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Investigation of Cadmium uptake and its Effect on the Composition of Nutrient Elements and some Quantitative Indices in Seedlings of two Species of *C. aronia* and *J. polycarpos* in the Western part of Iran

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ABSTRACT

Background: Phytoremediation is a method in which plants are used to absorb pollutants. Heavy metals, through competition with nutrients elements, have a significant effect on their distribution in the plant.
Methods: In this study, seedlings were exposed to different concentrations (0.50, 100 and 150 ppm) of cadmium nitrate during a growing period in a completely random design with three replications. Then the value of cadmium uptake was measured by atomic absorption device in different organs of *c.aronia* and *j. polycarpos* (root, stem and leaf).
Results: The obtained results of the analysis of variance showed traits such as stem length, basal diameter, and root length were significantly affected by cadmium metal ($P < 0.01$).It was also observed that cadmium accumulation in the root and aerial parts of the plant increased with increasing cadmium concentration, and cadmium accumulation in the root tissues was higher in all treatments than aerial parts. In addition, heavy metals reduced the concentration of all nutrientelements in leaves and stems of seedlings.
Conclusion: According to the results, Seedlings of *c.aronia* and *j. polycarpos* can be suggested as stabilizing varieties for remediation of Cadmium polluted soils. It is worth to note that *j. polycarpos*, in comparison with *c.aronia*, is better absorbent of Cadmium.

1. Introduction

Soil pollution to heavy metals is one of the most important environmental issues around the world [1]. Due to the fact that these metals are absorbed by plants and transported to humans, this is more serious about crop soils [2]. Although heavy metals are naturally occurring in the environment, the expansion of urbanization and the development of industrial and agricultural activities have led to the dispersion of these metals in the biosphere and as a result, soil, the atmosphere, and aquatic environments have become contaminated [3]. Cadmium is a heavy metal that naturally occurs in the soil.The total amount of this unnecessary metal in the soils is 0.01 up to 3 mg.kg-1 and its average allowable level in water is 0.5 μg.L^{-1} .The ability of some plants species to absorb contaminating elements and compounds makes it possible for plants to be used to remediate contaminated environments; in recent decades, this has been very considered [4].

In fact, phytoremediation, as one of the methods of bioremediation, is a natural process used by plants to stimulate detoxification microorganisms and destruction of pollution and has been very much considered for reasons of high impact, low cost, low degradation, coordinating with nature, and usability on a large scale [5-7].

In the phytoremediation process, one of the most important points for success and appropriate response is the selection of an effective plant that is capable of growing in a contaminated environment and during its growth, can do optimally, cheaply and easily bioleaching and biorefining, along with the transfer of harmful substances and pollutants to its organs [8].

Among the researches on the phytoremediation of some wooden species, Hosseinzadeh Monfared (2011), which studies the comparison of the absorption of cadmium and lead in the soil and leaves, and of one-year branch of Robinia and Platanus species, and the results showed that there were no significant differences between the two species in terms of the adsorption of lead and cadmium, while the ability to absorb these two species was different in combating the pollutants and each of them absorbed a certain amount of pollutants [9].

The aim of this study was to investigate the ability of three-year-old seedlings of *Crataegus aronia* and *Juniperus polycarpos* in the accumulation and transfer of cadmium in different organs, as well as to understand the effect of cadmium accumulation on some quantitative indices and nutrient composition of these two species and to study their mechanism against cadmium toxicity. The hypothesis of this research based on the specific system of these types is included: 1. The roots have more ability to absorb cadmium than the aerial parts; 2. The stress of cadmium on the absorption of nutrients by *C. aronia* and *J. polycarpos* seedlings is effectiveand this effect varies between these two species.In this research, it has been attempted to investigate the absorption of cadmium in *C. aronia* and *J. polycarpos* species and in order to achieve the appropriate result, the suitable species have been proposed for the recovery and cleaning of soils and contaminated lands with this metal.

C. aronia and *J. polycarpos* are among the species in Iran (Iran-Turanian region), which are used in forestry development and enrichment activities, so far, their potential in using phytoremediation has not been investigated.In this research, for the first time, the potential of resistance to cadmium contamination in the soil for *C. aronia* and *J. polycarpos* was studied and investigated at the seedling stage.

2. Materials and Methods

Two species of *Crataegus aronia* and *Juniperus polycarpos* from Kowshkan nursery (Zanjan province) were selected among the important Iranian-Turanian forest species from in the Nursery of the Natural Resources and Watershed Management Office of Zanjan Province in April 2018, The two species was planted in black plastic potsof size 15*25 cm^2 and the ratio of 2:1:1 (crop soil, dried animal fertilizer and sand) after 4 mm sieve (the soil weight of each filled pot was 2kg).After drying the soil, 2 mm sieve was used to remove the lesions. Then some physical and chemical properties of the soil were determined using standard laboratory methods (Table 1).

The three-year-old seedlings were sprinkled with solutions, cadmium nitrate that was prepared through dissolving in distilled water and different concentrations of 0, 50, 100 and 150 (ppm) were considered as 4 treatments.The foliar application was carried out with the help of a simple spraying device.The reason for choosing this method is to simulate deposition from the air and the rainfall containing these pollutants.

The time of foliar application was during mid-June and 100 ml of each prepared concentrations was sprayed on each potted seedlings during three stages with a time interval of seven days. Control pots were considered for both species and sprayed with distilled water. After the end of growing period, some quantitative indices of seedlings (in control and other treatments) such as root length, stem height and basal diameter (measured using digital caliper with accuracy of 0.01mm) and in term of mm were measured, as well as the rate of absorption of cadmium element in root, stem, and leaf was also determined.Then samples were placed in an oven drying for 48 hours at 70 °C and the samples were measured by the digital scales with a precision of 0.001gr.In the next step, 0.1 g of the powdered sample from each organ (stem, leaf, and root) isolated and using wet oxidation method, mixed with a ratio of 1:2:8 of nitric acid 65 %: concentrated sulfuric acid: perchloric acid and placed for 24 hours in the laboratory conditions.The samples were then digested at 120 °C for 30 minutes.The samples were reached to 50 ml with distilled water after cooling. The cadmium concentration was measured by the atomic absorption device GBC Avanta P model.In the case of nutrient elements, after the end of the growing season, the seedlings were cut from the basal and after washing with distilled water, they were divided into two groups of leaves and stems and were placed in an oven drying for 48 hours at 70 °C to reach constant weight.The biomass weight was recorded for each organ.Dried specimens were completely powdered and 0.5 g was used to measure the concentration of the elements.The amounts of phosphorus, potassium, and nitrogen were determined using the wet digestion method (using the SPECTRONIC 21D, Flame photometer 410, KjeltecTM2100) and iron and copper elements were measured using the dry digestion method (with the atomic absorption device GBC Avanta P model) [10].

For statistical analysis of the data, a completely random design was used. The number of potted seedlings required for the four treatments was used three replicates , for each species, 36 numbers were used. The total potted seedlings used in this study were 72 numbers, which was located in the safe place and their care and irrigation were continuously based on calculated crop capacity (60% crop capacity) until the end of the experiment. The safe place was selected to control the effect of possible rainfall, to prevent the effects of rain as an interferer agent.

Statistical analysis of measured traits was performed using SPSS, version 20, software.

Table 1: Physical and chemical traits of the studied soil

Features of control soil	Texture of the soil			Cadmium (ppm)	Organic carbon (%)	Total nitrite (%)	PH	EC Ds/m
	Clay (%)	Silt (%)	Gravel (%)					
Flowerpot`s soil	9	27	64	0.108	1.04	0.061	7.83	5.55

3. Result and Discussion

The results obtained from the analysis of variance showed that there was a significant difference between treatments for traits such as the height of stem, root and basal diameter. Also, the results of the analysis of variance showed that there was a significant difference in cadmium adsorption in stem, leaf, and root of two species of *J. polycarpos* and *C. aronia* at 1% probability level ($P< 0.01$) (Table 2).

The results of the comparison of absorption means in different organs of J. polycarpos also showed that there was a significant difference in leaf and root for all three treatments with control but there was no significant difference between the concentration of 50 ppm and the control in the stem of *J. polycarpos* species($P< 0.01$).

Therefore, it can be noted that increased concentrations have a negative effect on the growth of various plant organs.The results of the comparison of the absorption rates in different organs of *C. aronia* species showed that the absorption rate in the stem, leaf, and root only has a significant difference with the control at 100 and 150 ppm ($P< 0.01$), (Table 3).

Comparison of the means of the evaluated traits in *J. polycarpos* indicates that the length of the aerial and terrestrial parts in the three treatments has a significant difference with the control, but in *C. aronia*, only the concentrations of 100 and 150 ppm have a significant difference with the control ($P< 0.01$), (Tables 4).

Meanwhile, the study of the amount of cd transfer in the leaf, stem, and root shows that by increasing the cd concentration, the rate of transmission decreases and the most accumulation is in the root.

Among other cases, is the effect of cadmium on the absorption of nutrient elements by *C. Aronia* and *J. Polycarpos*. Nitrogen, phosphorus, and potassium are high-consumption nutrients, and iron and copper are low-consumption elements in the leaf and stem.Based on the results obtained from Tables 5 and 6, show that cadmium has an inhibiting role to the natural process of absorbing nutrients by both species seedlings. The highest absorption of nutrients in stems and leaves of both species was nutrients in control treatment and the lowest level of contamination was observed at 150 ppm Cd. The absorption of the nutrients in the leaf, the concentration of 50ppm for iron absorption in *J. polycarpos* seedlings was not significantly decreased ($P< 0.01$), while in *C. aronia* species there was a significant decrease in the absorption of all mentioned elements ($P < 0.01$). Cadmium contamination had a significant effect ($P< 0.01$) on all levels of absorption of nitrogen in the leaf in *J. polycarpos* and the absorption of nitrogen and copper in the leaf in *C. aronia*, which was not observed in any of the other nutrients (Table 5),(Figure1 and 2).

Similar alphabet in each row indicates no significant difference between the numbers at 1% level. The data are shown as mean ± SD of 3 repetitions.

The absorption of the nutrients in the stem was also indicative of a reduction in the presence of cadmium.At high levels of contamination (treatments 100 and 150 ppm), no significant difference was observed in the absorption of phosphorus, iron, and copper in *J. polycarpos* seedlings ($P< 0.01$).This observation for *C. aronia* seedlings occurred only in the case of phosphorus and copper (Table 6), (Figure 3 and 4).

Species and subspecies of the plant have different abilities and adaptations in the face of environmental stresses such as heavy metals [11]. In this research, the diameter and longitudinal growth of *J. polycarpos* and *C. aronia* seedlings were considered as growth indices. The results showed that the height of root, stem and basal diameter of *J. polycarpos* in concentrations of 50, 100 and 150 ppm cd had a significant difference with control ($P< 0.01$), but there was no significant difference between concentrations of 50 and 100 ppm which were the same results of the study, Khoramdar et al. (2013) on the height of the root and stem of the three-month-old Acacia victoria seedlings, after absorption of cadmium [12]. The increasing cadmium causes a significant reduction in the root, stem and basal diameter in both species, which indicates a negative effect of cadmium on plant growth.Therefore, it can be concluded that the growth inhibition is the typical response of different herbs to the stress of cadmium, is also one of the important indices of plant resistance to these metals.

Researchers such as Kaswan and Kidwai (2016), Zafar and Javed (2016), Abraham et al. (2015) , Hu et al. (2015) , Naz et al. (2015) , Aslam et al. (2014), Tito et al. (2014) , Gubrelay et al. (2013), Mondal et al. (2013), Subin and Steffy (2013), Wang et al. (2012), Hayat et al. (2011), Auda and Ali (2010), and Ghani (2010), found that with increasing cadmium, the length of root, stem and total plant decreased and the decrease in the root length of plants was greater than their stems [13-26].

The reason for a greater reduction in root length torather than to the stem by increasing the concentration of cadmium in these two species is the root of the plant is the first organ that is directly related to the contamination of the metal in the soil and is also the cause of higher cadmium toxicity in the root than the stem.This metal accumulates in the plasma of the root cells, leading to a slowing down of cell division and prolongation of the cells [21,22].

The accumulation of cadmium in all organs of the two species is more than the control species, especially in high concentrations, is significantly higher than that of the control.

Table 2: Analysis of variance of some of the morphological traits studied in the investigation of cadmium adsorption by *J. polycarpos* and *C. aronia* seedlings

Kind of species	Sources of change	Degrees of freedom	Average of squares					
			Stem length	Root length	Basal diameter	Stem absorption	Leaf absorption	Root absorption
J. polycarpos	Treatment	3	721417**	5126889**	5292**	576900**	243576**	5200025**
	Error	8	1.833	57,000	0.027	2.686	0.952	11.353
	Total	11	723.250	5183.889	5.319	579.586	244.528	5211.378
C. aronia	Treatment	3	1699000**	2010306**	5170**	1602010**	103367**	3294179**
	Error	8	10.500	35.250	0.100	4.440	4.028	18.552
	Total	11	1709.500	2045.556	5.270	1606.450	107.395	3312.731

** Significantly at the probability level of 0.01

Table 3: Comparison of the average cadmium absorption by *J. polycarpos* and *C. aronia* seedlings

Kind of species	Treatment (ppm)	Organ		
		Root	Leaf	Stem
J. polycarpos	Control	0.067 ± 0.033 [a]	0.033 ± 0.033 [a]	0.267 ± 0.266 [a]
	50	13.433 ± 1.021 [b]	2.367 ± 0.218 [b]	2.367 ± 0.218 [a]
	100	69.133 ± 4.952 [c]	8.033 ± 0.240 [c]	22.467 ± 1.811 [b]
	150	85.467 ± 4.454 [d]	20.200 ± 1.078 [d]	22.567 ± 0.425 [c]
C. aronia	Control	0.200 ± 0.057 [a]	0.033 ± 0.033 [a]	0.033 ± 0.033 [a]
	50	5.667 ± 0.433 [a]	5 ± 2.173 [ab]	1.100 ± 0.057
	100	53.667 ± 4.941 [b]	8.800 ± 0.550 [bc]	25.167 ± 0.693 [b]
	150	65.500 ± 0.360 [c]	13.900 ± 0.585 [c]	48.733 ± 2.331[c]

alphabet means no significant difference

Table 4: Comparison of Mean Morphological Properties of *J. polycarpos* and *C. aronia* Seedlings

Kind of species	Treatment (ppm)	Organs of *J. Polycarpos* seedlings		
		Root	Leaf	Stem
J. polycarpos	Control	109.330 ± 7.839 [a]	3.400 ± 0.100 [a]	38.670 ± 1.202 [a]
	50	52.670 ± 3.283 [b]	1.233 ± 0.145 [b]	11.670 ± 0.333 [b]
	100	47.670 ± 1.202 [b]	1.000 ± 0.057 [b]	10.670 ± 0.333 [b]
	150	9.000 ± 1.528 [c]	0.333 ± 0.033 [c]	3.330 ± 0.882 [c]
C. aronia	Control	80.330 ± 4.000 [a]	4.000 ± 0.288 [a]	76.670 ± 0.88 [a]
	50	55.000 ± 0.577 [a]	2.067 ± 0.088 [a]	31.670 ± 0.333 [a]
	100	51.670 ± 45.930 [b]	1.833 ± 0.088[b]	33.670 ± 1.764 [b]
	150	17.330 ± 5.590 [c]	0.867 ± 0.185[c]	13.000 ± 2.309 [c]

alphabet means no significant difference

The highest cadmium content of root, stem, and leaf of both species was observed at 150 ppm concentration. The highest concentration of cadmium was also observed in the root of seedlings so that in *J. polycarpos* species, root cadmium content at the treatment of 150 ppm was 4.2 and 3.1 times more than cadmium in leaf and stem and in C. *aronia* species at the same concentration was 4.7 and 1.3 times of leaf and stem, respectively.

Indeed, the difference in the accumulation of cadmium between the roots and the aerial parts of the plants indicates a limitation of the transfer of cadmium from root to aerial parts, as a result, the concentration of cadmium in the roots of plants is higher [27].

Also, the accumulation of cadmium in the roots of the plant relative to their stem can lead to the prevention of toxicity in the stem relative to the roots of the plant [22].According to researchers, the ability of a species to accumulate heavy metals in the root and preventing its transfer to the aerial parts indicates the resistivity mechanism of the species studied to the metal as well as its applicability in the phytoremediation process [28].

Another important work of heavy metals is their interactions with nutrient elements, so understanding these relationships is a common concern [29]. The results presented in this study showed that increased levels of cadmium contamination reduce nitrogen absorption by *C. aronia* and *J. polycarpos* seedlings. This decrease was significant at some levels of contamination and in some cases was not significant ($P < 0.1$). Reducing nitrogen absorption by increasing Cd concentration has been reported in numerous studies with other plants [10,30,31, 32], as well as Kapusta and Godzik (2013) pointed out that heavy metals increased the nitrogen absorption by the plant, which contrasts with the results of our study [33].

The phosphorus was the second most consumed element, which was determined, as showed at the Results table, It is revealed that phosphorus adsorption in both species is reduced after exposure to cadmium. As with increasing cadmium contamination levels up to 150 ppm, phosphorus absorption in the leaves decreased by 61% in *J. polycarpos* and 83% in *C. aronia* species.Yang et al. (2007), in their study, investigated the reduction of phosphorus absorption by the plant in the presence of heavy metal [31]. Karimi et al. (2013) also reported that phosphorus absorption in the presence of heavy metals is not significant, which differs from our research results [32].

Table 5: The effect of cadmium on the uptake of nutrient elements in the leaf of *J. polycarpos* and *C. aronia* seedlings

Kind of species	Nutrient element	Cadmium concentration (ppm)			
		0	50	100	150
J. polycarpos	Nitrogen (N) (g kg^{-1})	28.300 ± 1.153 [a]	17.200 ± 1.833 [b]	13.100 ± 1.493 [c]	6.900 ± 1.113 [d]
	Phosphorus (P) (g kg^{-1})	1.200 ± 0.100 [a]	0.900 ± 0.173 [b]	0.710 ± 0.17436 [b]	0.410 ± 0.175 [c]
	Potassium (K) (g kg^{-1})	9.800 ± 0.4 [a]	6.400 ± 0.529 [b]	6.100 ± 0.435 [b]	4.300 ± 0.655 [c]
	Iron (Fe) (mg.kg^{-1})	33.000 ± 1.734 [a]	28.700 ± 5.507 [a]	13.100 ± 1 [b]	9.800 ± 1.153 [b]
	Copper (Cu) (mg.kg^{-1})	5.800 ± 0.264 [a]	3.100 ± 0.556 [b]	2.700 ± 0.264 [b]	1.900 ± 0.264 [c]
C. aronia	Nitrogen (N) (g kg^{-1})	10.100 ± 0.264 [a]	7 ± 0.264 [b]	6 ± 0.173 [c]	3 ± 0.173 [d]
	Phosphorus (P) (g kg^{-1})	1.200 ± 0.264 [a]	0.500 ± 0.173 [b]	0.400 ± 0.020 [b]	-0.200 ± 0.100 [b]
	Potassium (K) (g kg^{-1})	10.300 ± 0.105 [a]	9.300 ± 0.173 [b]	7.100 ± 0.264 [c]	4 ± 1 [d]
	Iron (Fe) (mg.kg^{-1})	12 ± 1 [a]	10 ± 1.0104 [b]	9.300 ± 0.100 [b]	7.100 ± 0.264 [c]
	Copper (Cu) (mg.kg^{-1})	4 ± 0.100 [a]	2.900 ± 0.173 [b]	2.1 ± 0.173 [c]	1.800 ± 0.360 [c]

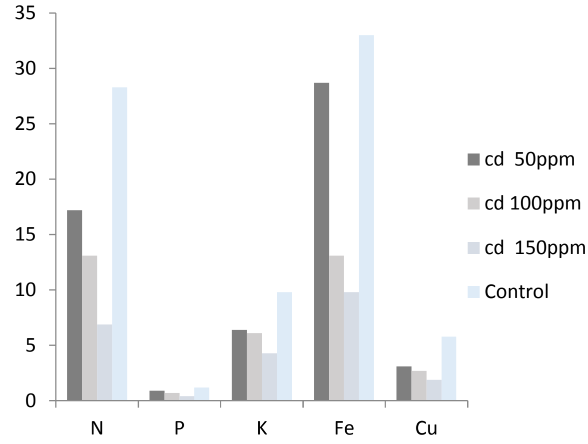

Figure 1: Mean Nutrient Content in *J. polycarpos* Leaves in Cadmium Treatment

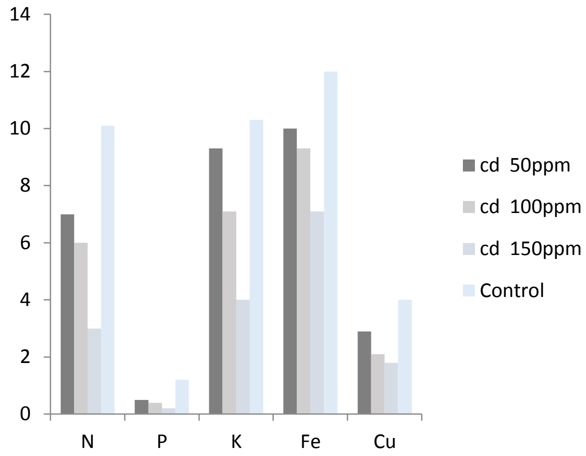

Figure 2: Average nutrient content in *J. polycarpos* leaf under cadmium treatment

Potassium measurements in the leaf and stem of C. aronia and *J. polycarpos* showed that cadmium had a decreasing effect on the absorption of this element. Cadmium concentration at 150 ppm reduced 56% and 61% in potassium absorption in leaves of *J. polycarpos* and *C. aronia* species respectively. Reduction of potassium absorption under heavy metal stress has been reported in other plant species [10,32,34].

Iron is one of the key micronutrients for the plant, and its deficiency causes metabolism disorders in the plants. Many studies have shown that heavy metals cause iron deficiency of the plants [10,35]. Results of this study also showed that Lead and Zinc stress reduced iron absorption in the stems and leaves of both species. This decrease was significant in some levels of contamination and in some cases was not significant ($P < 0.1$).

The other studied micronutrient which was measured, is the copper. The exposure to cadmium reduced the plant's ability to absorb copper [10]. Increasing the concentration of cadmium up to 150 ppm reduced 56% and 61% in copper absorption in leaves of *J. polycarpos* and *C. aronia* species respectively. Heavy metals prevent the absorption of food elements in various ways.The competition, the effect on the membrane of root (for example, the effect on root proteins and also the root membrane peroxidation), the effect on ATP of the testes and other carriers, the reduction of root respiration (which reduces the active transmission of the elements), root damage and postponement of its growth (which ultimately leads to a reduction in being suberose and damage to the permeability of the root cells) are some of the mechanisms by which heavy metals prevent the absorption of nutrients in the plant [35].

Table 6: Effect of Cadmium on Nutrient Uptake in Stems of *J. polycarpos* and *C. aronia* Seedlings

Kind of species	Nutrient element	Cadmium concentration (ppm)			
		0	50	100	150
J. polycarpos	Nitrogen (N) (g kg^{-1})	7.033 ± 1.069 ^{a}	17.200 ± 1.833 ^{a}	6 ± 0.264 ^{a}	4 ± 1.212 ^{b}
	Phosphorus (P) (g kg^{-1})	0.700 ± 0.264 ^{a}	0.590 ± 0.210 ^{ab}	0.440 ± 0.06 ^{ab}	0.356 ± 0.051 ^{b}
	Potassium (K) (g kg^{-1})	3.200 ± 0.435 ^{a}	3 ± 0.173 ^{a}	2.200 ± 0.264 ^{b}	1.200 ± 0.100 ^{c}
	Iron (Fe) (mg.kg^{-1})	8 ± 0.871 ^{a}	3 ± 0.173 ^{ab}	6.700 ± 0.435 ^{bc}	6.1 ± 0.519 ^{bc}
	Copper (Cu) (mg.kg^{-1})	2.200 ± 0.692 ^{a}	1.800 ± 0.346 ^{ab}	1.500 ± 0.173 ^{ab}	1.100 ± 0.458 ^{bc}
C. aronia	Nitrogen (N) (g kg^{-1})	10.100 ± 0.1732 ^{a}	9.300 ± 0.173 ^{b}	7 ± 0.264 ^{c}	3 ± 0.173 ^{d}
	Phosphorus (P) (g kg^{-1})	1.200 ± 0.1 ^{a}	0.700 ± 0.173 ^{b}	0.500 ± 0.1000 ^{bc}	0.39 ± 0.02 ^{c}
	Potassium (K) (g kg^{-1})	3.300 ± 0.173 ^{a}	0.200 ± 0.173 ^{b}	1.800 ± 0.100 ^{b}	0.87 ± 0.026 ^{c}
	Iron (Fe) (mg.kg^{-1})	10 ± 1.732 ^{a}	8 ± 0.264	7.100 ± 0.264 ^{b}	5.100 ± 0.173 ^{c}
	Copper (Cu) (mg.kg^{-1})	3.9 ± 0.2646 ^{a}	2.4 ± 0.36^{b}	1.900 ± 0.173 ^{c}	1.600 ± 0.173 ^{c}

Similar letters in each row indicate no significant difference between numbers at 1% level; data shown as mean ± SD of three replications

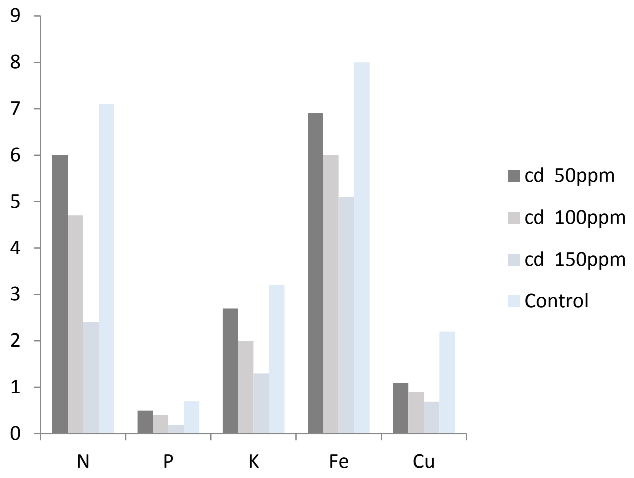

Figure 3: Mean Nutrient Content in *J. polycarpos* Stems in Cadmium Treatment

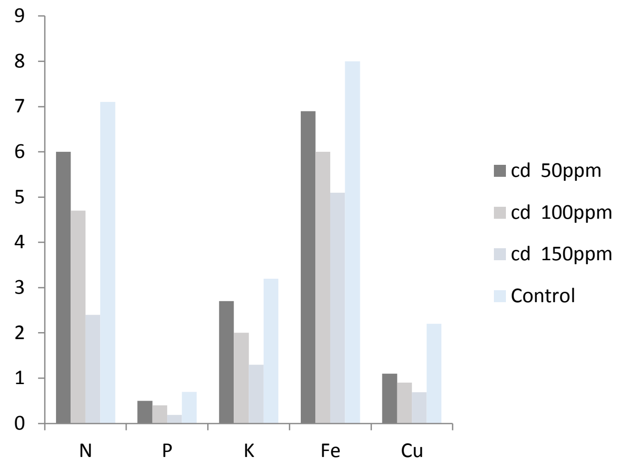

Figure 4: Mean Nutrient Content in *C. aronia* Stems in Cadmium Treatment

4. Conclusion

The mechanism of the interaction between heavy metals and nutrients are not known.Because plant viability is one of the key components of the success of phytoremediation projects, it certainly will increase the information about the circulation mechanism of heavy metals, and subsequently, using methods to maintain proper nutrient absorption conditions by the plant will help to solve the problems of phytoremediation projects. According to the results of the current study regarding the absorption of heavy metals such as cadmium by two species of *J. polycarpos* and *C. aronia* seedlings, it is recommended to develop their cultivation level in cadmium contaminated areas for phytoremedation.

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 themanuscript.

Conflict of Interest

The Authors declare that there is no conflict of interest.

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