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Uptake and accumulation of zinc and their effects on nutrient and chlorophyll content in seedlings

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ABSTRACT

Phytoremediation is a method in which plants are used to absorb contaminants. Heavy metals significantly affect plant distributions by competing with nutrients and limiting plant growth. In this study, three-year-old seedlings of *Crataegus aronia* and *Juniperus polycarpos* were exposed to different concentrations of zinc nitrate (0, 50, 250, and 500 ppm) in a completely randomized design with three replications. Then, the amounts of zinc uptake in different organs of *C. aronia* and *J. polycarpos* seedlings were measured by atomic absorption spectroscopy after acid digestion and total chlorophyll content was measured with a spectrophotometer after extraction. The results obtained from the analysis of variance showed that in both species, zinc accumulation increased in roots and stems by elevating the heavy metal concentration and that zinc accumulation was higher in root tissues than in stem tissues in all treatments ($p < 0.05$). In addition, zinc contamination decreased the concentration of all nutrient contents in leaves and stems of seedlings, and total chlorophyll decreased by 56% in *J. polycarpos* and 47% in *C. aronia*. Accordingly, it can be said that the seedlings of *C. aronia* and *J. polycarpos* have the ability to prevent the transfer of the metal to aerial parts and to lower its toxicity to the plant by accumulating zinc in the roots. It should be noted that *J. polycarpos* was a better accumulator of zinc than *C. aronia* by 33%.

Keywords: *Crataegus Aronia*, Heavy metals, *Juniperus Polycarpos*, Phytoremediation
Article type: Research Article.

INTRODUCTION

There are global concerns regarding heavy metal-induced pressures on soil ecosystem since past decades due to increasing urban, industrial and agricultural activities. Threat linked with heavy metals with respect to natural environment and humans is a function of their mobility and bio-accessibility (Kaur *et al.* 2024). The contamination of soil with heavy metals is a serious environmental threat and one of the most pressing environmental problems in the world (Xu *et al.* 2019). Heavy metals are highly toxic and their high concentrations in the soil can damage plant growth and reproduction (Ackova 2018). Zinc is an essential component for plants, but, in excessive amounts, it can cause toxicity (Kumar *et al.* 2017). The distribution of contaminants in a plant is determined by the physiological character of the plant. Some plants tend to accumulate contaminants in certain organs. The distribution depends on the mobility of the contaminant in the plant tissues, the type of plant, and the conditions of its growth (Štofejová *et al.* 2021). Most plant species cannot adapt to a high level of heavy metal content, but some plants survive, grow, and reproduce in soils contaminated with heavy metals. Vast majorities of these species tolerate heavy metal concentrations and retain most of the heavy metals in the roots with minimal translocation to the leaves. So, they can be used for soil remediation that is called "phytoremediation". Phytoremediation has some benefits including: Ecologically sound and sustainable, commercially viable, and various contaminants remediation (Fig. 1; Islam *et al.* 2024). Hyperaccumulators show the opposite behavior concerning the absorption and the distribution of heavy metals in the plant (Hall *et al.* 2021). Their activity consists of an active uptake of large amounts of heavy metals from the soil. Heavy metals are not retained in the roots, but translocated to other parts of the plant. Despite high concentrations of heavy metals, they do not show phytotoxicity (Kozhevnikova *et al.* 2017). Some studies reported that zinc has effect on photosynthetic rate and nutrient balance in plants. Some similar literature are as follows: the research on different concentrations of zinc on nutrient acquisition traits of basil (Tolay 2021), the effect of zinc on growth rate, chlorophyll, and mineral contents of mungbeans plant (Samreen *et al.* 2017), zinc influence and salt stress on photosynthesis (Tavallali 2009), the effect of zinc on growth and shoot concentrations of sodium and potassium in pepper plants (Aktaş *et al.* 2006), and the effect of zinc levels on growth, mineral composition, and biochemical changes of broad bean (Saleh *et al.* 2009).

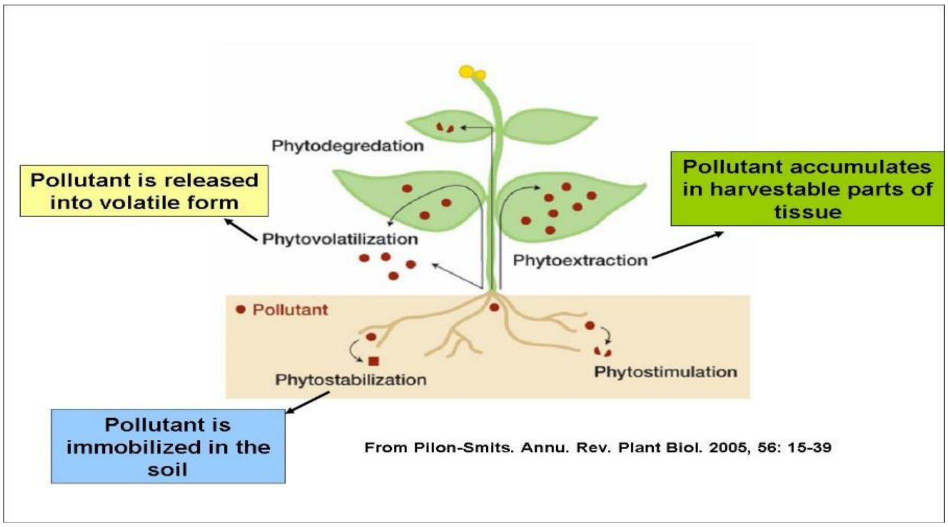

Fig. 1. Phytoremediation using green plant engineering (Sergeevna 2021).

The current research sought to assess the ability of three-year-old seedlings of *Crataegus aronia* and *Juniperus polycarpos* to store and transfer zinc in various parts, as well as the impact of zinc accumulation on nutrient and chlorophyll levels, to explore the plants defence mechanisms against zinc toxicity. Our hypothesis posited that roots have a greater ability to absorb zinc than stems, and that zinc-induced heavy metal stress affects nutrient uptake differently in the *C. aronia* and *J. polycarpos* seedlings, with varying effects between the two species. Our study focused on zinc absorption and resistance in these species to assess their potential for soil reclamation and decontamination in heavy metal-contaminated areas.

MATERIALS AND METHODS

Three-year old seedlings of *Crataegus aronia* and *Juniperus polycarpos* were selected from Kuchkan nursery, Zanjan Province, Iran. In early April, the plants were planted in black plastic pots (diameter = 15 cm, depth = 25 cm) filled with a 1:1:2 mixture of soil, dried animal manure, and sand which had been sifted with a 4-mm sieve (soil weight per filled pot was 2 kg). The experiments were conducted at the nursery of Department of Natural Resources and Watershed Management, Zanjan Province. After drying the soil, it was passed through a 2-mm sieve to remove waste, and physical and chemical properties of the soil were determined through standard laboratory methods (Table 1).

Table 1. Physical and chemical characteristics of the soil.

Properties of control soil	pH	Total nitrogen (%)	Saturation (%)	Organic carbon (%)	Zinc (ppm)	Soil texture			EC ds m^{-1}
						Sand (%)	Silt (%)	Clay (%)	
Pot soil	7.83	0.06	31	1.04	1.38	64	27	9	5.55

The seedlings were sprayed with zinc nitrate. In order to prepare the solution, zinc nitrate was dissolved in distilled water and concentrations of 0, 50, 250 and 500 ppm (T_0, T_{50}, T_{250} and T_{500}) were prepared. Spraying was carried out with the aid of a simple sprayer device. The number of potted seedlings needed for four treatments in three replications was 72 for each species. The total of 144 seedlings used in this study were placed in a sheltered location to control for the effects of possible rainfall as a confounding variable and their care and irrigation were carried out on a continuous basis according to the calculated crop capacity (60% of crop capacity) until the end of the experiment (Khodakarami *et al.* 2009). The zinc nitrate solution was sprayed in mid-June and 100 mL of each concentration was sprayed on each potted seedling in three rounds during seven days. The uptake of zinc in roots, stems and leaves was measured. The samples to be dried were put in an oven at 70 °C for 48 hours and then weighed with a digital scale with an accuracy of 0.001 g. For metals digestion, 0.1 g of the powdered sample was removed from each organ (stem, leaf and root) and mixed with 65% mixture of nitric acid, concentrated sulfuric acid, and perchloric acid in a ratio of 8:2:1 for 24 hours for *in vitro* wet oxidation. The samples were digested at 120 °C for 30 min. After the samples were cooled, distilled water was added to bring the volume to 50 mL. Then, zinc concentration was measured by a GBC P Avanta atomic absorption apparatus, USA (Vicentim & Ferraz 2007; Zaefarian *et al.* 2011). To measure nutrient concentrations, the seedlings were cut at the collar after the growing season. After washing with distilled water, leaves and stems were cut and dried in oven at 70 °C. Biomass was recorded for each organ. Dried samples were powdered and 0.5 g of the powder was taken to measure nutrient concentrations. The amounts of phosphorus, potassium and nitrogen were measured by the wet digestion method using SPECTRONIC 21D, Flame photometer 410, and KjeltecTM2100, respectively. Iron and copper were evaluated by dry digestion method followed by measuring absorbance with an atomic absorption spectrometer (GBC P Avanta atomic absorption, USA; Vicentim & Ferraz 2007; Zaefarian *et al.* 2011). In addition, total chlorophyll content was measured by the Arnon method (1949) using an ultraviolet spectrophotometer after extraction with acetone (Arnon 1949). All statistical analyses were performed in SPSS 20.

RESULTS

The results of ANOVA test revealed that there was a significant difference between treatments in terms of zinc uptake in stems, leaves and roots of *Crataegus aronia* and *Juniperus polycarpos* ($p<0.05$; Table 2). Comparing zinc uptake in different organs of *J. polycarpos* showed that there was a significant difference between treatments and control in stems and roots ($p<0.05$). Significant difference was found in the leaves between the 250 ppm-treatment (T_{250}) and the control (T_0; $p<0.05$). In the case of zinc uptake in organs of *C. aronia*, uptake varied significantly between all treatments ($p<0.05$). In the roots of *C. aronia*, transport rate decreased and maximum accumulation occurred as the concentration of contaminant increased (Table 2).

Table 2. Comparison of average zinc uptake by *C. aronia* and *J. polycarpos* seedlings.

Seedling	Treatments (ppm)	Evaluation factors (ppm)		
		Root	Leaf	**stem**
J. polycarpos	Control	7.80 ± 0.20 [a]	14.80 ± 0.10 [a]	12.03 ± 0.15 [a]
	50	50.38 ± 3.57 [b]	15.60 ± 2.52 [a]	20.19 ± 4.24 [b]
	250	58.34 ± 7.44 [c]	40.33 ± 2.08 [b]	43.96 ± 3.43 [c]
	500	171.10 ± 3.57 [d]	44.23 ± 2.13 [c]	48.63 ± 2.09 [d]
C. aronia	Control	6.23 ± 0.37 [a]	8 ± 0.62 [a]	7 ± 1.47[a]
	50	13.53 ± 0.40 [b]	19 ± 0.75[b]	21.06 ± 0.35 [b]
	250	37.93 ± 0.56 [c]	21.33 ± 0.40 [c]	76.28 ± 0.90 [c]
	500	112.73 ± 5.74 [d]	25.13 ± 2.17[d]	35.93 ± 2.25[d]

Note: Similar letters in each row indicate no significant difference between the numbers at the 5% level. Data are presented as mean ± SD of three replications.

In this study, the effect of zinc on the uptake of other nutrients by the *C. aronia* and *J. polycarpos* seedlings was investigated. Concentrations of nitrogen, phosphorus, potassium, iron, and copper in the leaves and stems were measured. According to the results, zinc inhibited the natural process of nutrient uptake by seedlings of both species. The highest nutrient uptake was found in the stems and leaves of both species in the control treatment while the lowest at the T_{500} (Tables 3 and 4). At high levels of contamination (T_{250} and T_{500}), there was no significant difference in Cu uptake in the leaves of *J. polycarpos* seedlings. Similarly, there were no significant differences between T_{250} and T_{500} for any nutrient in *C. aronia* seedlings ($p<0.05$). At low exposure concentration (T_{50}), no significant difference was observed between zinc treatments and control in terms of K uptake in *J. polycarpos* seedlings ($p<0.05$). In addition, in the cases of nitrogen, iron, and copper, no significant differences were observed in *C. aronia* seedlings (Table 3). In terms of phosphorus, iron, and copper uptakes in the stem, there was no significant difference among *J. polycarpos* seedlings ($p < 0.05$). In the case of *C. aronia* seedlings, only iron uptake did not exhibit a significant difference ($p < 0.05$). At high contamination levels (T_{250} and T_{500}), there was no significant difference in the uptake of P, Fe, and Cu in the *J. polycarpos* seedling stems ($p < 0.05$). Similarly, for *C. aronia* stems, nutrient uptake did not display any significant difference at high contamination levels, except for nitrogen ($p < 0.05$). In the low concentration treatment (T_{50}), no significant difference was observed between treatment and control in the *J. polycarpos* stems. However, it was not true for *C. aronia* seedlings (Table 4). As shown in Table 5, treatment with zinc reduced chlorophyll content in *C. aronia* and *J. polycarpos* seedlings. ($p < 0.05$). Significant differences were observed in both species under treatments, but there was no significant difference between treatments ($p < 0.05$). Also, total chlorophyll decreased by 56% in *J. polycarpos* and 47% in *C. aronia.*

Table 3. Effect of zinc on uptake of nutrients in leaves of *C. aronia* and *J. polycarpos* seedlings.

Species type	**Nutrients (g kg^{-1})**	**Zinc concentration (ppm)**			
		0	50	250	**500**
J. polycarpos	Nitrogen (N)	28.30 ± 0.66 ^{a}	19.10 ± 0.40 ^{b}	15.90 ± 0.25 ^{c}	11 ± 0.11 ^{d}
	Phosphorus (P)	1.20 ± 0.05 ^{a}	1 ± 0.05 ^{b}	0.80 ± 0.02 ^{c}	0.49 ± 0.01 ^{d}
	Potassium (K)	9.80 ± 0.23 ^{a}	6.70 ± 0.20 ^{a}	6.30 ± 0.15 ^{b}	5.10 ± 0.15 ^{c}
	Iron (Fe)	33 ± 1 ^{a}	30.20 ± 0.25 ^{b}	15.70 ± 0.40 ^{c}	12.20 ± 1.06 ^{d}
	Copper (Cu)	5.80 ± 0.15 ^{a}	3.90 ± 0.20 ^{b}	3.20 ± 0.10 ^{c}	2.90 ± 0.11^{c}
C. aronia	Nitrogen (N)	10.10 ± 0.26 ^{a}	10 ± 0.95 ^{a}	8.90 ± 0.10 ^{b}	8 ± 0.45 ^{b}
	Phosphorus (P)	1.20 ± 0.26 ^{a}	0.80 ± 0.02 ^{b}	0.60 ± 0.03 ^{bc}	0.50 ± 0.01 ^{c}
	Potassium (K)	10.30 ± 0.10 ^{a}	1.20 ± 0.10 ^{b}	1.10 ± 0.10 ^{bc}	1 ± 0.10 ^{c}
	Iron (Fe)	12 ± 1 ^{a}	11.70 ± 026 ^{a}	11 ± 0.65 ^{ab}	10 ± 0.26 ^{b}
	Copper (Cu)	4 ± 0.10 ^{a}	3.80 ± 0.10 ^{a}	3 ± 0.30 ^{b}	2.56 ± 0.55 ^{b}

Note: Similar letters in each row indicate no significant difference between numbers at 5% level. Data are presented as mean ± SD of three replicates.

Table 4. Effect of zinc on uptake of nutrients by stems of *C. aronia* and *J. polycarpos* seedlings.

Species type	***Nutrients (g kg^{-1})***	***Zinc concentration (ppm)***			
		0	**50**	**250**	***500***
J. polycarpos	Nitrogen (N)	7.10 ± 0.88 ^{a}	7.20 ± 0.26 ^{a}	6.90 ± 0.17^{a}	*5.46 ± 0.40 ^{b}*
	Phosphorus (P)	0.70 ± 0.26 ^{a}	0.69 ± 0.01 ^{a}	0.60 ± 0.01 ^{a}	*0.50 ± 0.02 ^{a}*
	Potassium (K)	3.20 ± 0.43 ^{a}	3 ± 0.01 ^{a}	2.90 ± 0.01 ^{a}	*2.10 ± 0.10 ^{b}*
	Iron (Fe)	8 ± 0.87 ^{a}	7.80 ± 0.26^{ab}	7.60 ± 0.10 ^{ab}	*7 ± 0.26 ^{b}*
	Copper (Cu)	2.20 ± 0.69 ^{a}	2 ± 0.26^{a}	1.70 ± 0.20 ^{a}	*1/60 ± 0.20 ^{a}*
C. aronia	Nitrogen (N)	10.10 ± 0.17 ^{a}	9.40 ± 0.20 ^{b}	9 ± 0.10 ^{c}	*8 ± 0.17 ^{d}*
	Phosphorus (P)	1.20 ± 0.10 ^{a}	1 ± 010 ^{b}	0.90 ± 0.01^{bc}	*0.80 ± 0.09 ^{c}*
	Potassium (K)	3.30 ± 0.17 ^{a}	3 ± 0.17 ^{b}	2.60 ± 0.17 ^{c}	*2.50 ± 0.10 ^{c}*
	Iron (Fe)	10 ± 1.73 ^{a}	9.80 ± 0.45 ^{b}	9.50 ± 0.10 ^{c}	*8.70 ± 0.55 ^{c}*
	Copper (Cu)	3.90 ± 0.26 ^{a}	3.60 ± 0.10 ^{b}	3 ± 0.10 ^{c}	*2.90 ± 0.10 ^{c}*

Note: Similar letters in each row indicate no significant difference between numbers at 5% level. Data are presented as mean ± SD of three replicates.

Table 5. Comparison of mean total chlorophyll content in *C. aronia* and *J. polycarpos* seedlings.

Treatment	***Concentration (ppm)***	***Species***	
		C. aronia	***J. polycarpos***
zinc	Control (0)	0.73 ± 0.15 ^{a}	*2.46 ± 0.41 ^{a}*
	50	1.56 ± 0.26 ^{b}	*1.32 ± 0.24 ^{b}*
	250	1.35 ± 0.32 ^{b}	*1.12 ± 0.18 ^{b}*
	500	1.14 ± 0.14 ^{b}	*1.08 ± 0.06 ^{b}*

Common letters indicate no significant difference. Data are shown as mean ± SD of three replicates.

DISCUSSION

Plants have three basic strategies for growing in soils contaminated with heavy metals. These strategies include: Metal excluder: These plants prevent metals from entering the aerial parts. Metal indicators: Species that actively accumulate metals in aerial tissues and generally reflect the surface of metals in the soil. Metal accumulators: These plants can concentrate metals in their aerial parts to levels higher than those in the soil. Most hyperaccumulators absorb high levels of contaminants and concentrate them in their roots, stems, or leaves. Phytoremediation can be employed as a sustained, eco-friendly and cost-effective technology to rehabilitate toxic pollutants using green plants (Khan *et al.* 2023). The overall objective of any soil remediation approach is to develop a solution that is protective of human health and the environment (Martin & Ruby 2004). In this study, zinc concentrations in roots, stems and leaves increased in both species under all treatments. Maximum concentration of zinc in roots, stems, and leaves was observed in T_{500}. We found higher zinc concentrations in roots similar to the results of Baum *et al.* (2006) on *Salix dasyclados*. Pulford & Dickinson (2003) stated that compared to other heavy metals, zinc shows a stronger tendency to accumulate in the roots. In a study on grey mangrove in Australia, MacFarlane *et al.* (2003) reported that zinc uptakes in roots and leaves were 295 mg g^{-1} and 25 mg g^{-1}, respectively. HeeHan *et al.* (2010) studied the zinc and cadmium accumulation in *Salix caprea*, reporting that the highest accumulations of both metals were found in the roots. Stabilization and accumulation of heavy metals in the roots and prevention of transfer to the stem are among the mechanisms used by plants to neutralize the toxicity of heavy metals. This solution protects organs involved in plant metabolism from heavy metal damage (Erenoglu *et al.* 2011).

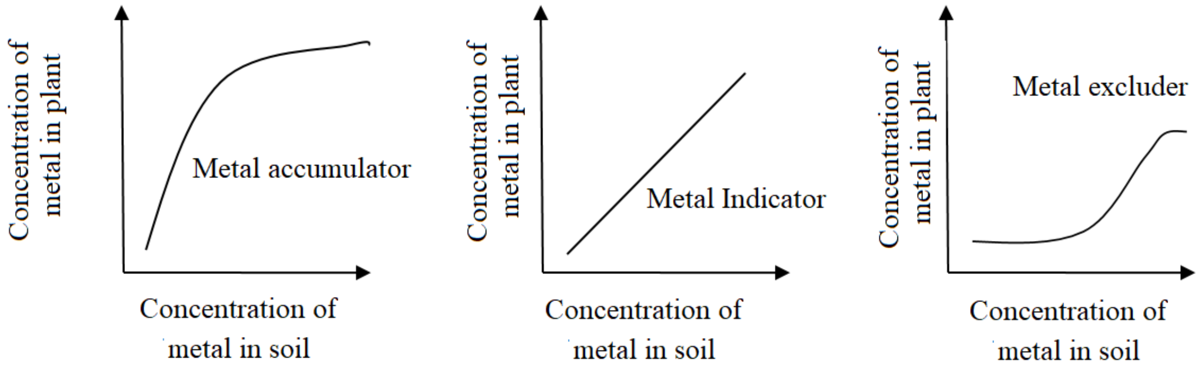

Fig. 2. Plant reaction in relation to increasing the concentration of total metals in the soil (Sergeevna, 2021).

In the present study, zinc concentrations were higher in stems than in leaves at all levels of zinc treatment. In a study by Mrnka *et al.* (2012), by examining *S. alba* and *S. nigra* seedlings grown in heavy metal-contaminated soil (often cadmium, lead, and zinc), reported that zinc accumulation was higher in stems than in leaves. In general, greater heavy metal accumulation in the stem rather than in leaves is critical since the stem plays a less significant role in metabolism. Consequently, the damage to the plant's physiological functions is reduced. Another critical impact of heavy metals is their interaction with nutrients. Therefore, understanding these relationships is a public concern (Liu *et al.* 2003). The results of this study revealed that increasing levels of zinc contamination reduced nitrogen uptake by *Crataegus aronia* and *Juniperus polycarpos* seedlings. This decrease was significant at some levels of contamination and non-significant at others. A decreased nitrogen uptake with increasing levels of contamination has been reported in numerous studies on other plants. However, Arduini *et al.* (2006), Yang *et al.* (2007), Zaefarian (2011), and Kapusta & Godzik (2013) reported that heavy metals increased nitrogen uptake by the plant, which is not in line with the results of this study. The second most-consumed element was phosphorus, which is presented in Tables 3 and 4. Phosphorus uptake decreased in both species in the presence of zinc. By increasing zinc contamination to 500 ppm, phosphorus uptake reduced by 60% and 58% in leaves of *J. polycarpos* and *C. aronia*, respectively. Yang *et al.* (2007) reported a decrease in phosphorus uptake in the presence of a heavy metal. Karimi *et al.* (2013) reported that changes in phosphorus uptake in the presence of heavy metals were not significant, which is not in according with the results of this study. Measurements of potassium in the leaves and stems of *C. aronia* and *J. polycarpos* showed that zinc had an inhibitory impact on potassium uptake. Zinc contamination at 500 ppm decreased leaf potassium content by 48% and 90% in *C. aronia* and *J. polycarpos*, respectively. Decreased potassium uptake under heavy metal stress has been reported in other plant species (Zornza *et al.* 2002; Zaefarian *et al.* 2011; Karimi *et al.* 2013). Iron is a key micronutrient for plants and its deficiency can impair plant metabolism. Many studies have shown that heavy metals cause iron deficiency in plants (Siedlecka & Baszynki 1993; Zaefarian 2011; Schmidt *et al.* 2020). Measurements in this study revealed that zinc stress reduced iron uptake in the stems and leaves, which was significant only at some levels of contamination. Similarly, zinc stress decreased the ability of plants to absorb copper (Zaefarian *et al.* 2011). Increasing zinc concentration up to 500 ppm decreased copper uptake by 50% and 36% in leaves of *C. aronia* and *J. polycarpos* respectively. Heavy metals inhibit nutrient absorption in different ways including competition, effects on root membrane (such as effect on proteins and membrane peroxidation), effects on ATP and other carriers, reduced root respiration (which reduces active transport of elements), and damage to the root and delaying its growth (which eventually results in impaired root cell permeability; O'Lexy *et al.* 2018). The mechanisms of interactions between heavy metals and nutrients are not well known. Because plant vitality is one of the key components of the success of phytoremediation projects, information on changes in nutrient content under the influence of heavy metals can guide appropriate nutrient retention practices, and thus significantly contribute to phytoremediation projects. The results of this study indicated that zinc treatment decreased total chlorophyll content. This decrease can be explained by the fact that heavy metals reduce metabolic processes by inhibiting enzymes. The reduction in chlorophyll content due to heavy metal stress can be the result of inhibition of the enzymes responsible for chlorophyll biosynthesis (Zengin & Munzuroglu 2005). The results of this study are similar to previous works, which reported a drop in photosynthetic pigments such as total chlorophyll in response to heavy metals such as zinc (Van Assche & Clijsters 1990). Bonnet *et al.* (2000) studied ryegrass (*Lolium perenne* L.) and found that excessive amounts of zinc decrease chlorophyll synthesis. Replacing the central magnesium ion in chlorophyll by heavy metals is another effect of heavy metal damage which prevents trapping of light, thus destroying chlorophyll and decreasing photosynthetic activity (Prasad & Strazalka 1999). High concentrations of zinc in soil led to higher concentrations of the metal in the *C. aronia* and *J. polycarpos* seedlings and reduced total chlorophyll content. It should be noted that *J. polycarpos* was a better accumulator of zinc than *C. aronia* by 33%. It should be noted that the accumulation of zinc was higher in the roots of *J. polycarpos* than *C. aronia*. Although both species can adsorb zinc in nature, they are more appropriate choice for zinc absorption, especially *J. polycarpos*. In addition, the survival of all seedlings of both species shows the remarkable capability of the species in phytoremediation. Undoubtedly, complementary studies with higher zinc nitrate treatments over longer study periods can help with making better decisions. Present findings confirm the phytoremediation ability of both experimental species that can be a sustainable environmental technique thereby combining the advantages of low-cost nature-based and eco-friendly solutions in order to eradicate the zinc pollution problem from the soils around the mines in the studied area and elsewhere.

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