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Evaluation of Cd phytoremediation by *Portulaca oleracea* irrigated by contaminated water

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Abstract

Background: Worldwide, more than 10 million regions with an area of more than 20 million hectares are considered contaminated soils. The expansion in industrial activities and urbanization has led to accumulation of high concentration of cadmium (Cd) in soil and water resources, which is a serious danger for environment and human health. Phytoremediation is a technique recommended for the removal of heavy metals from contaminated soils, sediments, and waters. This study aimed to investigate the *Portulaca oleracea* in the removal of Cd from contaminated soil.

Methods: To investigate the effect of *P. oleracea* phytoremediation on the removal of Cd, in 5 pots, 3 kg of soil was poured. And in each pot, 10 seeds were pursued. The pots were irrigated twice a week for 8 weeks with concentrations of 25, 50, 75, and 100 mg/L Cd. After 8 weeks, the amount of Cd of roots, shoots, and leaves was measured by atomic absorption spectrometry.

Results: The results showed that Cd accumulation in purslane plants was significant ($P = 0.008$) and the highest Cd accumulation was in leaves and shoots, and increasing Cd concentration prevented plant growth and led to lower growth at higher concentrations.

Conclusion: According to the results, *P. oleracea* can be used for the phytoremediation of Cd.

Keywords: Bioaccumulation, Cadmium, Purslane, Phytoremediation, Soil pollutants

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Introduction

With the expansion of urbanization and the development of industry, environmental pollution with heavy metals has become a global problem. High levels of heavy metals in water adversely affect human health and make the water unusable (1). Metals in water sources enter water sources either naturally or due to pollution (2). Humans are permanently and temporarily exposed to 35 toxic metals, 23 of which are heavy metals. Heavy metals do not decompose and gradually accumulate in the bodies of living things such as plants and animals. These metals are deposited and accumulated in adipose tissue, muscles, bones, and joints (1). Heavy metals are of particular importance in environmental pollution due to their non-degradability and harmful physiological effects on organisms, even at low concentrations (3,4).

Different kinds of industries release heavy metals like cadmium (Cd) and nickel (Ni) in wastewaters at various concentrations, which prove highly toxic for human health. After entering the aquatic ecosystems, bioaccumulation of heavy metals in different organisms varies in the food chain and different organisms show different tolerance levels (5).

As a result of urbanization and increased anthropogenic activity, pollution caused by heavy metals in soil, water, and mesosphere indicates the growth of environmental problems affecting food quality and human health in cities (6).

Heavy metals are highly noxious for all biotic components of the environment. Heavy metal contamination results either from the direct water source or through biomagnification. Sometimes in mining areas, high air concentrations also become a source of heavy metal contamination (7)

According to the definition of the World Health Organization (WHO), drinking water is water that is suitable for human consumption and all household uses (8). It is important to remove heavy metals from drinking water because they are dangerous to health, especially lead salts that enter the water through pipelines. Organic matter delivery to water supply lines due to fractures, reverse siphons, microleakage, and analysis or lack of disinfectants and the formation and growth of biofilms in the walls of pipes, water wastage, interaction of water and pipes and the presence of microorganisms and organic matter that has undergone treatment processes and factors such as all provide the conditions for the growth of microbial populations and changes in the chemical composition of water in the distribution network. There are three types of contaminants in water including bacteria and viruses, toxic chemical compounds, and heavy metals. Heavy metals include metals such as lead, copper, zinc, Cd, mercury, etc., which are often found to be carcinogenic and dangerous (9).

Cd is a heavy metal that is very stable in the environment. The increased risk of lung cancer is also one of the effects of Cd inhalation. The excessive accumulation of Cd in the body of animals and humans causes disorders such as bone fatigue, bronchitis, kidney damage, hypertension, and atherosclerosis (10,11).

The biggest source of Cd release into the environment is the burning of fossil fuels such as coal and oil and municipal garbage burning. Also, Cd may enter the air during the extraction of zinc, lead or copper. It can also enter the water through the irrigation of agricultural lands around the cities. Chemical fertilizers mainly contain Cd. Food and smoking are the biggest sources of pollution for humans. The average daily intake for each person during a day is 30 mg orally. Smokers also receive 1-3 mg daily (12).

Any accumulation in plants may not show any toxicity, however, their consumption by the other living beings may in turn show toxicity in the consuming organisms. In fact, Cd contamination affects humans more than animals, because they are at the top of food chain. Generally, high levels of Cd in plants lead to a reduction in photosynthesis, water uptake, and to some extent, nutrient uptake as well (5).

When Cd enters the body, it accumulates in the kidneys and liver, reproductive organs, nervous system, respiratory system, gastrointestinal tract, and heart muscle, and when its amount exceeds a certain limit, due to its long-term effects, its complications in the form of diseases. These effects are more severe in children (1,13). Cd is often combined with metallothionein and accumulates in the cortex of the kidney. Kidney dysfunction is the first sign of Cd poisoning (14). The biochemical effects of Cd include the breakdown of phosphorus oxides, interference with enzyme activity, as well as the ability to react with nucleic acids (15), and the development of chromosomal abnormalities and carcinogenesis in the lung (1,13). Continuous uptake of Cd may cause anemia by reducing ion uptake by the intestine. In addition, Cd uptake may cause itai-itai (14).

If the drinking water is too soft, some Cd will enter the water due to corrosion of the pipes. Ceramic or metal containers can also add some Cd to the water. The short-term effects of Cd consumption are nausea, vomiting, diarrhea and muscle contraction, sensory disturbances, seizures, and shock, and the long-term effects of Cd consumption are damage to the kidneys, liver, bones, and blood (9).

When heavy metals such as lead and Cd are dispersed in water, soil, and air, they are absorbed by plants (16). Ardakani et al reported that the elements lead and mercury had the least ability to reach the shoots in vegetables and alfalfa. Kabata-Pendias reported that Cd and lead had the highest and lowest post-adsorption capacity, respectively. The amount of lead accumulation in plant organs is different and is often more in the leaves than in other parts of the plant (17).

Many methods have been developed to clean soils contaminated with heavy metals. Among these techniques, plant extraction (phytoremediation) has been proposed as an effective purification method, in which the uptake and accumulation of contaminants in plant-based textures are considered (18).

Phytoremediation is a technique recommended for the removal of heavy metals from contaminated soils, sediments, and water (19).

Phytoremediation or the use of plants to purify pollutants from soil, water, and sediment as a relatively new technology through filtration root, stabilization plant, absorption plant, sublimation plant, and destructive plant causes the removal, destruction, or confinement of pollutants and the best approach to plant pollution, absorption, and transport of pollutants from soil to plant without destroying the soil structure and changing its fertility. The efficiency of the adsorption plant in soil purification depends on two factors, biomass and metal concentration in biomass. Therefore, by applying appropriate treatments and good crop management, biomass production and bioavailability of metal, soil uptake, and uptake by plants can be increased (20).

Portulaca includes over 100 annual and perennial species that are distributed worldwide (mostly in the tropic and subtropic area) with most species' diversity in South America and Africa. The number of Portulaca species is not clear it is estimated to be from a few to over 100 morphologically variable species (21).

Portulaca oleracea is an annual, thermophilic, and carbonaceous plant with herbaceous shoots, a dormant and fleshy plant that has high adaptability in arid and semi-arid areas, which grows easily in acidic and saline soils and is, therefore, one of the halophyte plants. Although this plant is known as a weed, its use as an edible and medicinal plant has a long history by absorbing large amounts of water in the organs. This plant can enter high concentrations of metals into its tissues and play a role in their extraction from the soil. Research has shown that this plant can accumulate high concentrations of heavy metal chromium in its textures (22). Considering special characteristics of *P. oleracea* and its high tolerance in arid and saline regions such as MENA (Middle East and North Africa) countries, the study of its tolerance to growing mineral pollutants such as lead and Cd is valuable, because it may be used as an alternative plant in contaminated areas to eliminate pollution (19,23).

The main factors account for soil salinization have been the low quality of irrigation waters, excessive fertilization and the deficient drainage of some soils. As a result, the yield of crops decreases and it is no longer possible to grow more salt-sensitive species. During the evolution, plants have developed complex strategies to ensure their survival and reproduction especially under adverse environment conditions. Indeed, purslane grows in soils that may be arid and saline. Hence, it is listed as halophyte in the eHALOPH database. Considering its tolerance to especially chlorine salinity, Parnian et al suggested purslane as a promising candidate for the use in drainage water reuse system, because of not only its survivability and water use, but also its usefulness as a vegetable and oil seed crop (21).

Due to the increasing probability of heavy metals contamination, it is necessary to investigate the uptake of these elements by the *P. oleracea* plant. In this research, the phytoremediation properties of *P. oleracea* in soils contaminated with Cd were investigated. The effect of different plants on the removal of heavy metals was investigated to some extent in the effluents, but the effect of *P. oleracea* on contaminated soil environments was not considered.

Materials and Methods

This research was conducted as a pilot project in the scientific-research laboratory of the Faculty of Health of Mazandaran University of Medical Sciences. To start the experiment, 10 plastic pots with an opening diameter of 25 cm and a height of 30 cm were prepared and 3 kg of soil was poured into each pot with a depth of 25 cm.

In order to measure soil pH, 20 g of soil was poured into a 50-g jar and 40 mL of distilled water was added to it and stirred with a glass stirrer until mixed well. After filtering, the pH of the solution was measured with a pH meter, and the pH 6.3 was obtained.

The soil was gathered from garden and sieved. Each pot was considered as a test unit and 10 seeds of *P. oleracea* prepared from Mazandaran Agricultural and Natural Resources Research and Education Center were planted in it. Greenhouse conditions were maintained at a temperature of approximately 25 to 35°C and the pots were irrigated each time to the capacity of the field. To prepare contaminated water with concentrations of 25, 50, 75, and 100 mg/L, Cd nitrate ($Cd(NO_3)_2.4H_2O$) with a molecular weight of 308.48 g and a purity of 100% was used. The groups in this study include soil containing Cd, and healthy soil without Cd (control group). Ten *P. oleracea* seeds were sown evenly in 5 pots uniformly (Figure 1).

The pots were irrigated 16 times (twice a week) with a sprinkler and evenly with solutions of Cd nitrate and control pots with distilled water 16 times (twice a week) during the growth period of the plant (22). It was frequently inspected and the seeds that were poor in terms of growth were removed from the pot and thinned. The environmental conditions were the same for each pot. After 60 days, healthy specimens of *P. oleracea* were harvested after 8 weeks of growth and its different parts (roots, shoots, and leaves) were separated and weighed separately. After measuring the fresh weight, they were powdered, and then, their dry weight was measured and the results are presented in Table 1.

The root and shoot samples were dried separately at 85°C for 2 hours. After drying, the leaf, shoots, and roots

Table 1. Fresh and dry weight of purslane plant (shoot, leave, and root) (g)

Plant parts	Irrigation concentration (mg/L)	Fresh weight of purslane plant (g)	Dry weight of purslane plant (g)
Root		0.33	0.03
Shoot	25	1.49	0.05
Leave		1.57	0.14
Root		0.26	0.03
Shoot	50	0.33	0.04
Leave		0.95	0.09
Root		0.27	0.03
Shoot	75	1.4	0.09
Leave		1.84	0.12
Root		0.32	0.03
Shoot	100	0.79	0.07
Leave		1.41	0.09
Root		0.32	0.03
Shoot	Control	0.77	0.07
Leave		1.87	0.18

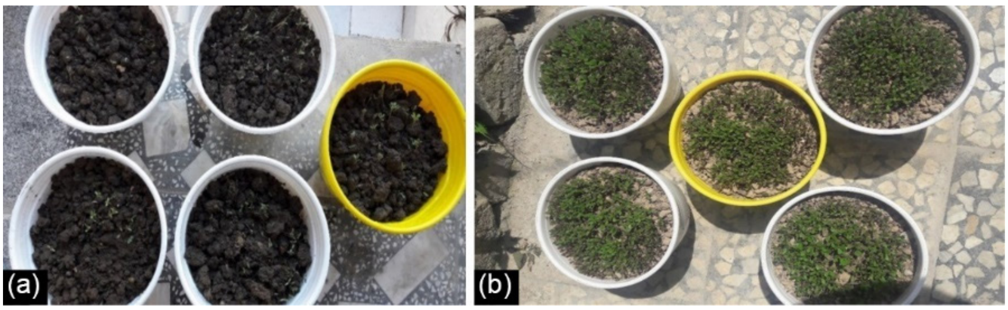

Figure 1. The growth of *Portulaca oleracea* in the third week (a), *Portulaca oleracea* at harvest (b).

were ground separately and turned into a soft powder.

0.03 g of each shoot, root, and leaves sample was put into special containers, and then, 20 mL of 0.1 N nitric acid was added to the container and heated on a hot plate for 20 minutes to ensure proper acidic digestion. The digested material was filtered using Whatman 42 filter paper, and then, the containers containing shoots, leaves, and roots were reduced to a volume of 10 mL, respectively. At this stage, the extract was ready to be presented to PerkinElmer atomic absorption spectroscopy (Model AAnalyst™ 700) so that the Cd level of each sample could be measured separately by this device.

To measure the amount of Cd in the samples, after preparing the device, a Cd lamp with a wavelength of 228 nm was used. After the thermal adjustments, the prepared standard Cd solutions were measured in order from low to high concentrations.

The injection volume was the same in all standards and samples (about 3 cc). Then, the concentration of the standards and the number of times they were read were entered. After the standards were read and due to the correct value of the correlation coefficient (r), the device was allowed to read the samples, and finally, the concentration of Cd (ppm) in the samples was read.

Metal removal was determined by quantifying the concentration of the leftover in the medium after incubation with plants. 2-mL water samples were taken daily and the removal of metals was then calculated using the following Eq. (1):

$$R\ (\%) = [(C0 - Ct) / C0] \times 100 \quad (1)$$

where *C0* and *Ct* represent the residual concentration of metal at the beginning of the experiment and at time *t*, respectively.

The data obtained from the experiment were analyzed using SPSS version 25 and the Kruskal-Wallis test.

Results

The average amount of cadmium in Portulaca oleracea

The amount of Cd in the primary soil and the amount absorbed by different parts of the *P. oleracea* as well as the average of Cd after converting the raw data to mg/kg of *P. oleracea* are shown in Table 2.

Table 2 shows the highest and lowest Cd accumulation in *P. oleracea* in shoots of *P. oleracea* irrigated with 75 mg/L (4.467 mg/kg) and root of *P. oleracea* irrigated with 100 mg/L (0.154 mg/kg), respectively.

The effect of Portulaca oleracea on cadmium uptake

According to the significance level for Cd ($P = 0.008$), it was shown that *P. oleracea* has a significant effect on Cd uptake ($P \leq 0.05$). However, according to the average rankings, it was determined that the highest amount of Cd accumulation is in leaves, shoots, and roots, respectively.

According to the results of measurement of Cd in different parts of *P. oleracea*, as shown in Table 2, the lowest mobility of Cd was in the roots of the control group and the highest mobility was at a concentration of 100 mg/L, indicating the lowest accumulation of Cd in the roots and vice versa.

As shown in Table 2, the highest amount of Cd accumulation in the shoot was at a concentration of 75 mg/L and the lowest one was at a concentration of 50 mg/L. However, at concentrations of 25, 50, 100 mg/L and in the control group, the amount of Cd accumulation did not differ much.

Measurement of Cd in the leaf of *P. oleracea* showed the highest accumulation of Cd at concentrations of 25 and 50 mg/L (Table 2).

As shown in Table 2, the amount of Cd absorption by *P. oleracea* was significant and the highest amount of Cd absorbed in *P. oleracea* was in the leaf of this plant and only in pots irrigated with Cd solution at a concentration of 75 mg/L, the highest amount of Cd absorbed was accumulated in the shoot. In the control group, the amount of Cd absorbed in the shoot was slightly higher than that in the leaf.

Removal Percentage of cadmium by Portulaca oleracea

As shown in Table 3, the efficiency of Cd uptake in the aerial parts of *P. oleracea* (stem and leaf) was higher than that in underground organs (roots).

The percentage of uptake at irrigation concentrations of 25, 50, and 100 mg/L from the root to the leaf has increased regularly and the highest uptake was observed in leaf, shoot, and root, respectively. In this case, at an irrigation concentration of 75 mg/L, this pattern was the highest as shoot > leaf > roots.

Discussion

Phytoremediation, a method to remove pollutants from the environment by using plants and is a cost-effective and eco-friendly technique for remediating soils. Notably, phytoremediating plants uptake and accumulate Cd inside their roots, shoots, leaves, and vacuoles. Still, it takes a long time to provide fruitful results because phytoremediation

Table 2. Soil cadmium concentration absorbed by different parts of *Portulaca oleracea* (mg/kg)

Irrigation concentration (mg/L)	Control			25 mg/L			50 mg/L			75 mg/L			100 mg/L		
Plant parts	Root	Shoot	Leave	Root	Shoot	Leave	Root	Shoot	Leave	Root	Shoot	Leave	Root	Shoot	Leave
Cd (mg/kg)	0.3693	0.4418	0.4369	0.2844	0.4061	3.9472	0.2934	0.3059	3.8950	0.2139	4.4675	1.5427	0.1541	0.3720	0.5065

is still under the investigation

and progress phase, and several technical barriers have to be overcome (24).

Cd is a mobile metal that is easily absorbed from the plant surface, moves to their woody tissue, and accumulates in the upper part of the plant (25). According to the results of this study, the highest amount of Cd is accumulated in the shoots of *P. oleracea*, which is consistent with the results of above-mentioned studies.

In the present study, the highest amount of Cd uptake was in the shoot of *P. oleracea* and the concentration of Cd irrigation solution was 75 mg/L (4.47 mg/kg) and the data were statistically significant.

The highest and lowest levels of Cd (4.47 and 0.154 mg/kg) were obtained in the shoot of the irrigated plant with a concentration of 75 mg/L and the root irrigated with a concentration of 100 mg/L, respectively. The Cd mean concentration was in the *P. oleracea* plant (1.175 mg/kg) (Table 4).

In the present study, the highest amount of Cd accumulation was in the upper organs of *P. oleracea*. In 2008, Tiwari et al examined the phytoremediation efficiency of *P. oleracea* grown in industrially irrigated areas of Gujarat, India. They found that the highest Cd accumulation was in *P. oleracea* root, and concluded that *P. oleracea* is a biomass. It is good and has a high reconstruction property (26), which is not consistent with the results of the present study. The reason for this discrepancy can be attributed to the difference in soil pH, because the soil irrigated with industrial effluent has an alkaline pH at which the movement of heavy metals to the upper extremities is slow and due to the acidity of the soil in the present study, a contradictory result was obtained compared to the study of Tiwari et al.

Table 3. The percentage removal efficiency of *Portulaca oleracea* in removing cadmium

Irrigation concentration (mg/kg)	Part of plant	The percentage removal efficiency for Cd	The percentage removal efficiency of plant for Cd
25	Root	0.3	
25	Shoot	0.4	4.6
25	Leave	4	
50	Root	0.3	
50	Shoot	0.4	2.3
50	Leave	4	
75	Root	0.15	
75	Shoot	0.15	2.1
75	Leave	2	
100	Root	0.07	
100	Shoot	1.5	0.25
100	Leave	0.5	

Table 4. Cd means in *Portulaca oleracea* (mg/k)

	N	Min	Max	Mean	SD
Cd	15	0.1541	4.4675	1.1757	1.5530

According to the reports provided by the National Standard of Iran, the World Health Organization (WHO) allows Cd concentration for leafy and grain vegetables to be 0.1 mg/kg (25). In 2018, Vatanian et al studied the levels of Cd, arsenic, nickel, and lead in mint and purslane vegetables in Dezful and Hamidiyeh soils in Khuzestan province and concluded that the levels of Cd, lead, arsenic, and nickel in mint and *P. oleracea* were below the standard level, which may be due to soil characteristics, irrigation conditions, and low soil organic matter (27). According to the results of the present study, the amount of Cd accumulation in all parts of *P. oleracea* and different concentrations irrigated in this study is higher than the allowable limit of the WHO, which indicates that *P. oleracea* is a good adsorbent for removing Cd metal from contaminated soil.

In a study conducted by Ziarati and Alaedini on measuring the amount of heavy metals and pesticides in the soil and agricultural products of Zanjan, pesticides were analyzed by GC/MS and the amount of heavy metals by atomic absorption apparatus. The results showed that the amount of copper and zinc in soil samples was not higher than the allowable limit, but the amount of Cd and lead in 70% of soil samples was higher than the allowable limit. Also, in leaf samples, the amount of lead and Cd was higher than the limit set by the WHO (28), which is consistent with the findings of the present study.

Abu-Muriefah et al showed that Cd by reducing enzymatic activities reduces protein production and reduces or stops plant growth (29). In the research conducted by Yousefi et al. with the reed plant in the hybrid vertical-horizontal wetland system, the average removal percentage for the two metals cadmium and lead from the water environment was 66 and 67%, respectively, which showed the successful performance of the phytoremediation (30). In the present study, with increasing Cd concentration, less growth of *P. oleracea* was observed, which is consistent with the findings of the study by Abu-Muriefah.

Conclusion

According to the reports of the WHO regarding the permissible amount of Cd (0.2 mg/kg) in plants (31), the results of this study showed that the amount of Cd present in the purslane plant in this study is higher than the global permissible limit and is in the range of contamination. As a result, this plant can be used as an environmental method to purify Cd-contaminated soils.

Based on the obtained results, it should be noted that heavy metals, especially Cd, enter the body from other sources, so it is necessary to continuously monitor human consumables such as water, vegetables, and aquatic products based on the existing standards.

In the end, it is suggested to plant purslane plant in soil already contaminated with a specific concentration and irrigation with distilled water, to measure the amount of lead and Cd in different intervals of plant sampling, and to use purslane in wetlands to absorb Cd and lead.

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Ethical issues

This article is a research project and simultaneous thesis of Mrs. Hajar Nouri Esbokolaee, approved by the Vice Chancellor for Research and Technology of Mazandaran University of Medical Sciences. (Approval code: 6803) and the ethical code of the Ethics Committee will be IR.mazums.rec 99.6803.

Competing interests

The authors declare that they have no conflict of interests.

Authors' contribution

Conceptualization: Zabihollah Zousefi.
Data curation: Zabihollah Zousefi.
Formal analysis: Rezaali Mohammadpour.
Funding acquisition: Zabihollah Yousefi.
Investigation: Zabihollah Zousefi, Hajar Nouri Esbokolaee.
Methodology: Zabihollah Zousefi, Esmaeel Babanejad, Hajar Nouri Esbokolaee.
Project administration: Zabihollah Zousefi.
Resources: Zabihollah Zousefi.
Software: Rezaali Mohammadpour.
Supervision: Zabihollah Zousefi, Esmaeel Babanejad.
Validation: Zabihollah Zousefi.
Visualization: Zabihollah Zousefi, Esmaeel Babanejad.
Writing – original draft: Hajar Nouri Esbokolaee, Zabihollah Zousefi, Hajar Nouri Esbokolaee.

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