



Lead Uptake and Its Effects on the Quantitative Indices of *C. aronia* and *J. polycarpus* Seedlings in the Environment

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ABSTRACT

Background: Removal of mineral pollutants from polluted soil is considered to be major environmental concern in the modern era. The present study aimed to investigate the capability of lead uptake and transfer in various organs of *Crataegus aronia* and *Juniperus polycarpus* seedlings and determine the effects of lead accumulation on some of the quantitative indices of these plant species.

Methods: Three-year-old saplings were exposed to various concentrations of lead (zero, 200, 300, and 400 ppm) (NO_3)₂ in the sprouting period in a completely random project in triplicate. Afterwards, the lead uptake value was measured in various organs of *C. aronia* and *J. polycarpus* (roots, stems, and leaves) using an atomic absorption device.

Results: The analysis of variance indicated that the features of *C. aronia* and *J. polycarpus* saplings (e.g., stem length, basal diameter, and root length) were significantly influenced by lead contamination ($P < 0.05$). Moreover, the saplings of both varieties had high capacity for lead uptake in their roots, stems, and leaves.

Conclusion: According to the results, the seedlings of *C. aronia* and *J. polycarpus* could be used as stabilizing varieties for the removal of lead from polluted soil. It is also notable that *J. polycarpus* is a more effective lead absorbent compared to *C. aronia*.

1. Introduction

Presence of heavy metals in soil not only endangers various animal and plant species, but it also leads to the inefficiency of a large portion of land soil. Phytoremediation is the process through which plants are used to remove, decrease, transfer or stabilize soil pollution and is considered to be a highly effective, economical approach in this regard [1]. In total, 57 elements are classified as heavy metals, the density of which is more than 5 g/cm³. Heavy metals are among the most alarming pollutants in industrial areas worldwide [2].

Lead is a heavy metal that could enter the ecosystem through a wide range of resources; for instance, the gas fuel of automobiles is a major source of lead. Moreover, considerable amounts of lead are found in the effluents of

various industries, such as dyeing and textile industries. Military industries and petrochemical plants are also the other leading sources of lead [3,4].

According to the data of the Conservation of Environment Agency [5], lead is the most important heavy metal pollutant in the environment. Some of the main adverse effects of lead on plants include the reduction of the germination, length, diametric growth, and biomass of various dried plant parts [6].

Recent studies have applied plant species to remove heavy metals (e.g., zinc, manganese, and lead) from polluted natural resources. Some plants that have proven effective in this regard include *Brassica juncea*, *Ambrosia artemisiifolia*, *Thlaspi caerulescens*, and *Helianthus* [7]. However, data is scarce regarding the use of arboreal species for such purposes, and a general literature search indicates that

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several related studies have been mainly focused on the Salicaceae family [8].

Although plant species have been extensively applied for the removal of soil pollutants, the roots of most of these species have limited depth, and their phytoremediation is limited to shallow depths [9]. As such, arboreal species with high resistance and deep root development systems may be proper alternatives in some areas [10].

Several studies have been focused on phytoremediation using wooden species, some of which include white poplar (*Populus alba L.*) in the soil polluted with lead Salehi et al. (2014); Acacia (*Acacia victoriae Benth*) for the removal of lead and zinc by Mahdavi and Khermendar (2015); and Avicennia (*Avicennia marina [Forks]*) for the removal of lead and nickel by Mansouri et al. (2015) [11-13].

The present study aimed to investigate the capacity of three-year-old seedlings of *Crataegus aronia* and *Juniperus polycarpus* for the collection and transfer of lead to their organs, determine the effects of lead concentration on the quantitative indices of these plant species, and evaluate their mechanisms against lead toxicity. Considering the specific system of these species, the hypothesis of the study was that the roots of the plants have higher capacity for lead uptake compared to their aerial organs.

In Iran, *C. aronia* and *J. polycarpus* are used in forest development and enrichment activities (Iran-Turonian region). However, their potential for phytoremediation has not been experimented yet. This was the first study to examine their resistance potential to lead toxicity in soil in their sapling period.

2. Materials and Methods

To carry out the experiments, three-year-old varieties of *C. aronia* and *J. polycarpus* were obtained from the plant center of Koushkan, located in Zanjan province, Iran. The obtained samples are among the important forest varieties available in Iran-Turonian region. In early April, the plant species were planted in black plastic pots (size: 15 * 25 cm) in the Nursery of the Natural Resources and Watershed Management Office of Zanjan Province at the ratio of 2:1:1 (crop soil, dried animal fertilizer, and sand) after filtering through a four-millimeter sieve. The soil weight in each of the filled pots was two kilograms. After the drying of the soil, a two-millimeter sieve was used to remove the lesions, and some of the physical and chemical properties of the soil were determined using standard laboratory methods (Table 1).

At the next stage, lead nitrate was dissolved in distilled water in order to prepare a solution, and the concentrations of zero, 200, 300, and 400 ppm were considered as the treatment doses. Following that, the three-year-old seedlings were sprinkled with the obtained solution, and solution sprinkling was performed using a simple spray device. This method was selected for the simulation of rainfall with these pollutants. Solution sprinkling was carried out in the middle of May, and 100 milliliters of each concentration was sprinkled on each pot three times at seven-day intervals.

Some of the quantitative indices of the saplings were measured, such as the root length, stem length, and basal diameter (control samples and other treatments), in millimeters using a digital caliper with the accuracy of 0.01 at the end of the sprouting period. Afterwards, the lead uptake value was measured in the roots, stems, and leaves of the plants. The samples were placed in an oven at the temperature of 70 °C for 48 hours to be dried and weighed afterwards using a digital scale with the accuracy of 0.001.

At the next stage, 0.1 gram of the powdered samples of each organ (stems, leaves, and roots) was separated and mixed with 65% nitric acid, dense sulfuric acid, and perchloric acid (ratio: 1:2:8) using the wet oxidation method and preserved in laboratory conditions for 24 hours. Following that, the samples were digested at the temperature of 120 °C for 30 minutes. After becoming cold, the samples were distilled with water twice (volume: up to 50 ml), and the lead density value was measured using the GBC atomic absorption device (model: Avanta P, made in Australia).

The obtained data were analyzed using completely randomized methods. The number of the potted seedlings for the mentioned treatments in triplicate was 36 per each species. In total, 72 potted seedlings were used in the study, all of which were placed in a protected location, treated, and irrigated continuously based on the calculated farming capacity (60% of farming capacity) until the end of the experiments. The protected location was selected so as to prevent and control the effects of possible rainfall as an intervening factor.

Data analysis was performed in SPSS version 20 using one-way analysis of variance (ANOVA) and comparison of means based on the least significance difference (LSD) test at the significance level of 5%.

3. Results and Discussion

The results of ANOVA at the significance level of 5% ($P < 0.05$) indicated significant differences in the lead uptake between different stem lengths, root lengths, and basal diameters of *C. aronia* and *J. polycarpus* (Table 2). Furthermore, the results of the comparison of means regarding the lead uptake value in various organs of *J. polycarpus* demonstrated that the uptake value in stems had a significant difference at the three treatment concentrations compared to the control samples ($P < 0.05$). However, no such difference was observed in the leaf and root lead uptake of *J. polycarpus* at the treatment concentration of 200 mg/kg compared to the controls ($P > 0.05$). With regard to the leaves of the studied plant species, no significant difference was observed between the treatment concentrations of 200 and 300 ppm in terms of lead uptake ($P > 0.05$) (Table 4).

Comparison of the mean evaluated features of *C. aronia* and *J. polycarpus* in terms of the land and aerial organs indicated a significant difference between various treatment concentrations (200, 300, and 400 ppm) as opposed to the control samples (Tables 5 & 6).

Table 1: Physical and Chemical Properties of Control Soil Used in Research

Features of control soil	Texture of soil			Pb ppm	Organic carbon (%)	Total nitride (%)	PH	EC Ds/m
	Clay (%)	Silt (%)	Sand (%)					
Soil in vase	9	27	64	0.768	1.04	0.061	7.83	5.55

Table 2: Variance Analysis of Some Morphological Parameters in Lead Uptake Level by *J. polycarpus* and *C. aronia* Saplings

Species	The sources of changes	Degree of freedom	Mean Square					
			Stem length	Basal diameter	Root length	Uptake in stem	Uptake in leaf	Uptake in root
<i>J. polycarpus</i>	Treatment	3	753.556*	6535.639*	5.202*	402.046*	130.943*	8905.221*
	Error	8	1.75	53.250	0.012	2.673	9.642	46.139
<i>C. aronia</i>	Treatment	3	2606.306*	2308.750*	6.481*	300.220*	166.810*	1677.331*
	Error	8	1.500	39.333	0.103	6.500	0.744	12.444

*Significant at probability level of 0.05

Table 3: Comparison of Mean Lead Uptake by *J. polycarpus* Saplings

Treatment (mg/kg)	Parameters of evaluation		
	Stem length	Basal diameter	Root length
Control	0.733 ± 0.082 ^a	0.767 ± 0.882 ^a	2.933 ± 2.2341 ^a
200	4.867 ± 0.4410 ^b	3.433 ± 0.4055 ^{ab}	12.800 ± 1.5308 ^a
300	17.133 ± 0.5925 ^c	8.233 ± 0.4372 ^b	90.133 ± 4.1127 ^b
400	26.100 ± 1.7349 ^d	15.800 ± 3.5346 ^c	111.400 ± 6.2132 ^c

*Same letter in every column shows lack of significant difference

Therefore, it could be concluded that increased treatment concentration resulted in adverse effects on the growth of various organs of these plant species. Moreover, the comparison of lead uptake levels by various organs of *C. aronia* demonstrated that the lead uptake in the stems, leaves, and roots of this species had a significant difference only at the treatment concentrations of 300 and 400 ppm compared to the control samples ($P < 0.05$) (Table 7). On the other hand, the assessment of the pollutant levels that were transferred through the leaves, stems, and roots of the species indicated that the increase level of the pollutant decreased its transfer, while the highest accumulation level was observed in the roots (Tables 3 & 7).

Various species and sub-species of plants have different capacity and adaptation in the face of environmental tensions, such as contamination with heavy metals [14]. In this regard, the present study was focused on the length and diametric growth of the *J. polycarpus* and *C. aronia* seedlings as the main growth indices. According to the obtained results, the length and diametric growth and root growth of the *J. polycarpus* and *C. aronia* species reduced at the low-to-medium solution concentrations, while in the treatment with the highest pollution concentration (400 ppm), both species showed higher reduction compared to the control samples. These findings are consistent with the studies by Abbasi et al. (2015) conducted on the *Fraxinus excelsior* and *Acer cappadocicum* species [15].

In the current research, lead salt had no positive effects on the growth of the seedlings, and its adverse effects in this regard were confirmed at high concentrations. In contrast, Begonia et al. (1998) and Kadukova et al. (2008) reported the positive effects of lead on the growth and biomass of *Brassica juncea L.* and *Tamarix smyrnensis Bunge*, respectively [9,16]. On the other hand, some studies have reported the adverse effects of higher lead concentrations on the growth of *Acacia farnesiana* [13].

According to the results of the present study, lead concentration in all the organs of both species was higher compared to the control samples, and the concentration

increased significantly at the higher concentrations compared to the controls. The highest levels of lead in the roots, stems, and leaves were also observed at the lead concentration of 400 ppm. On the other hand, the highest lead concentration was observed in the roots of the plants, so that the level of lead in the roots of *J. polycarpus* at the concentration of 400 ppm was seven and 4.2 times higher than the lead levels in the leaves and stems of this species, respectively.

In the roots of *C. aronia*, the lead levels were observed to be three and 2.2 times higher than the leaves and stems, respectively. Higher lead concentration in the roots compared to the other plants organs has also been reported by Salehi et al. (2014) in *Populus alba*, Kamalpour et al. (2012) in *Eucalyptus camaldulensis*, Baum et al. (2006) in *Salix dasyclados*, Borisev et al. (2009) in *Salix alba*, *S. nigra*, and *S. matsudana*, and Bissonnette et al. (2010) in *S. viminalis* and *Populus × generosa*. Furthermore, Pulford and Dickinson (2005) reported that compared to other heavy metals, lead could accumulate more in the roots of plants. In fact, the stabilization and accumulation of heavy metals in plant roots and its prevention from reaching the aerial organs due to the sequestration of metal pollutants in the cells and vacuoles of roots is a mechanism used by some plants to confront the toxicity caused by heavy metals. This mechanism protects plant organs and metabolism of plants against the damage caused by heavy metal contamination [11,17-21].

According to the findings of the current research, lead concentration in the stems of the assessed plant species was higher compared to their leaves at all the treatment concentrations of lead, which is in congruence with the findings of Arriagada et al. (2005) in the *Eucalyptus globulus* saplings planted in lead-polluted soil [22]. Furthermore, this finding is consistent with the study by Mrnka et al. (2012) on *Salix alba* and *Salix nigra* saplings planted in soil polluted with heavy metals (often cadmium, lead, and zinc) [10].

Table 4: Comparison of Mean Morphological Parameters in *J. polycarpus* Seedlings

Treatment (mg/kg)	Parameters of evaluation		
	Stem length	Basal diameter	Root length
Control	38.67 ± 1.202 ^a	3.400 ± 0.1000 ^a	109.33 ± 7.839 ^a
200	10.33 ± 0.667 ^b	0.0577 ± 1.200 ^b	22.33 ± 2.4047 ^b
300	9.00 ± 0.577 ^b	1.033 ± 0.0333 ^b	21.000 ± 1.732 ^{bd}
400	3.33 ± 0.333 ^c	0.367 ± 0.0333 ^c	7.67 ± 0.882 ^d

*Same letter in every column shows lack of significant difference

Table 5: Comparison of Mean Morphological Parameters in *C. aronia* Seedlings

Treatment (mg/kg)	Parameters of evaluation		
	Stem length	Basal diameter	Root length
Control	76.67 ± 0.882 ^a	4.000 ± 0.2887 ^a	80.33 ± 6.119 ^a
200	29.33 ± 0.882 ^b	2.200 ± 0.1528 ^b	51.67 ± 2.963 ^b
300	29.00 ± 0.577 ^b	2.167 ± 0.1667 ^b	49.67 ± 2.333 ^b
400	6.67 ± 0.333 ^c	0.400 ± 0.0577 ^c	12.67 ± 0.882 ^c

*Same letter in every column shows lack of significant difference

Table 6: Comparison of Mean Lead Uptake by *C. aronia* Seedlings

Treatment (mg/kg)	Parameters of evaluation		
	Stem length	Basal diameter	Root length
Control	0.033 ± 0.333 ^a	0.733 ± 0.1453 ^a	0.300 ± 0.3000 ^a
200	2.933 ± 0.7638 ^a	1.867 ± 0.3528 ^a	5.867 ± 1.3445 ^a
300	10.600 ± 0.7638 ^b	8.100 ± 0.8622 ^b	30.367 ± 3.7790 ^b
400	22.433 ± 2.8050 ^c	17.000 ± 0.3786 ^c	51.600 ± 0.6429 ^c

*Same letter in every column shows lack of significant difference

4. Conclusion

Higher concentration of heavy metals in plant stems compared to their leaves is considered important for two reasons. First, heavy metal accumulation occurs in the plant organs that are less involved in their metabolism, which in turn decreases the subsequent damage affecting the morphological features of plants. Second, heavy metal accumulation in the stems of plants is considered to be greatly important throughout the phytoextraction process [3].

According to the results of the study, lead concentration in the roots of *J. polycarpus* was twice higher than the roots of *C. aronia*. Although both these species could act as lead absorbent in nature, *J. polycarpus* is more efficient in terms of lead uptake compared to *C. aronia*. Furthermore, lack of mortality in these species confirms their tolerance against lead pollution, as well as their capacity for phytoremediation. Undoubtedly, complementary studies with higher concentrations of lead nitrate are required in this regard, in which the exposure time is longer for the reliable estimation of the maximum endurable concentration of lead in each of these plant species.

Authors' Contributions

M.M., and M.M.F., designed the manuscript; M.M., and M.M.F., managed the analyses of the study literature searches; F.Sh., managed the acquisition of Data. M.M., Performed the statistical analysis; M.M., wrote the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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