters) and influence of roots (without plants  $\times$  lupine, lowercase letters)



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Table 2. Values of correlation analyses of soil enzyme relationships and the toxic elements content in the soil without plants and in the soil with sown *Lupinus angustifolius* (lupine)

	Without plants	Lupine
$\beta$ -glucosidase	<b><math>r = -0.84, P = 0.039</math></b>	$r = -0.79, P = 0.062$
Acid phosphatase	<b><math>r = -0.88, P = 0.019</math></b>	<b><math>r = -0.96, P = 0.002</math></b>
Arylsulphatase	<b><math>r = -0.91, P = 0.012</math></b>	<b><math>r = -0.97, P = 0.001</math></b>
Lipase	$r = 0.50, P = 0.318$	$r = 0.67, P = 0.146$
Chitinase	$r = -0.55, P = 0.257$	$r = 0.53, P = 0.283$
Cellobiohydrolase	<b><math>r = -0.92, P = 0.009</math></b>	<b><math>r = -0.83, P = 0.039</math></b>
Alanine aminopeptidase	<b><math>r = -0.94, P = 0.006</math></b>	$r = 0.47, P = 0.344$
Leucine aminopeptidase	<b><math>r = -0.94, P = 0.006</math></b>	$r = -0.59, P = 0.222$

Bold font represents the significant relationship between soil enzyme and toxic elements content in soil ( $P < 0.05$ )

trend was observed in the non-hyperaccumulating fern *P. straminea*. Among the evaluated activities of five soil hydrolases ( $\beta$ -glucosidase, urease, arylsulphatase, acid and alkaline phosphatase) in the root zone soil of *Plantago* spp., arylsulphatase activity was identified as the most sensitive to soil TE pollution (Milosavljevic et al. 2020). According to Bowles et al. (2014), the highest activity of arylsulphatase is linked to the decomposition of organic matter, the presence of vegetation, and the abundance of microbial biomass in soil.

The TEs presence played an important role in modifying the nitrification process – an important process in the N cycle in soil. During nitrification, ammonium N is transformed into nitrate N, which plants then utilise. Toxic elements in soil inhibit the enzymatic activities of ammonium oxidation bacteria (Bai et al. 2023). In addition, Lu et al. (2022) have published the inhibition of nitrification by long-term TE pollution (Cu, As, Cd and Pb). According to Li et al. (2024), nitrification microorganisms are sensitive to the bioavailable portions of TEs. Our results

confirmed these findings. Over a 14-day incubation period,  $\text{N-NH}_4^+$  content declined in the control soil without plants to 5.5% (from 7.5 to 0.4 mg  $\text{N-NH}_4^+$ /kg). A significant reduction of  $\text{N-NH}_4^+$  content was not shown in the contamination. The nitrification rate is closely related to soil contamination and plant activity through the stimulation of microbial growth and multiplication *via* root exudates (He et al. 2024). The significant difference in nitrification rate was determined in soil from the contamination with lupine. Ammonia nitrogen content decreased to 23% over 14 days. The close correlations of our results confirmed the relationship between the TE content in the soil and the content of  $\text{N-NH}_4^+$  ( $r = 0.99, P < 0.0001$ ). A lower correlation for this relationship was calculated on soils with lupine ( $r = 0.73, P < 0.0001$ ), confirming that plants play an important role in stimulating nitrification through root exudates. Root exudates provide bioavailable C and act as signalling substances, promoting the growth and activity of microorganisms (He et al. 2024). Our results provide direct evidence of a priming effect of

Table 3. Toxic element content (mg/kg dry weight) and dry weight in roots and leaves of *Lupinus angustifolius* in control and contamination

	Roots		Leaves	
	control	contamination	control	contamination
DW (g/plant)	$0.09 \pm 0.03^A$	$0.06 \pm 0.002^A$	$0.2 \pm 0.03^A$	$0.2 \pm 0.04^A$
As (mg/kg)	nd	$14.3 \pm 2.8$	nd	nd
Cd (mg/kg)	$0.3 \pm 0.1^A$	$31.8 \pm 1.7^{Bb}$	nd	$1.1 \pm 0.1^a$
Pb (mg/kg)	$1.1 \pm 1.9^A$	$311.0 \pm 45.3^{Bb}$	nd	$6.5 \pm 1.3^a$
Zn (mg/kg)	$53.6 \pm 3.0^{Ab}$	$144.7 \pm 13.3^{Bb}$	$39.2 \pm 4.5^{Aa}$	$52.3 \pm 1.4^{Ba}$

The values represent the mean  $\pm$  standard deviation ( $n = 3$ ). Different letters indicate significant differences ( $P < 0.05$ ) among soils (control  $\times$  contamination, uppercase letters) and part of plants (leaves  $\times$  roots, lowercase letters). DW – dry weight; nd – not detectable (detection limits: As:  $< 0.03$ ; Cd:  $< 0.001$ ; Pb:  $< 0.02$  mg/L)

plants on nitrification in the root zone. Plant roots respond to TE toxicity by creating barriers to TEs or sequestration of elements in vacuoles accompanied by changes in root growth and branching. The primary effect of TE toxicity in plants is rapid inhibition of root growth, probably due to the inhibition of cell division in the root tip. It was demonstrated that TEs caused inhibition of cell division (Kaur et al. 2013).

Reduced biomass yield and the visible symptoms of stress in response to TEs toxicity are commonly observed trends (Wang et al. 2015). For example, Wyszowski and Modrzeska (2015) reported that soil Zn contamination reduced the growth and development of yellow lupine, as a dose of 300 mg Zn/kg soil caused wilting of plant seedlings. Dahlawi et al. (2023) observed a significant reduction in the growth of *Brassica* cultivars and their yield in pots spiked by TEs (Cd, Pb, and Ni) compared to the control. A change in yield was observed in the contamination compared to the control in our experiment, showing a reduction of 33% for roots and 5% for leaves of lupine (Table 3). No significant difference was observed between the contamination and the control. Also, correlations did not confirm a significant relationship between biomass yield and TEs contents. Despite there being no significant difference in the biomass of roots and leaves between the contamination and control, the contents of TE confirmed differences between the treatments, highlighting the major role of roots in the uptake and accumulation of TEs.

The content of TEs in roots is influenced by the soil, especially by the total content and forms of TEs present, as well as by the plant itself (Clemens and Ma 2016, Antoniadis et al. 2017). Moreover, translocation into the shoots depends on the rate of uptake in different root cell compartments, mobility in the root symplast and across barriers such as the endodermis, loading into the xylem, and upward movement in the xylem (Clemens and Ma 2016). A significant role of soil contamination and roots in the accumulation and translocation of TEs for lupine was confirmed in our experiment. In the lupine roots of the contamination, a 106-fold, 283-fold, and 2.7-fold higher accumulation of Cd, Pb, and Zn was observed compared to the control, respectively (Table 3). Content of As in the roots of the control, as well as As, Cd, and Pb in the leaves in the control, was confirmed to be undetectable. Arsenic content was also undetectable in lupine leaves growing in contaminated soil. A significant increase of Zn content (by 25%) was confirmed in the leaves in the contami-

nation compared to the control. Additionally, in the contamination, a significantly lower content of TEs was observed in leaves (Cd by 96%, Pb by 98%, Zn by 64%) compared to roots. These results indicate low translocation of TEs from roots to leaves in lupine, which is in accordance with the findings reported by Dahlawi et al. (2023). The authors observed accumulation of Cd, Pb, and Ni in *Brassica* cultivars in the following order: soil > root > shoot > seed. Correlations confirmed the relationship between the TEs content in the soil and the roots (As:  $r = 0.97$ ,  $P < 0.0001$ ; Cd:  $r = 0.99$ ,  $P < 0.0001$ ; Pb:  $r = 0.98$ ,  $P < 0.0001$ ; Zn:  $r = 0.98$ ,  $P < 0.0001$ ) and leaves (Cd:  $r = 0.99$ ,  $P < 0.0001$ ; Pb:  $r = 0.96$ ,  $P < 0.0001$ ; Zn:  $r = 0.89$ ,  $P < 0.0001$ ). Similarly, Alia et al. (2015) observed an increasing trend in the accumulation of TEs (Cd, Pb, and Zn) in *Spinacia oleracea* L. as the doses of TEs increased.

Soil contamination with TEs has adverse effects on the root system architecture. The response of roots to TE multicontamination is more severe than the toxic effect caused by each element alone (van Dijk et al. 2022). Plant hormones play a crucial role in regulating root growth and development under stress conditions. The contents of specific phytohormones in our experiment – auxins, abscisic acid (ABA), salicylic acid (SA), and bioactive cytokinins (bCKs), were lower in the contamination compared to the control (Table 4). Correlations confirmed a significant negative relationship between the TE content in the roots and the contents of phytohormones (auxins:  $r = -0.96$  to  $-0.97$ ; ABA:  $r = -0.83$  to  $-0.86$ ; SA:  $r = -0.95$  to  $-0.99$ ; bCKs:  $r = -0.87$  to  $-0.93$ ).

Toxic elements are known to cause various morphological defects in plants, accompanied by an alteration in the content of auxins in plant organs. According to Moeen-Ud-Din et al. (2023), the redistribution of

Table 4. Phytohormone contents in roots of *Lupinus angustifolius* in control and contamination (pmol/g fresh weight)

	Control	Contamination
Bioactive cytokinins	3.2 ± 0.1 <sup>B</sup>	2.7 ± 0.1 <sup>A</sup>
Absciscic acid	57.4 ± 5.1 <sup>B</sup>	45.4 ± 0.5 <sup>A</sup>
Auxins	38.1 ± 1.3 <sup>B</sup>	30.2 ± 0.2 <sup>A</sup>
Salicylic acid	358.7 ± 22.2 <sup>B</sup>	166.1 ± 13.5 <sup>A</sup>

The values represent the mean ± standard deviation ( $n = 3$ ). Different letters indicate significant differences among soils ( $P < 0.05$ )

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auxins by TEs directly affects the inhibition of the primary root by disrupting auxin biosynthesis and transport. Our results confirmed a 21% reduction in auxin content in the contamination compared to the control (Table 4). The level of auxins in root cells plays a crucial role in regulating primary root growth and lateral root branching (Mathur et al. 2022). Toxic elements tested by Wang et al. (2015) in their experiments indicate differences in the effects of TEs on auxin distribution in the root meristem. Similarly, in *Arabidopsis thaliana* (L.) Heynh., exposure to Cd caused a significant decrease in primary and lateral root elongation (Sofo et al. 2017). Piacentini et al. (2020) reported that Cd and As impact the auxin metabolism and distribution in the lateral and adventitious root apices. The results of the study by Sofo et al. (2022) confirmed that the low concentrations of Pb, Cu, Ni, and Zn decreased the primary root length compared to control *Arabidopsis* plants. Additionally, these low concentrations stimulated the development and growth of lateral roots by regulating auxin homeostasis.

An increase in TE content in lupine roots decreased the accumulation of bCKs in this plant part by 16% (Table 4). A similar result was found for the non-hyperaccumulator fern *P. straminea* growing under As stress (Pavlíková et al. 2020). Zhang et al. (2024) confirmed a decrease in CK content in soybeans growing under TE stress, which was associated with its oxidation and subsequent destruction. Cytokinins are mainly synthesised in root apical meristems (Sosnowski et al. 2023). Their contents are controlled by other phytohormones, which significantly affect their biosynthesis and their degradation. For example, auxin plays an important role in decreasing the biosynthesis of bCKs in the roots (Cammarata et al. 2023, Sosnowski et al. 2023). The ratio of auxin to CKs regulates cell proliferation and branch formation. Auxin and CKs act antagonistically. Auxin stimulates lateral root branching, while CKs inhibit this process. According to Cammarata et al. (2023), the ratio of these two hormones (not their absolute values) is the determining factor for the regulation of root development. Our results show that the ratio of auxin/CKs in the control (11.9) increased by 6% compared to the contamination (11.2). Therefore, this ratio could affect root branching in contaminated soil.

Additionally, auxin exhibits crosstalk with ABA, which protects the plants from oxidative stress. ABA plays a significant role in the long-distance root-to-shoot signalling process, which regulates root de-

velopment under stress. According to Vishwakarma et al. (2017), TEs such as Cd, Cu, Cr, Ni, Zn, and Al increase the ABA content in plants. Our results show an opposite trend – a significant 21% decrease in ABA (Table 4). Parwez et al. (2022) and Singh and Roychoudhury (2023) published the antagonistic effect of auxin and ABA and their interaction controlling primary root development. The results of Mao et al. (2018) demonstrated that a higher auxin/ABA ratio leads to the induction of root initiation. Our results did not confirm any differences in the auxin/ABA ratio between the variants. Besides the mentioned phytohormones, SA plays a role in the response of plants to toxicity caused by TEs.

Low SA content can enhance plant tolerance and defence mechanisms by inducing antioxidant enzyme activities and decreasing oxidative stress (Emamverdian et al. 2020). This finding corresponds with our results (Table 4). The content of SA in the roots was significantly reduced in the contamination compared to the control (by 54%). López-Orenes et al. (2020) reported a positive feedback interaction between SA and ABA.

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