



# Phytoremediation potential of seedlings: comparing heavy metal accumulation in *Ailanthus*, *Acer*, and *Fraxinus* species

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**Abstract** This study investigates the phytoremediation potential of non-productive seedlings of *Ailanthus altissima*, *Acer pseudoplatanus*, and *Fraxinus excelsior* for lead, cadmium, and zinc accumulation in contaminated soils of Zanjan Province, an industrial area with significant pollution. The evaluation employed a completely randomized design, with three treatment levels for each element, alongside a control treatment, replicated three times over a two-year period. A total of 810 one-year-old seedlings from the three species were involved in the

study. Soil contamination levels, ranging from 0 to 2000 mg/kg for lead and zinc and from 0 to 200 mg/kg for cadmium, were administered through soil pot irrigation. Sampling of seedling stems and pot soils was conducted in November of 2021 and 2022. The absorption levels of elements in the samples were determined using the dry acid digestion method and an ICP-OES atomic absorption spectrometer. Results indicate species-specific variations in metal absorption, with *Ailanthus* showing the highest accumulation rates. Findings suggest *Ailanthus* as a promising candidate for soil improvement in polluted environments, particularly in contaminated soils of Zanjan Province.

## Highlights

- Comparative analysis of heavy metal accumulation in three plant species for phytoremediation.
- Novel insights into differential absorption patterns of lead, zinc, and cadmium.
- Investigation of phytoremediation potential in non-productive seedlings.
- Contribution to environmental science through innovative research methods.
- Implications for sustainable pollution management and ecosystem health.

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## Introduction

The expansion of industrial activities has led to widespread pollution, particularly in ecosystems, with heavy metals emerging as significant pollutants. These metals, present in various organic and inorganic compounds, pose serious environmental threats even in trace amounts, as they permeate the food chain, escalating pollution levels (Jokar et al., 2017; Mohammadian et al., 2016; Sasan et al., 2017). Their persistence and adverse physiological impacts, even at minimal concentrations, pose

substantial risks to ecosystem health (Maddah et al., 2013; Selahvarzi et al., 2021; Mustafawi et al., 2020; Sasan et al., 2017; Sybhashini & Swamy, 2013; Ahmad Zuhaidi & Jeyanny, 2018). Nevertheless, the accumulation of heavy metals in plant tissues offers valuable insights into ecosystem dynamics. Woody plants, in particular, are pivotal in absorbing and sequestering heavy metals, rendering them crucial indicators of environmental contamination and potential agents for phytoremediation.

The entrance of heavy metals into the human body, either directly or indirectly through food consumption, can lead to severe health consequences (Liu et al., 2013; Rafati et al., 2012; Sharma & Sachdeva, 2015). While certain metallic elements in soil are essential for plant growth and physiological activities at typical concentrations, an excessive increase in their levels can be detrimental. This is due to the formation of free radicals and their rapid displacement in pigments or enzymes, disrupting functionality and adversely affecting plant presence and growth, thus considered toxic (Askari et al., 2012; Panah et al., 2016; Sharma & Sachdeva, 2015; Tangahu et al., 2011; Liu et al., 2013; Sybhashini & Swamy, 2013; Ahmad Zuhaidi & Jeyanny, 2018; Čudić et al., 2016; El-Amier & Alghanem, 2018; Wegiel et al., 2018).

Environmental remediation of areas contaminated with heavy metals and organic pollutants through plant absorption, known as phytoremediation, has gained significant prominence (Hashemi et al., 2020; Yavarian et al., 2021; Daghestani et al., 2023). Given the costly and challenging nature of traditional methods for removing heavy elements from soil and water, the need for pollution-reducing plants is evident. Phytoremediation offers a promising approach for purifying soil and water, by either absorbing pollutants or trapping them within plants (Babak Baharvand et al., 2022; Azzarello et al., 2012; Liu et al., 2013; Tangahu et al., 2011; Wegiel et al., 2018; Sybhashini & Swamy, 2013; Ahmad Zuhaidi & Jeyanny, 2018; Čudić et al., 2016; Guerra et al., 2011; Din Ahmad et al., 2018; Bandyopadhyay & Maiti, 2019; Kaewtubtim et al., 2016). Various phytoremediation techniques exist as emerging green technologies, including phytostabilization, phytoextraction, phytodegradation, rhizofiltration, and phytovolatilization. The first two mechanisms are the most reliable (Azzarello et al., 2012; Cheraghi et al., 2015; Laghlimi

et al., 2015; Liu et al., 2013; Liu et al., 2013; Neisi et al., 2014; Tangahu et al., 2011).

Various plant species have been investigated and recommended for heavy metal removal from soil, with most phytoremediation projects focusing on herbaceous plants and their leaves and roots. However, when the impact of trees on element absorption has been explored, the concentration of these elements in leaves, roots, and surrounding soil has been assessed. Trees can be successfully utilized in phytoremediation due to their ability to thrive in nutrient-poor soils, extensive root systems, rapid growth rates, metal resistance, and cost-effective secondary use. These characteristics enable trees to immobilize, extract, degrade, or evaporate soil pollutants (Salehi, 2019; Mohammadi et al., 2020; Tangahu et al., 2011; Sybhashini & Swamy, 2013; Ahmad Zuhaidi & Jeyanny, 2018; Guerra et al., 2011; Din Ahmad et al., 2018; Bandyopadhyay & Maiti, 2019; Kaewtubtim et al., 2016; El-Amier & Alghanem, 2018; Čudić et al., 2016; Mleczek et al., 2017; Pulford & Watson, 2003). Based on reviewed literature, numerous tree and shrub species such as *Morus alba*, *Citrus limetta*, *Platanus orientalis*, *Fraxinus excelsior*, *Olea europaea*, *Robinia pseudoacacia*, *Quercus alba*, *Eucalyptus camaldulensis*, *Alnus subcordata*, *Acacia dealbata*, *Ricinus communis*, *Calotropis procera*, *Salix babylonica*, and *Populus nigra* have been identified for phytoremediation purposes, capable of absorbing multiple heavy metals and removing them from the soil (Guerra et al., 2011; Pulford & Watson, 2003; Salehi, 2019; Wegiel et al., 2018).

Mining activities in Zanjan have resulted in significant heavy metal pollution, particularly in soils, as evidenced by studies conducted by Saba et al. (2015) and Yari et al. (2017). The industrial activities related to lead in Zanjan, owing to the presence of the Anguran lead and zinc mine, one of Iran's largest lead and zinc mines, have experienced remarkable progress and expansion (Moammeri et al., 2017; Mohammadi et al., 2020). The exploitation of mines and extraction of metals constitute a significant source of employment and income for the region's residents, leading to the proliferation of heavy metal pollution in the natural resources of the province, especially in soil, surface water, groundwater, and sediments (Afshari & Khademi, 2017; Majidi et al., 2016). Mining operations and associated industrial activities' waste can lead to soil contamination with heavy metals in

agricultural and urban areas. Several studies have examined this pollution in the soil, water, and plants of this province (Mosalii et al., 2014a, b). In 2008, Mohammadian et al. investigated the concentrations of lead, zinc, and cadmium in 14 drinking water wells near the Zanjan lead and zinc factory, showing that the concentrations of lead and cadmium exceeded the World Health Organization standards by 59% and 53%, respectively. Abdollahi et al. (2013) demonstrated that the concentrations of heavy metals in the soils around industrial factories in the Anguran area have increased. The results of Afshari et al. (2015) showed severe soil contamination with metals such as lead, zinc, and cadmium in soil samples adjacent to lead and zinc production and processing factories, with pollution levels increasing closer to the factories. Additionally, the analysis of plant samples indicated high concentrations of lead, zinc, and cadmium in the fodder and fruit produced in the orchards near the lead and zinc factory. Afshari and Khademi (2017) further showed that the concentrations of these elements in the soils around Zanjan city, Sultanieh, and industrial factories are highest, decreasing with distance from these centers of pollution. They identified the main factors contributing to the relative increase in lead and zinc concentrations in the soils around Zanjan city as the operation of the lead and zinc factory in the western part of Zanjan city and traffic (Afshari & Khademi, 2017). The study by Yari et al. (2017) on the determination of the heavy metal concentrations of zinc, copper, lead, and cadmium in soil samples from different locations using X-ray fluorescence spectroscopy demonstrated that the soil contamination with heavy metals in the study area is considerable.

Despite the extensive research on phytoremediation, there is still a need to explore the effectiveness of various tree species in removing heavy metals from contaminated soils. Soil contamination in the industrial areas of Zanjan directly impacts the region's water contamination (agricultural and drinking water). Thus, one of the primary objectives of this research was to clean the soils of the extensive industrial areas. Our main goal was to eliminate contamination.

In this study, we focused on phytoextraction. To prevent heavy metal contamination from entering the food chain, we used non-productive trees. After determining their optimal absorption capability, these

trees can be introduced to the industrial zone to clean the area's soil without contaminating the food chain. The primary focus was on storing contaminants in the wood rather than the leaves, as the leaves could return the contamination to the soil through leaf fall. Therefore, this study aims to investigate the potential of several tree species in Zanjan Province, Iran, for phytoremediation purposes. The goals include assessing the ability of selected tree species to absorb and accumulate heavy metals from contaminated soils and evaluating the factors influencing their efficacy in phytoremediation. The hypothesis is that certain tree species, due to their physiological and ecological characteristics, will exhibit higher efficiency in removing heavy metals from contaminated soils compared to others. This research is of paramount importance as it contributes to expanding our understanding of tree-based phytoremediation techniques and identifying practical solutions for mitigating heavy metal pollution in the environment.

## Methods and materials

### Research method

The research was conducted at the greenhouse of the Agricultural Research and Natural Resources Center of Zanjan, Zanjan County, Zanjan Province, Iran. Initially, a specific volume of soil (equivalent to two truckloads) was transported from the Qareh Chiryan area to the greenhouse. Prior to its transfer, the soil underwent evaluation at the Water and Soil Laboratory of the Research Center. The suitability of the soil type was confirmed, ensuring it had low nutrient content consistent with soils found around industrial areas where tree planting was intended. Superphosphate fertilizer was recommended to enrich the soil, ensuring the seedlings received necessary nutrients.

Since the soil in all the pots was the same (see Table 1), the soil variable was not considered in measuring heavy metal absorption. However, soil type is undoubtedly a factor that influences absorption levels. We did measure lead, zinc, and cadmium concentrations in the soil before starting the research. These initial measurements revealed that all values were below 1 mg/kg, which indicates they did not

**Table 1** Soil properties and initial heavy metal concentrations

Soil property	Depth	Clay	Silt	Sand	Phosphorus (P)	Potassium (K)	Organic carbon (OC)	Total neutralizing value (T.N.V.)	pH	Electrical conductivity (EC)	Saturation percentage (S.P.)
Unit	cm	%	%	%	mg/kg	mg/kg	%	%		Ds/m	%
Value	0–30	30	36	34	30	234	0.747	16.43	7.55	0.862	47

impact the analysis results. As these values were not substantial enough to affect the study, they were not reported in a separate table, since the control sample in the analyses is the soil without initial contamination, and its values are specified.

The following are the permissible heavy metal contamination levels in agricultural soil in Iran (unit: mg/kg):

- Lead: 50 mg/kg for acidic soil; 75 mg/kg for non-acidic soil
- Zinc: 200 mg/kg for acidic soil; 500 mg/kg for non-acidic soil
- Cadmium: 1 mg/kg for acidic soil; 5 mg/kg for non-acidic soil

Based on these standards and the prevalent contamination levels in the Zanjan industrial area, the treatment levels for this study were selected accordingly.

#### Preparation of treatment solutions

Considering the soil contamination standards in the region for non-acidic soils (lead 75 mg/kg, zinc 500 mg/kg, and cadmium 5 mg/kg) and the actual contamination levels in the area, as well as previous studies, the treatment levels were determined as follows.

Treatment solutions for the experiment included lead (Pb), zinc (Zn), and cadmium (Cd) at three experimental levels: lead at 1000, 2000, and zero (control); zinc at 1000, 2000, and zero; and cadmium at 100, 200, and zero. These solutions were prepared from metal nitrates. After preparation, the soil was sprayed in different batches to be used in the seedling treatment operations. *Acer pseudoplatanus*, *Ailanthus altissima*, and *Fraxinus excelsior* were selected among the tree species, with approximately 810 seedlings obtained from the Koushken Nursery, affiliated with the Zanjan Department of Natural Resources.

#### Experimental design

A randomized complete block design with three replications, spanning two years, was implemented. The research focused on three heavy metal elements: lead, zinc, and cadmium, at three experimental levels. A

total of 810 seedlings were transferred to pots filled with soil enriched with the specified elements and arranged in their designated positions.

The 810 seedlings were distributed as follows:

- Two hundred seventy *Ailanthus altissima* seedlings
- Two hundred seventy *Fraxinus excelsior* seedlings
- Two hundred seventy *Acer pseudoplatanus* seedlings

Each species' seedlings were split equally between the two years of study, resulting in the following:

- One hundred thirty-five *Ailanthus altissima* seedlings per year
- One hundred thirty-five *Fraxinus excelsior* seedlings per year
- One hundred thirty-five *Acer pseudoplatanus* seedlings per year

For each year, the 135 seedlings per species were further divided:

- Forty-five seedlings for each species allocated to the three experimental treatments
- Each treatment group consisted of 15 seedlings, divided into 3 repetitions, with 5 seedlings per repetition

The experimental design used is illustrated in Table 2.

Cultivation and maintenance

A drip irrigation system was set up to irrigate the seedlings up to 60% of their agricultural capacity, with pots irrigated three times a week. Necessary operations, including weed removal and irrigation, were performed throughout the year. Sampling of dormant seedlings commenced in the month of Aban (October–November) according to a predetermined pattern. Wood sections of the trunk and soil samples from each pot were collected, transferred to designated bags, and promptly sent to the laboratory for further analysis.

Sample preparation and heavy metal analysis

Sample preparation for determining the concentration of heavy metals in plant samples utilized the dry acid digestion method. The concentration of heavy metals in all samples was determined using an ICP-OES device, and statistical analysis of the data was performed using SPSS and R software. Mean comparisons were made using the LSD method, and graphs were plotted using Excel software.

For sampling, three equal parts were taken from the base, middle, and top of each seedling and combined. To determine the concentration of heavy

**Table 2** Experimental design

$R_I$	$S_1$			$S_2$			$S_3$		
	$E_1$	$E_2$	$E_3$	$E_1$	$E_2$	$E_3$	$E_1$	$E_2$	$E_3$
	$D_1$	$D_1$	$D_1$	$D_1$	$D_1$	$D_1$	$D_1$	$D_1$	$D_1$
	$D_2$	$D_2$	$D_2$	$D_2$	$D_2$	$D_2$	$D_2$	$D_2$	$D_2$
$R_{II}$	$S_3$			$S_1$			$S_2$		
	$E_2$	$E_3$	$E_1$	$E_2$	$E_3$	$E_1$	$E_3$	$E_2$	$E_1$
	$D_2$	$D_1$	$D_1$	$D_3$	$D_2$	$D_2$	$D_3$	$D_2$	$D_2$
	$D_1$	$D_2$	$D_3$	$D_2$	$D_1$	$D_3$	$D_2$	$D_1$	$D_3$
$R_{III}$	$S_2$			$S_3$			$S_1$		
	$E_2$	$E_3$	$E_1$	$E_2$	$E_1$	$E_3$	$E_1$	$E_2$	$E_3$
	$D_2$	$D_1$	$D_3$	$D_3$	$D_1$	$D_2$	$D_2$	$D_3$	$D_1$
	$D_1$	$D_3$	$D_2$	$D_2$	$D_3$	$D_1$	$D_1$	$D_1$	$D_3$
	$D_3$	$D_2$	$D_1$	$D_1$	$D_2$	$D_2$	$D_3$	$D_2$	$D_2$

$R$  repetitions,  $S$  tree species,  $E$  heavy metal elements,  $D$  concentration levels

metals in the seedling stem samples, the dry acid digestion method was employed.

First, 1–2 g of each sample was placed in an Erlenmeyer flask (Alizadeh et al., 2015). To obtain the dry weight, the flasks were placed in an oven at 105 °C for 24 h. The dried samples were then ashed in a furnace (zinc at 550 °C, lead and cadmium at 250 °C) for 24 h.

Next, for acid digestion, approximately 5 ml of 65% nitric acid were added to each flask. The resulting solution was then placed on a hot plate to complete the dry digestion process. The hot plate temperature was initially set at 25 °C and gradually increased to 60 °C, 105 °C, and 120 °C. Heating continued until all the acid had evaporated.

After acid evaporation, the flasks were brought to the desired volume with deionized water (double distilled) containing 1% nitric acid. The prepared solutions were filtered through a Whatman 42 filter paper and transferred to pre-weighed bottles, which were then re-weighed.

Finally, the concentration of heavy metals in all samples was determined using an ICP-OES atomic absorption spectrometer, and the heavy metal content of the samples was extracted.

Regarding the calculation of the bioaccumulation coefficient, since the selected seedlings had only two years to absorb contaminants in the artificially contaminated pots, this time was insufficient to assess the plants' real performance and determine the BAF and BCF indices accurately. Therefore, these coefficients were not calculated to avoid providing unrealistic information.

The translocation factor (TF) was calculated, and the results are presented in Table 3. Due to the high costs of laboratory analyses and budget limitations for this research, the heavy metal absorption by roots was measured only in the second year and in treatment number 3. The translocation factor (TF) is determined as follows:

## Results

The analysis of interactive effects using the LSD test in R software for the first and second years revealed significant differences in lead uptake levels in the stems among species and pollution concentration levels ( $P < 0.01$ ) (Table 4). Similar results were observed for the lead concentration in the soil of the seedlings in both the first and second years ( $P < 0.01$ ).

Comparison of mean lead accumulation in the stems among examined pollution concentrations in the first year showed significant differences at a 95% confidence level among the three species. The highest accumulation was observed at a concentration of 2000 mg/kg, while the lowest was in the control samples. *Ailanthus altissima* exhibited the highest lead uptake, followed by *Acer pseudoplatanus* and *Fraxinus excelsior*. Similar patterns were observed for zinc and cadmium, with significant differences among concentration levels for each species ( $P < 0.05$ ), and the highest uptake seen at a concentration of 2000 mg/kg. *Ailanthus altissima* consistently exhibited the highest accumulation of these metals across both years.

In the second year, results mirrored those of the first year, with significant differences among concentration levels for each species ( $P < 0.05$ ) and the highest accumulation observed at a concentration of 2000 mg/kg. The order of accumulation among species remained consistent with that of the first year.

Regarding zinc, significant differences in zinc uptake in the stems among species and pollution concentration levels were observed in both the first and second years ( $P < 0.01$ ) (Table 5). Similarly, significant differences were noted for the zinc content in the soil of plant pots across different species in the second year ( $P < 0.01$ ). However, in the first year, no significant differences were found among species; only differences among observed pollution levels were noted.

**Table 3** Heavy metal analysis in seedling roots in the second year and translocation factors

Species	Heavy metal analysis (mg/kg)			Translocation factors		
	Cd	Zn	Pb	Cd	Zn	Pb
<i>Ailanthus</i>	19	29	38	2.63	2.69	1.76
<i>Acer</i>	12	63	45	1.16	0.93	1.46
<i>Fraxinus</i>	26	33	39	0.54	0.72	0.69

**Table 4** Lead absorption in seedling stems and soil residue: species and pollution levels

Source of variations	Degrees of freedom	Sum of squares	Mean square	<i>f</i>	<i>P</i> -value	Sig
Variation in lead accumulation in seedling stems in the first year						
Species	2	2421.5	1210.7	74.472	1.97E−09	***
Pollution concentration level	2	15,539.2	7769.6	477.899	2.52E−16	***
Species × pollution concentration level	4	1339.4	334.9	20.596	1.61E−06	***
Residuals	18	292.6	16.3			
Variation in lead accumulation in seedling stems in the second year						
Species	2	3433.7	1716.9	85.466	6.47E−10	***
Pollution concentration level	2	22,772.5	11,386.2	566.806	<2.2e−16	***
Species × pollution concentration level	4	1740.8	435.2	21.665	1.11E−06	***
Residuals	18	361.6	20.1			
Variation in residual lead content in seedling soil in the first year						
Species	2	183,903	91,952	48.44	5.69E−08	***
Pollution concentration level	2	1,579,877	789,938	416.16	8.54E−16	***
Species × pollution concentration level	4	91,849	22,962	12.1	5.99E−05	***
Residuals	18	34,167	1898			
Variation in residual lead content in seedling soil in the second year						
Species	2	151,656	75,828	60.17	1.07E−08	***
Pollution concentration level	2	1,309,853	654,927	519.69	<2e−16	***
Species × pollution concentration level	4	77,485	19,371	15.37	1.25E−05	***
Residuals	18	22,684	1260			

Significant results were observed for the heavy metal cadmium among species and pollution concentration levels in both the first and second years of the study ( $P < 0.01$ ) (Table 6). Similarly, the same outcomes were repeated for the residual cadmium content in the soil of plant pots across different species in both the first and second years.

The analysis revealed significant differences in cadmium uptake among the three examined species at a 95% confidence level ( $P < 0.05$ ), with the highest accumulation observed at a concentration of 200 mg/kg (Fig. 1). *Ailanthus altissima* exhibited the highest uptake, followed by *Acer pseudoplatanus* and *Fraxinus excelsior*, respectively, in the first year. Similarly, the results for lead and zinc accumulation in the second year mirrored those of the first year, with significant differences observed between pollution concentration levels for all three species ( $P < 0.05$ ). The highest accumulation was observed at the concentration of 2000 mg/kg, while the lowest amounts were in control samples. *Ailanthus*, *Acer pseudoplatanus*, and *Fraxinus excelsior* were ranked in terms of absorption levels for all

three metals. These results highlight the species-specific variations in heavy metal absorption and reaffirm *Ailanthus altissima* as the most effective species, particularly evident in its notably higher cadmium uptake compared to the other two species in the second year.

Comparing the remaining heavy metal lead levels in the pot soils of first-year seedlings across various pollution concentrations revealed significant differences for *Ailanthus* and *Fraxinus* at a 95% confidence level ( $P < 0.05$ ), with the highest residue levels detected at a concentration of 2000 mg/kg (Fig. 2). Conversely, no significant difference was observed for *Fraxinus* between the 1000 and 2000 mg/kg levels ( $P < 0.05$ ). Among the species, *Fraxinus* exhibited the highest lead residue levels, while *Ailanthus* showed the lowest. Significant differences were also noted for zinc and cadmium residue levels among pollution concentration levels across all three species ( $P < 0.05$ ), with the highest residue levels observed at a concentration of 2000 mg/kg. *Acer* and *Fraxinus* displayed the highest zinc residue levels, while *Ailanthus* exhibited the lowest. Similarly, *Acer* had the



**Table 5** Zinc absorption in seedling stems and soil residue: species and pollution levels

Source of variations	Degrees of freedom	Sum of squares	Mean square	<i>f</i>	<i>P</i> -value	Sig
Variation in zinc accumulation in seedling stems in the first year						
Species	2	3340.9	1670.5	83.585	7.75E−10	***
Pollution concentration level	2	13,560.9	6780.4	339.272	5.14E−15	***
Species × pollution concentration level	4	1718.1	429.5	21.492	1.18E−06	***
Residuals	18	359.7	20			
Variation in zinc accumulation in seedling stems in the second year						
Species	2	3909	1954	105.23	1.17E−10	***
Pollution concentration level	2	20,848	10,424	561.28	<2e−16	***
Species × pollution concentration level	4	2014	503	27.11	2.10E−07	***
Residuals	18	334	19			
Variation in residual zinc content in seedling soil in the first year						
Species	2	59,035	59,035	3.6293	0.06935	
Pollution concentration level	2	1,353,956	1,353,956	83.2381	4.19E−09	***
Species × pollution concentration level	4	19,373	19,373	1.191	0.28642	
Residuals	18	374,119	16,266			
Variation in residual zinc content in seedling soil in the second year						
Species	2	83,536	41,768	37.19	4.05E−07	***
Pollution concentration level	2	1,331,621	665,810	592.74	<2e−16	***
Species × pollution concentration level	4	48,426	12,107	10.78	0.000123	***
Residuals	18	20,219	1123			

highest cadmium residue levels, followed by *Fraxinus* and *Ailanthus*.

Additionally, the comparison of the mean residual lead levels in the soil among pollution concentrations in the second year demonstrated significant differences between concentration levels for *Fraxinus* and *Acer* species, while no difference was observed for *Ailanthus* between the second and third pollution levels ( $P < 0.05$ ). Consistently, the highest residual levels were observed at a concentration of 2000 mg/kg. *Fraxinus*, *Acer*, and *Ailanthus* were ranked in terms of residual levels, respectively. Regarding zinc, significant differences were found between pollution concentration levels for *Ailanthus* and *Acer* species, while no significant difference was observed for *Fraxinus* between the 1000 and 2000 mg/kg levels ( $P < 0.05$ ). The highest residual zinc levels were observed at a concentration of 2000 mg/kg, with *Acer pseudoplatanus* showing the highest levels, followed by *Fraxinus excelsior* and *Ailanthus altissima*, respectively. Similarly, significant differences were found for cadmium among the three examined species at a 95% confidence level, with each species placed

in separate groups ( $P < 0.05$ ). However, in the second year, *Acer* exhibited higher residual cadmium levels compared to the other two species, while *Ailanthus* and *Fraxinus* were ranked next.

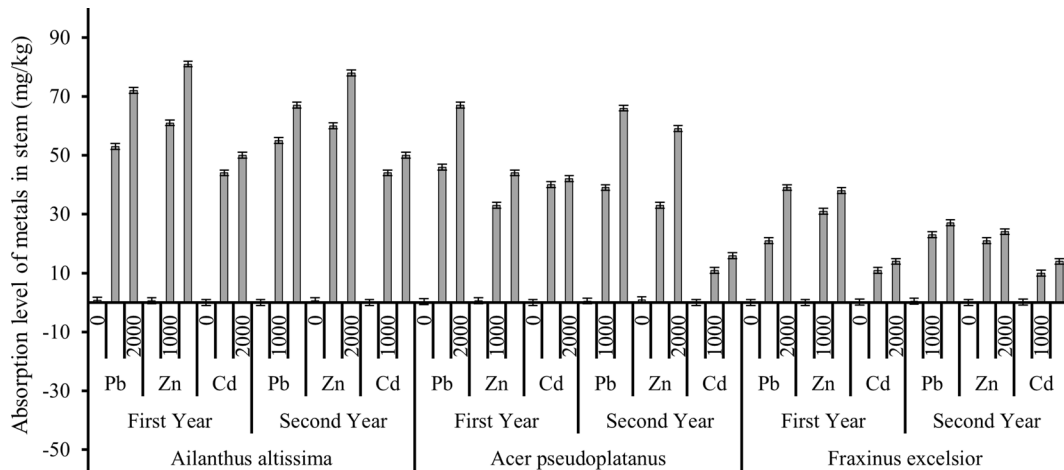
**Temporal trends in absorption rates** Comparison of absorption trends in the stems over two years revealed varying patterns. Lead absorption decreased consistently in *Fraxinus*, while overall metal absorption declined with increasing age across all species. Zinc uptake in stem tissues generally decreased at concentration level 2 but increased at level 3 in the second year. Conversely, no clear trend was observed for cadmium uptake.

**Residual levels in soil** Residual lead levels in soil decreased with seedling age, especially notable in *Fraxinus* and *Acer* species. Zinc residue levels exhibited no distinct trend across species, although *Ailanthus* showed a decreasing trend while *Acer* exhibited an increasing trend. Cadmium residue levels decreased in *Acer pseudoplatanus* and *Fraxinus excelsior*.



**Table 6** Cadmium absorption in seedling stems and soil residue: species and pollution levels

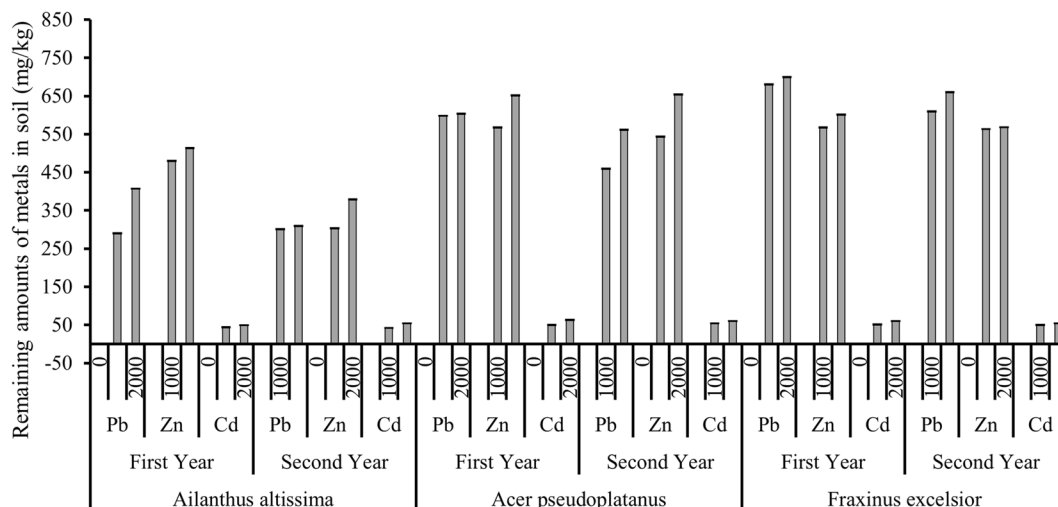
Source of variations	Degrees of freedom	Sum of squares	Mean square	<i>f</i>	<i>P</i> -value	Sig
Variation in cadmium accumulation in seedling stems in the first year						
Species	2	1791.9	1791.9	18.3992	0.000274	***
Pollution concentration level	2	5217.8	5217.8	53.5755	1.90E-07	***
Species × pollution concentration level	4	696.2	696.2	7.1489	0.013562	*
Residuals	18	2240	97.4			
Variation in cadmium accumulation in seedling stems in the second year						
Species	2	633	316	6.409	0.00791	**
Pollution concentration level	2	11,577	5788	117.29	4.74E-11	***
Species × pollution concentration level	4	406	102	2.057	0.12915	
Residuals	18	888	49			
Variation in residual cadmium content in seedling soil in the first year						
Species	2	1791.9	1791.9	18.3992	0.000274	***
Pollution concentration level	2	5217.8	5217.8	53.5755	1.90E-07	***
Species × pollution concentration level	4	696.2	696.2	7.1489	0.013562	*
Residuals	18	2240	97.4			
Variation in residual cadmium content in seedling soil in the second year						
Species	2	247	124	16.095	9.81E-05	***
Pollution concentration level	2	10,057	5029	654.48	<2e-16	***
Species × pollution concentration level	4	242	61	7.886	0.000742	***
Residuals	18	138	8			



**Fig. 1** Mean absorption levels of lead (Pb), zinc (Zn), and cadmium (Cd) at different concentrations in the stems of the studied species in the first and second years

**Species-specific absorption and residual levels** Across species and pollution levels, significant differences were observed in lead, zinc, and cadmium absorption in stems. *Ailanthus* exhibited

the highest absorption, while *Fraxinus* showed the lowest. Conversely, soil residue levels varied among species, with *Fraxinus* having the highest lead levels, *Acer* having the highest zinc levels, and



**Fig. 2** Mean residual amounts of lead, zinc, and cadmium at different concentrations in the soil of the studied species in the first and second years

cadmium showing the lowest residual levels across all species.

**Comparison of absorption rates at different pollution levels** Comparing mean absorption rates at different pollution levels showed lead absorption decreasing in *Acer pseudoplatanus* over two years, while zinc absorption increased, and cadmium absorption displayed no distinct trend.

In summary, temporal trends in absorption rates and residual levels varied among species and elements, highlighting the complex dynamics of heavy metal uptake and accumulation in both stem tissues and soil across different pollution levels.

## Discussion

Phytoremediation may serve as a short- and long-term therapeutic option for rehabilitating lands exposed to heavy metal pollution. However, in most cases and studies, it is recommended that native species be used to ensure compatibility with local water, air, and soil conditions. Only a few plant species have been identified as effective in phytoremediation. This does not imply that many plants are ineffective, as only a small number of them have been accurately assessed. Mirbagheri et al. (2017) noted that “biosand” played a

significant role in reducing heavy metal contamination in polluted soils, generally showing good adaptation to local water and soil conditions. If a specific plant is considered for phytoremediation of heavy metals in a project, its capability in the particular soil where phytoremediation is to occur must be tested. This study aimed to determine the extent to which the stems and wood of non-productive seedlings of *Ailanthus*, *Acer pseudoplatanus*, and *Fraxinus excelsior* accumulate lead, cadmium, and zinc in soils contaminated with lead, cadmium, and zinc for phytoremediation purposes. This study was conducted in Zanzjan Province, which is an industrial hub in the country and has significant soil pollution.

The results of this research indicate that the stems of the studied species of seedlings showed significant differences in the accumulation of heavy metals at different pollution levels. Significant differences were also observed among different species in the absorption of elements in stems and their residues in soil. Higher contamination levels of each metal resulted in greater accumulation in the seedling stems over the two years of the study. Therefore, it can be stated that the higher the soil contamination with heavy metals, the greater the accumulation of these elements in the plant stems. In line with the findings of this study, the comparison of average lead accumulation in seedling stems of spruce trees showed that the trend of lead accumulation increased significantly with pollution

levels across all levels (Alizadeh et al., 2015). In line with the results of this study, a comparison of the average lead accumulation in alder seedling stems showed that the trend of lead accumulation increased significantly with pollution levels across all levels (Jokar et al., 2017). Similarly, significant differences were observed between different lead pollution levels in *Robinia* seedlings in the study by Askary et al. (2012).

In general, the concentration of residual elements in the soil has been higher than in the woody seedling stems. Significant differences have been observed in the absorption of lead, zinc, and cadmium in the stems of the three species; among the three species, *Ailanthus* demonstrated the highest levels of lead and zinc absorption in the stems across different pollution levels during the two years of the study, followed by *Acer pseudoplatanus* and then *Fraxinus excelsior*, which exhibited the lowest levels. Similar results have been repeated for cadmium as well. However, the absorption of cadmium has been significantly lower in *Fraxinus excelsior* compared to the other two species. Therefore, *Ailanthus* is reported as the most successful species in absorbing heavy metals in the seedling stems in this study. These differences may be attributed to the physiological characteristics of the species, as some plant species, known as hyperaccumulators, can absorb heavy metals from the environment to a considerable extent without being significantly harmed themselves, while other plant species may have lower absorption capacity and may suffer damage or perish in environments contaminated with heavy metals due to toxicity.

The absorption of cadmium was lower in the tree species compared to lead and zinc. However, since the cadmium treatment was only one-tenth of the levels for lead and zinc, the cadmium absorption ratio was relatively higher. This indicates that the *Ailanthus altissima* species has a particularly high capacity for cadmium uptake. The high translocation factor observed in *Ailanthus altissima* suggests that this species is highly effective for soil remediation, and given that this study focused on soil remediation and phytoextraction, *Ailanthus altissima* stands out as the most promising species among the three tested.

Mattina et al. (2003) stated that the translocation factor (TF) can be used to identify hyperaccumulator species. In accumulator plants, the TF is greater than one, whereas in excluder plants, it is less than

one (Cooper et al., 1999). Since the translocation factor for *Ailanthus altissima* is above one for all three elements and considering its high growth potential in highly contaminated and even acidic environments, along with its favorable reproductive characteristics and ease of cultivation, *Ailanthus altissima* emerges as an excellent candidate for establishment in industrial zones and lead–zinc processing facilities.

In a comparison conducted by Majidi et al. (2016) in the same province, the absorption of zinc, copper, cadmium, and lead in the bark of ten different species including *Robinia pseudoacacia*, *Fraxinus excelsior*, *Populus alba*, *Populus nigra*, *Rosa damascene*, and *Salix babylonica* were investigated in zinc industrial place in the fifty-kilometer radius southeast of the city of Zanjan. They reported *Populus alba* as having the highest absorption rates for lead and cadmium in the bark, respectively, while *Rosa* exhibited the lowest absorption levels for both lead and cadmium. However, Mohammad et al. (2016) demonstrated in their research around the specialized industrial town of Zanjan that the best species for lead and cadmium absorption were the *Populus alba*, while for copper, *Rosa*, *Salix babylonica*, and *Populus nigra* were found to be the most efficient. Moreover, *Robinia* and *Fraxinus* were also identified as suitable for lead absorption. These findings contradict the results of the current study.

Furthermore, Heidari et al. (2014) showed in their study on trees surrounding a lead and zinc factory in Zanjan that the leaves of *Salix* and *Populus* had a high potential for phytoremediation, with significant differences observed in the metal concentration among the branches of the studied trees. They found that the branch of *Populus* for lead and of *Cupressus arizonica* for zinc had a higher potential absorption, while *Salix* was more efficient for zinc extraction, suggesting them as the most suitable options. Another investigation on heavy metals in the leaves of eight tree species in the metallurgical industries of Zanjan indicated that *Populus nigra* was the best accumulator for manganese, lead, and cadmium, *Thuja orientalis* was identified as the best extractor for iron, and *Cupressus sempervirens* var *arizonica* was recognized as the best species among the native plants studied for lead accumulation (Saba et al., 2015). Similarly to the current study, *Fraxinus* did not exhibit an ideal status in heavy metal absorption.

Panah et al.'s study (2016), conducted around the Ilam cement factory, revealed that broadleaf species outperform needleleaf species in terms of heavy metal deposition. They found that eucalyptus had the highest lead deposition, juniper had the highest manganese and cadmium deposition, oak had the highest zinc deposition, and sparrow's tongue had the lowest deposition. Similarly, in the current study, juniper exhibited the highest cadmium absorption, while sparrow's tongue showed the lowest zinc absorption in the stem, aligning closely with the present study's findings. Rafati et al. (2012) determined that mulberry species are effective for nickel removal, while sapidar is suitable for removing cadmium and nickel from the soil. However, they noted that none of these species are ideal for fixing these metals in the soil. Abbasi et al.'s (2017) research in Nishtarud, Mazandaran, compared seedlings of *Acer cappadocicum*, *Fraxinus excelsior*, and *Platycladus orientalis* over 6 months. They found that only *Platycladus orientalis* could accumulate a large amount of lead in the stem, causing biomass reduction in vane and maple species. Similar to the present study, sparrow's tongue was not effective in lead purification in plants. However, Aksoy and Demirezen's study (2006) in Turkey recognized sparrow tongue as a suitable species for this purpose. Ćudić et al. (2016) investigated the phytoremediation potential of 5 species in soils contaminated with heavy metals in Belgrade. They found that *Ailanthus* could increase heavy metal bioconcentration over time but ranked intermediate compared to other species. They suggested its use for plant stabilization in contaminated loamy soil. Mleczek et al. (2017) in Poland reported that certain broad-leaved species, compared to *Acer platanoides*, had the highest bioconcentration factor (BCF) and the lowest translocation factor (TF), making them promising for plant stabilization. Dadea et al. (2017) compared 9 broadleaf species in Central Europe and found *Betula pendula* and *Robinia pseudoacacia* to be better candidates for phytoremediation compared to *Acer pseudoplatanus* and *Ailanthus altissima*.

The comparative analysis of the first and second-year graphs revealed no specific trend of decrease or increase in the absorption levels for elements in different levels in the tree stems. However, the absorption of lead decreased with the age of the seedlings in all three species. Conversely, there was an increasing trend in the absorption of zinc in the stem of the

seedlings with age. Therefore, the seedlings were successful in zinc absorption but unsuccessful in lead absorption over time. Lead absorption in the stem decreased over time, especially with increased pollution levels in all three species, and they were unable to absorb higher amounts. Regarding cadmium, no specific trend was observed. The absorption of cadmium in the stem was lower than the other two elements because the selective pollution levels in the experiments were lower compared to lead and zinc.

In comparing the absorption of three elements, *Ailanthus* has been successful in absorbing zinc in the stem, while *Acer* did not show a significant difference in lead absorption, and *Fraxinus* did not show a significant difference in zinc absorption. The effect of tree species and the region of growth on the accumulation of heavy metals such as lead, zinc, and cadmium has been confirmed in the study by Mostafavi et al. (2020). In all three species, cadmium had the lowest absorption in the seedling stems. One reason for this is the lower concentration of the solution compared to the other two elements in this study. However, cadmium is a metal that tends to accumulate more in the roots due to its lower mobility and solubility, making it less transferable to aerial parts (Jokar et al., 2017; Sharma & Sachdeva, 2015; Yan et al., 2020).

Heavy metals exhibit different behavioral patterns and mobility in trees. Lead, chromium, and copper are mainly immobilized and retained in the roots. This has significant implications for controlling the movement of heavy metals in broader environments, which is one of the primary goals of phytoremediation (Pulford & Watson, 2003; Sharma & Sachdeva, 2015; Tangahu et al., 2011).

Lead has a tendency to accumulate in the roots, and in the case of heavy pollution, it is transferred to the above-ground organs, particularly in the leaves, leading to toxicity (Aslam et al., 2021). It should also be noted that cadmium is a non-essential and toxic element, while zinc is a vital micronutrient for plants. However, high concentrations of cadmium can be toxic to plants, causing functional and structural disturbances (Askary et al., 2012; Laghlimi et al., 2015; Liu et al., 2013; Mahdavi & Khermandar, 2015; Tangahu et al., 2011).

Lead pollution primarily induces plant toxicity by inactivating enzymes and inducing physiological changes. The presence of heavy metal lead in soil can

have inhibitory effects on plant physiological and biochemical parameters. Lead can create physical barriers and disrupt root absorption physiology, thereby inhibiting the absorption of many essential ions. Lead toxicity also interferes with the uptake of other macro (calcium, magnesium, and sodium) or trace elements (iron, copper, and zinc), which are essential for plant growth (Abbasi et al., 2017; Tangahu et al., 2011).

The overall numbers related to the absorption levels of metals in plants in this study are relatively low. One reason for this could be the short duration of the experiment and the young age of the seedlings. Typically, roots accumulate higher levels of heavy metals due to their contact with the soil. This phenomenon is actually a form of plant adaptation to protect the aerial parts from toxicity. Additionally, most of these plants generally have limited ability to transfer pollutants from roots to aerial parts, which results in the sequestration of pollutants in vacuoles and root cells (Ang et al., 2010; Marques et al., 2009; Yavarian et al., 2021).

It should be noted that in this study, only the stems of seedlings were studied, and undoubtedly, adding the absorbed amounts in other plant organs (leaves, roots) will increase the total absorption of these heavy metals in the plant. The translocation of elements from the root to the shoot is one of the main pathways for the uptake of soil elements (Khodakarami et al., 2009). This issue has been addressed in most studies. In the study by Alizadeh et al. (2015), the biomass of roots, leaves, and stems decreased with increasing pollution concentration in pine seedlings, but the uptake efficiency of lead and cadmium in the tissues of the plant increased. The effect of cadmium on some physiological parameters in eucalyptus and the comparison of cadmium accumulation and translocation in the study by Hashemi et al. (2016) on *Pinus deltooides* showed that the absorption of this metal in the roots was higher than the amount in the stems and leaves. Khodakarami et al. (2009) believe that at low concentrations, lead is not accumulated in the stems of oak seedlings. Therefore, the necessity of examining the accumulation levels in other organs and comparing them with the stem is felt.

In discussions regarding phytoremediation of heavy metals, attention should be paid to plant tolerance factors, root system of plants, translocation ability from underground organs to aerial parts (translocation factor), high growth rate, and biomass. On the

other hand, the efficiency of accumulation and distribution of heavy metals in plants not only is related to plant factors but also depends on soil factors such as the accessibility of heavy metals. This accessibility is largely influenced by the nature of the metal and soil properties such as pH, organic matter, and cation exchange capacity. Thus, decreasing the soil pH can increase the bioavailability of heavy metals, including lead (Mahdavi & Khermandar, 2015). Various studies have shown that the higher the soil contamination with heavy elements, the greater the likelihood of accumulation of these elements in plants (Mahdavi & Khermandar, 2015).

The remaining amounts of lead, zinc, and cadmium in the soil of the pots related to seedlings of tree species have shown significant differences among the three species. The highest amount of residual lead in the soil in both years at different pollution levels was observed with *Fraxinus*, while the lowest was with *Ailanthus*. Similarly, the highest levels of residual zinc were observed in *Acer* and *Fraxinus*, while the lowest was in *Ailanthus*. Regarding cadmium, similar results were observed. The amount of residual lead in the soil decreased with the age of the seedlings in all three species. The decreasing trend was only observed in *Ailanthus*, while an increasing trend was observed in *Acer*. The trend for zinc and cadmium was unclear and contradictory from the first to the second year. *Fraxinus* had the highest levels of residual lead in the soil, followed by *Acer*. In terms of residual zinc, *Fraxinus* and *Acer* showed the highest levels in the soil. *Acer* had the highest residual cadmium in the soil pots. This study has shown that the amount of pollutant absorption depends on the species and the concentration consumed, as demonstrated in the study by Khodakarami et al. (2009). However, increasing phytoremediation efficiency (increasing transpiration rate, resistance, soil pH alteration, etc.) can increase absorption (Mahdavi & Khermandar, 2015).

The analysis indicated significant differences in the residual amounts of lead, zinc, and cadmium in the soil pots related to seedlings of tree species. *Ailanthus* had the highest accumulation of cadmium, lead, and zinc in the soil, followed by the sparrow's tongue. *Ailanthus*, also known as Tree of Heaven, is a highly resilient species suitable for afforestation, with a wide ecological distribution and geographic range. It is easy to cultivate and maintain, making

it cost-effective. Due to its high tolerance to various environmental conditions, *Ailanthus* is widely planted in the arid soils of Zanjan Province, where it contributes to soil remediation efforts, particularly in areas affected by industrial pollution. Therefore, considering the potential of *Ailanthus* for soil improvement in polluted environments, it could be a suitable candidate for reforestation programs in contaminated areas.

## Conclusion

This study demonstrates the potential of phytoremediation as an effective method for addressing heavy metal contamination in soils. Our research focused on the accumulation of lead, cadmium, and zinc in the stems of non-productive seedlings from three tree species: *Ailanthus altissima*, *Acer pseudoplatanus*, and *Fraxinus excelsior*. The results reveal notable differences in heavy metal absorption among these species.

Specifically, *Ailanthus altissima* showed superior efficiency in absorbing lead and zinc across varying levels of contamination. This finding positions *Ailanthus altissima* as a particularly promising species for phytoremediation applications aimed at reducing lead and zinc pollution.

The study underscores the critical role of selecting appropriate plant species for effective phytoremediation and the necessity for long-term monitoring to assess and ensure the success of remediation efforts. The observed differences in heavy metal uptake among species and variations in soil contamination levels over time highlight the complex dynamics of phytoremediation processes.

Further research is needed to explore the physiological and biochemical mechanisms underlying heavy metal uptake and tolerance in different plant species. Understanding these mechanisms will aid in developing more targeted and efficient phytoremediation strategies, ultimately contributing to the sustainable management of heavy metal-contaminated soils.

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**Author contribution** The authors contributed to this work. Their individual contributions were as follows: writing—original draft preparation, MD and MK; collecting and coding data, MD; and reviewing and editing, MD and MK. All authors have read and agreed to the published version of the manuscript.

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**Data availability** On reasonable request, data may be provided by the corresponding author.

## Declarations

**Ethical approval** None is declared.

**Conflict of interest** The authors declare no conflict of interests.

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