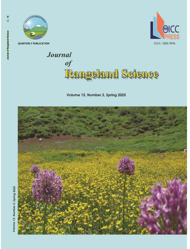

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Comparative investigation of heavy metals in soil and fruit of medicinal plant *Capparis spinosa* L. (Case Study: Hashtgerd Region, Iran)

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Original Research

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Abstract:

Capparis spinosa L. has a variety of uses, including preventing soil erosion, land restoration, edible consumption, and medicinal benefits. The pollution caused by municipal waste and factory effluents can affect the growth and medicinal properties of this plant in nature. This study aimed to investigate the morphological traits and heavy metals of soil and fruit of *C. spinosa* in contaminated and uncontaminated natural habitats in Hashtgerd region, Alborz province, Iran in June 2020. For this purpose, stem length, leaf length and width, fruit length and width, coverage and number of rootstocks were measured in 10 random plots of 4 m^2. Five samples of soil and fruit of *C. spinosa* were taken from the plots to measure the heavy metals contents (Fe, Cu, Ni, Mn, Zn, Cd and Pb). T-test analysis demonstrated significant differences between two contaminated and uncontaminated sites for the amounts of stem length, fruit length and width, leaf length and width, coverage and density of rootstocks ($P < 0.01$). The highest amounts of stem length (162.5 cm), leaf length and width (2.4 and 2.7 cm), fruit length and width (3.7 and 2.5 cm), coverage (17.1%) and density (219 plant/ha) were found in the uncontaminated site. There was the higher heavy metals content in the fruit as Zn > Fe > Mn > Pb > Cd > Cu > Ni and in the soil as Zn > Fe > Mn > Ni > Cu > Pb > Cd. The results of the T-test showed that there was a significant difference between the amounts of Cu, Ni and Mn, Fe, Cd and Pb ($P < 0.01$) and Zn ($P < 0.05$) in contaminated and uncontaminated sites. Based on the obtained results, it was suggested to cultivate this species in contaminated areas with heavy metals for phytoremediation and soil stabilization, and in uncontaminated areas, for medicinal, food and fodder purposes.

Keywords: Rangeland; Industrial pollution; Contaminated soils; Toxic metals; Phytoremediation

Introduction

Medicinal plants are used as a common way of treating diseases worldwide. *C. spinosa* has a special status in traditional medicine because of its compounds such as flavonoids, phenol and antioxidant activity in different organs (Saberi et al., 2022a). Environmental conditions affect the therapeutic properties of this species by affecting the amounts of these compounds. For example, in this salinity-tolerant species, different salinity treatments had no significant effects on the activity of four antioxidant enzymes SOD, POD, CAT, and APX while the activity of these enzymes significantly increased under drought stress conditions (Afzali et al., 2023).

Plants that grow in industrial areas have different amounts of heavy metals such as Fe, Cu, Ni, Mn, Zn, Pb, and Cd while the accumulation of these elements in species of the same area is also different. The plants are good sources of heavy metals bioaccumulation. On the one hand, the accumulation of metallic elements is a desired feature of phytoremediation. However, it may be dangerous when the plants are used as food or as a therapeutic agent in traditional medicine (Barthwal et al., 2008). It seems potential maximum of heavy metals concentration in hyperaccumulator plants to be the main factor phytoremediation of contaminated sites. However, phytoremediation also depends on plant dry matter yield, and soil-to-plant transfer coefficient (Antoniadis et al., 2017). The dangerous effect of metals on human's decreases approximately in the following order: Hg > Cu > Zn > Ni > Pb > Cd > Cr > Sn > Fe > Mn > Al (Melkaoui et al., 2023).

Phytoremediation process causes the toxicity of hyperaccumulators due to the accumulation of rare elements in plant organs and the reduction of plant growth in the presence of these elements in the soil. As a result, the efficiency of plants to remove heavy metals from the soil decreases. Nevertheless, this method has been widely used due to the high rate of absorption of heavy metals, lower cost compared to traditional methods, maintenance of soil structure and less adverse environmental effects (Rostami and Azhdarpoor, 2019). There are two strategies in phytoremediation. The first strategy is phytostabilization, in which resistant plants are used to stabilize heavy metals. Phytostabilization involves reducing the mobility, toxicity and bioavailability of heavy metals within a limited area through root accumulation or precipitation within the rhizosphere (Martínez-López et al., 2014). Using this technology, metal pollutants are reduced without direct removal from the site and do not enter the water cycle and food chain. Phytoextraction is another strategy in which heavy metals are absorbed from soil by plant roots. In this method, hyperaccumulators are used to clean pollutants. Then, these plants are collected from the contaminated area and moved to non-usable areas (Anoopkumar et al., 2020; Siyar et al., 2022).

Pollutants have a great effect on the accumulation of heavy metals in plants. Some heavy metals at low concentrations stimulate biological processes but become toxic at the threshold concentration. Heavy metals can cause severe phytotoxicity and may act as a powerful force for the evolution of a tolerant plant community (Moameri et al., 2017). These metals accumulate at different levels through food chains and can cause problems for human health (Rai et al., 2019). Therefore, medicinal plants have to be tested to determine heavy metal contents before use in traditional medicine and/or pharmacy.

According to the World Health Organization (WHO) research, about 80% of people use medicinal plants to treat their diseases directly and indirectly (WHO, 1998). In developing countries, people use medicinal plants to treat many diseases because of lacking medical facilities. Many essential drugs are natural products or derived from natural products. Over one-third (39.1%) of all Food and Drug Administration (FDA)-approved drugs have a natural origin (Boy et al., 2018).

Today, with the increasing urbanization, agricultural and industrial activities, the concentration of heavy metals in the environment has increased. These metals enter the human food chain through various ways such as extraction and melting of metallic mineral stones, volcanic dust, foundry industries, automobile battery sludge and sediment, paint, landfills, and accumulation of waste and sewage (Fan et al., 2003; Alloway, 2013; Onakpa et al., 2018). Some elements such as Pb, Hg, Sn, As, and Cr do not eliminate in the human body and accumulate in living tissues. The most critical point about heavy metals is that they could change during digestion or stored in animal tissues but could not metabolize.

Various methods have been used to treat soils contaminated with heavy metals (Alaboudi et al., 2018), but most of them are expensive and sometimes have adverse effects on the surrounding environment (Ent et al., 2012). Phytoremediation is a widely used, cost-effective and environmentally friendly method that increases biodiversity by reducing the impact of pollutants on the environment and improving soil structure (Sut-Lohmann et al., 2023). Since different plant species are very different in their ability to accumulate metals, the most important issue in phytoremediation is the selection of the appropriate species with environmental conditions (Monei et al., 2021).

In Iran, there are 875 native and planted medicinal plant species. About 1,200 aromatic species are also known, but their medicinal properties are unknown (Mozaffarian, 2013). *Capparis spinosa* is one of the most widely used medicinal plants in most parts of Iran. It is a valuable species of Capparaceae family with medicinal, edible, forage and ornamental properties. According to Flora of Iran, the *Capparis* genus in Iran has five species: *C. cartilaginea*, *C. decidua*, *C. mucronifolia*, *C. parviflora* and *C. spinosa* (Saghafi Khadem, 2000). *C. spinosa* is a herbaceous perennial with very long roots $10-12$ m long and numerous prostrate branches up to 1.5 m long, which sometimes lives up to thirty years. The leaves are ovate or oval, sometimes pointed, $10-60$ mm long and $10-40$ mm wide; flowers $5-8$ cm in diameter, solitary, white, pink, or rarely pale yellowish; and the oblong-ovoid capsule is $25-50$ mm long (Bussmann et al., 2020). This species is found in the semi-arid climate of the Mediterranean region at altitude of less than 1000 m.a.s.l, with annual temperature of more than 14 °C and annual precipitation of more than 200 mm (Chedraoui et al., 2017), and in Irano-Turanian and Saharo-Sindian regions at altitude of $0-2500$ m with annual temperature $11-27$ °C and annual precipitation $45-700$ mm (Najafian et al., 2021). This species can withstand absolute minimum temperature of -25 °C and absolute maximum temperature of 50 °C.

There are a lot of flavonoids, alkaloids, phenolic acids, fatty acids, and antioxidants in *C. spinosa* (Annaz et al., 2022). One of the main reasons for these biochemical studies is its medicinal properties such as treating inflammation, rheumatism, diabetes, pain and fever, cancer, infection, and hypertension. It is a blood lipid-lowering agent, liver protective, antiallergic, anticoagulant, diuretic, and immune system regulator (Lansky et al., 2013; Vahid et al., 2017).

There are few studies on the accumulation of heavy metals in different organs of *C. spinosa*, including Ghaderian et al. (2007), Özcan (2008), Niaz et al. (2013), Shah et al. (2013), Vaidya et al. (2017), Al Khateeb (2018), Randive and Jagtap (2019), Lashkari Sanami et al. (2023), and Mohaisen and Sirhan (2023). These studies indicated that plants in contaminated areas accumulated more heavy metals compared to uncontaminated areas. In these studies, the amounts of heavy metals in aerial organs and roots of *C. spinosa* were measured, but the concentration of metals in

its fruits was not determined.
This study aimed to investigate the potential of *C. spinosa* for phytoremediation of soils contaminated with heavy metals in the rangelands around the industrial factories of Hashtgerd, Iran. For this purpose, the concentrations of metals lead, zinc, nickel, cadmium, manganese, copper and iron in the soil and its fruit were measured in contaminated and uncontaminated areas where this species was present. Although all the organs of *C. spinosa* have medicinal properties (Upadhyay, 2011; El-Ansari et al., 2018), the medicinal and nutritional value of this species is mainly in its fruit. Therefore, it is important to determine the concentration of heavy metals in its fruit, because it is directly related to the health of local people.

Materials and methods

Study area

This study was carried out in the Hashtgerd region at longitude 50º44′ E and latitude 35º58′ N located in Alborz province, Iran in 1500 m.a.s.l. elevation (figure 1). The mean annual precipitation and temperature are 248 mm and 14 ºC, respectively. The climate of this region is cold and dry in the Emberger classification and dry in the De Martonne classification (Shirazi and Nateghi, 2020). Hashtgerd industrial town has many factories such as the construction industry, automotive parts, industrial pumps and wastewater, which have influential factors in increasing the soil pollution of the region with heavy metals.

Sampling method

The contaminated area (Site 1) in the south of Hashtgerd Industrial Town and the uncontaminated area (Site 2) in north of Chaharbagh, Savojbolagh district (Hashtgerd) of Alborz province were selected for sampling (figure 1). At each site, ten plots of 2 m × 2 m (4 m^2) were randomly established. Plot size was determined based on at least twice the canopy cover of the largest rootstock of *C. spinosa* in the habitat (Saberi et al., 2022b). In each plot, the number of rootstock of this species was counted and the density was calculated in terms of number per hectare. The length of the stem and two perpendicular diameters of the rootstock of *C. spinosa* were measured using a tape measure, and the canopy cover was determined by circle area ratio. From each plot, five leaves and fruits were randomly picked and mixed with samples selected from other plots. Then, among these samples, 20 leaves and fruits were randomly selected and their length and width were measured with a ruler. Five soil and fruit samples were taken from the plots to measure the heavy metal contents. The soil samples were collected from depth of $8-10$ cm. Field sampling was done in June 2020. Soils were air-dried and ground to pass through a -80 mesh sieve (< 190 μm). Then, they were oven-dried at 70 °C to constant weight.
Heavy metals were analyzed using Atomic Absorption Spectrophotometry. The standard chemicals including 65% nitric acid (HNO_3), 30% hydrogen peroxide (H_2O_2), and 70% perchloric acid ($HClO_4$) were used to prepare the required

Figure 1. Geographical location of the study area in Iran and Alborz province; Contaminated and uncontaminated areas are marked with red and black stars, respectively.

solution for the analysis of *C. spinosa* fruit and soil. All the glass containers were immersed in the nitric acid solution overnight to reduce the likelihood of a trial error. The containers washed with ionized water and twice with distilled water. To clean the fruits from dust, pollution, and other additives, they were washed with running water and then with ionized water. The fruits were dried in the shade and at room temperature (22 – 25 °C) for 2 weeks and then powdered with agate mortar. One gram of fruit powder dissolved in a uniform solution of HNO_3 and H_2O_2 (2:1 ratio). To increase the solubility, the solution heated to 130 °C to reduce the volume to 3 mL. After cooling, the residual solution transferred to a 25 mL container using a Whitman 42 filter paper and diluted (Shah et al., 2013). In addition, 1 g of soil samples dissolved in 10 mL HNO_3 and kept for 24 hours. After adding 5 mL of $HClO_4$ to the soil solution, it was heated to decrease the volume of the solution to 3 mL. The prepared solution was transferred to a 25 mL container and diluted (Adelekan and Abegunde, 2011). The obtained solutions were analyzed by Atomic Absorption Spectrophotometry (model PG-900) using acetylene flame and nitrogen oxide (N_2O) at 2700 °C temperature.
T-test was used to determine statistically significant differences between the mean of two sites for morphological traits, and heavy metals contents of soil and fruit of *C. spinosa* by SPSS ver.26 software.

Results

T-test for morphological traits showed that there were significant differences between the two studied sites for stem length, fruit length and width and coverage, leaf length and width and density of rootstocks ($P < 0.01$). Means comparison of the morphological traits demonstrated that their amounts are higher in the site-2 compared to the site-1 (Table 1). For example, the maximum and minimum stem lengths with an average of 162.5 and 92.4 cm were obtained in site-2 and site-1, respectively. Also, the coverage and density of rootstocks in site-2 were determined as 18.1% and 219 rootstocks per hectare, respectively while in site-1, it was 13.2% and 136 rootstocks per hectare (Table 1).
T-test for heavy metals in the fruit of *C. spinosa* indicated that there were significant differences between the two studied sites for Cd and Zn, Cu, Fe, Mn and Ni ($P < 0.01$), and Pb ($P < 0.05$). This means comparison of the heavy metals showed that their amounts are higher in site-1 compared to site-2 (Table 2). There were the most amounts of heavy metals in the fruit of *C. spinosa* as Zn > Fe > Mn > Pb > Cd > Cu > Ni, so Zn with the highest content, 118 and 102.4 mg/kg, and Ni with the lowest content, 1.49 and 0.32 mg/kg were found in site-1 and site-2, respectively (Table 2). Therefore, *C. spinosa* stores these essential and non-essential elements at different concentrations.
T-test for heavy metals in the soils of *C. spinosa* showed that there were significant differences between the two studied sites for Cu, Fe, Mn and Ni ($P < 0.001$), Zn ($P < 0.01$), and Cd and Pb ($P < 0.05$). Means comparison of the heavy metals demonstrated that their amounts are higher in the site-1 compared to the site-2 (Table 3). There were the most amounts of heavy metals in the soil of *C. spinosa* as Zn > Fe > Mn > Ni > Cu > Pb > Cd. Therefore, Zn with the highest content, 1346.8 and 1180.3 mg/kg, and Cd with the lowest amount, 12.2 and 7.5 mg/kg were found in site-1 and site-2, respectively (Table 3).

Discussion

Capparis spinosa is known as a very drought-resistant plant in arid and semi-arid regions. This species has an extensive and deep root system so in some areas, the length of the root is reported to be 1.5 – 2.3 meters (Izadi Khajeloo et al., 2016; Saberi et al., 2022b). In the previous report, it was mentioned that the root length was 6 – 10 meters, which constituted 65% of the total biomass of the plant (Sozzi and Vicente, 2006). Therefore, the root of the plant penetrates deep into the soil to access water and deal with drought, and as a result, it can withstand harsh environmental conditions. Although the height of the plant is about 0.4 – 1 m (Izadi Khajeloo et al., 2016; Khoshsima et al., 2017; Saberi et al., 2022b), according to the results of the present research, its long and creeping stems in site-2 to site-1 are 1.1 – 2.1 and 0.6 – 1.3 m, respectively, and 2 – 4 m has been reported in other studies (Chedraoui et al., 2017). The density of *C. spinosa* in site-2 to site-1 of Hashtgerd region is relatively high so that the number of its rootstocks in these regions is 110 – 340 and 70 – 200 rootstocks per hectare, respectively. The density of this species is reported to be higher in Ardabil region than in Hashtgerd region due to the increase in annual rainfall and decrease in annual temperature. In this region, the density of this species is 700 – 1700 (Izadi Khajeloo et al., 2016) and 215 – 1415 (Motamedi et al., 2020) rootstocks per hectare. The long stems of *C. spinosa* along

Table 1. Mean and standard deviation amounts of morphological traits of *C. spinosa* in site-1 and site-2.

Morphological traits	Site-1	Site-2	P-value
Stem length (cm)	92.4 ± 22.5^b	162.5 ± 32.2^a	**
Leaf length (cm)	2.76 ± 0.86^b	4.22 ± 1.19^a	**
Leaf width (cm)	1.56 ± 0.38^b	2.76 ± 0.93^a	**
Fruit length (cm)	2.30 ± 0.46^b	3.74 ± 0.46^a	**
Fruit width (cm)	1.59 ± 0.26^b	2.52 ± 0.39^a	**
Coverage (%)	13.2 ± 2.15^b	18.1 ± 2.79^a	**
Density (n/ha)	136 ± 44.05^b	219 ± 75.78^a	**

** = significant at 0.01 probability level.

Table 2. Mean and standard deviation of heavy metals contents of fruit in site-1 and site-2 in mg/kg.

Heavy metals	Site-1 (mg/kg)	Site-2 (mg/kg)	P-value
Cd	2.79 ± 0.15^a	1.29 ± 0.16^b	**
Cu	1.59 ± 0.31^a	0.42 ± 0.15^b	**
Fe	92.9 ± 2.92^a	84.6 ± 2.35^b	**
Mn	19.51 ± 1.60^a	7.59 ± 0.62^b	**
Ni	1.49 ± 0.18^a	0.32 ± 0.11^b	**
Pb	8.11 ± 0.72^a	5.39 ± 0.56^b	*
Zn	118.00 ± 4.10^a	102.39 ± 3.72^b	**

*, ** = significant at 0.05 and 0.01 probability levels.

with the large number of shoots and their sub-branches as well as the high density of the rootstocks have increased its coverage in natural habitats so that the coverage of this species in site-2 to site-1 of Hashtgerd region is $15-22$ and $11-18\%$, respectively. The coverage in other areas such as Ardabil (Motamedi et al., 2020) and Sistan Plain (Saberi et al., 2022b) was reported as $13-30$ and $7.7-10.7\%$, respectively. Therefore, *C. spinosa* covers a relatively large area of soil in natural habitats so that it provides good protection against water and wind erosion. As a result, this species can be used in contaminated and uncontaminated areas to prevent soil erosion, and in uncontaminated areas as a medicinal and forage plant.

C. spinosa is an important medicinal plant, but due to developmental projects in the Hashtgerd region, pollution and the detrimental effects of industry, agriculture, and urban waste on the growth and composition of the essential oil in this species are out of acceptance level. The study of morphological traits of *C. spinosa* showed that the mean of these characteristics significantly reduced from site-2 to site-1. The plant's growth in site 1 has been less due to the presence of toxic heavy metals.

Cadmium, as a non-essential heavy metal, is highly toxic even at low concentrations. This element causes neurotoxin, hypertension, cancer, and liver and kidney dysfunction (Adelekan and Abegunde, 2011; ATSDR, 2012). Cd content of *C. spinosa* fruit and soil in site-1 indicated a significant increase compared to site-2. The maximum Cd concentration in the soil of site-2 and site-1 was 7.7 and 12.4 mg/kg, and in the *C. spinosa* fruit, 1.3 and 2.9 mg/kg, respectively. Some researchers reported that the Cd content of *C. spinosa* leaves increased from $0.07-0.3$ mg/kg in uncontaminated areas to $0.1-1.4$ mg/kg in contaminated areas (Niaz et al., 2013; Shah et al., 2013; Al Khateeb, 2018). These results are in agreement with the findings of the present study. While WHO and Chinese Pharmacopeia have declared the maximum permissible level of cadmium in medicinal plants as 0.3 and 1 mg/kg, respectively (Luo et al., 2021), the amount of this metal in *C. spinosa* fruit and soil is very high in Hashtgerd region. The high amount of Cd in *C. spinosa* fruit indicates its excessive absorption by this plant, and using it is very dangerous for the health of local people.

Copper is an essential element for many enzymes, but in high concentrations, it causes various diseases in the human digestive system and liver (ATSDR, 2022). The maximum Cu concentration in the soil of site-2 and site-1 was 21.3 and 29.2 mg/kg, respectively. Due to increased Cu uptake by the plant root in the contaminated soil, Cu concentration in the *C. spinosa* fruit increased from 0.5 mg/kg in site 2 to 1.7 mg/kg in site 1. Some researchers showed that the Cu content of *C. spinosa* leaves increased from 12.5 and 6.4 mg/kg in uncontaminated areas to 15.5 and 15.4 mg/kg in contaminated areas (Niaz et al., 2013; Shah et al., 2013). It was also found that the Cu content of *C. spinosa* leaves increased from $2.4-3.8$ mg/g in uncontaminated or low-contaminated areas to 16.2 mg/g in contaminated areas (Al Khateeb, 2018). These results are in agreement with the findings of the present study. The permissible limit of Cu in medicinal plants is 10 mg/kg, and human requirement is 3

Table 3. Mean and standard deviation of heavy metals contents of soil in site-1 and site-2 in mg/kg.

Heavy metals	Site-1 (mg/kg)	Site-2 (mg/kg)	P-value
Cd	12.19 ± 0.96^a	7.52 ± 0.81^b	*
Cu	29.11 ± 1.03^a	21.22 ± 0.82^b	**
Fe	1149.37 ± 59.77^a	1022.29 ± 55.13^b	**
Mn	696.39 ± 28.97^a	594.3 ± 30.88^b	**
Ni	32.19 ± 1.92^a	30.39 ± 1.91^b	**
Pb	17.39 ± 1.26^a	10.81 ± 0.89^b	*
Zn	1346.81 ± 55.13^a	1180.31 ± 47.82^b	**

*, ** = significant at 0.05 and 0.01 probability levels.

mg/day (WHO, 2007).

Iron is the most abundant and essential element for all plants and animals, but in high concentrations, it causes oxidative damage and cell death in humans (Ward et al., 2014). The maximum Fe concentration in the soil of site-2 and site-1 was 1123.3 and 1158.1 mg/kg, respectively. As a result, the concentration of Fe in fruit increased from a maximum of 85.9 mg/kg in site 2 to 93.7 mg/kg in site 1. Some researchers reported that the Fe content of *C. spinosa* leaves increased from 47.5 and 49.9 mg/kg in uncontaminated areas to 48.8 and 50.3 mg/kg in contaminated areas (Niaz et al., 2013; Shah et al., 2013). It was also found that the Fe content of *C. spinosa* leaves increased from 24 – 88 mg/g in uncontaminated or low-contaminated areas to 308 mg/g in contaminated areas (Al Khateeb, 2018). These results are in agreement with the findings of the present study. The permissible limit of Fe in medicinal plants is 20 mg/kg, and the human requirement is 10 – 28 mg/day (WHO, 1998).

Manganese is an essential element for the synthesis and activation of lots of enzymes, accelerating the synthesis of protein, vitamins C and B, and improving the immune system in the human body. Mn deficiency causes diseases, including growth disorders, skeletal defects, reduced fertility, congenital disabilities, and changes in the metabolism of lipids and carbohydrates in humans and animals (Li and Yang, 2018). Mn content of soil and fruit of *C. spinosa* demonstrated a significant increase in site-1 compared to site-2. So the maximum Mn concentration in the soil of site-2 and site-1 was 599.4 and 703.4 mg/kg, and in the *C. spinosa* fruit, 7.8 and 19.8 mg/kg, respectively. Some researchers showed that the Mn content of *C. spinosa* leaves increased from 4.9 and 8.1 mg/kg in uncontaminated areas to 6.1 and 12 mg/kg in contaminated areas (Niaz et al., 2013; Shah et al., 2013). Meanwhile, the amount of Mn in the soil of the uncontaminated area increased from 111 to 134.8 mg/kg in the contaminated areas (Shah et al., 2013). These results are in agreement with the findings of the present study. The permissible limit of Mn in medicinal plants is 200 mg/kg, and the human requirement is 11 mg/day (WHO, 1998).

Nickel is an essential element for plants and animals, but it is very toxic at high concentrations. It can cause severe diseases in humans such as allergies, cardiovascular and kidney diseases, lung fibrosis, and lung and nasal cancer (Genchi et al., 2020). The maximum Ni concentration in the soil of site-2 and site-1 was 30.9 and 33.1 mg/kg, and in the *C. spinosa* fruit, 0.3 and 1.5 mg/kg, respectively. Some researchers indicated that the Ni content of *C. spinosa* leaves increased from 1 and 0.9 mg/kg in uncontaminated areas to 3.7 and 1.7 mg/kg in contaminated areas (Niaz et al., 2013; Shah et al., 2013). In addition, the amount of Ni in the soil of the uncontaminated area increased from 3.9 to 5.3 mg/kg in contaminated areas (Shah et al., 2013). These results are in agreement with the findings of the present study. The permissible limit of Ni in medicinal plants is 1.5 mg/kg, and human requirement is 1 mg/day (WHO, 2007).

Lead has no biochemical or physiological importance for humans and it is a toxic pollutant. This element causes anemia with increased blood pressure, severe damage to the brain and kidneys, tumor formation, abortion, reduced fertility in men, blood disorders, and damage to the nervous system (Wani et al., 2015). Pb content of soil and fruit of *C. spinosa* demonstrated a significant increase in site-1 compared to site-2. Sothat maximum Pb concentration in the soil of site-2 and site-1 was 10.9 and 17.6 mg/kg, and in the *C. spinosa* fruit, 5.5 and 8.2 mg/kg, respectively. Some researchers reported that the Pb content of *C. spinosa* leaves increased from 4.7 and 5.2 mg/kg in uncontaminated areas to 5 and 8.8 mg/kg in contaminated areas (Niaz et al., 2013; Shah et al., 2013). It was also found that the Pb amounts of *C. spinosa* leave increased from 0.5 – 0.8 in uncontaminated or low-contaminated areas to 1.6 mg/g in the contaminated areas (Al Khateeb, 2018). These results are in agreement with the findings of the present study. WHO and Chinese Pharmacopeia have declared the maximum permissible level of Pb in medicinal plants to be 10 and 5 mg/kg, respectively (Luo et al., 2021).

Zinc is an essential element in some enzymes and it has structural, regulatory, and catalytic roles. Deficiency of Zn has a detrimental effect on growth, neurodevelopment, and immunity. However, it interferes with the absorption of Cu in high concentrations (Plum et al., 2010; Adelekan and Abegunde, 2011). The maximum Zn concentration in the soil of site-2 and site-1 was 1187.9 and 1251.9 mg/kg, respectively. Due to increasing Zn uptake by root in the contaminated soil, Zn concentration in the *C. spinosa* fruit increased from a maximum of 103.1 mg/kg in site-2 to 121.1 mg/kg in site-1. The study showed that the Zn content of *C. spinosa* leaves in uncontaminated areas increased from 26.5 to 29 mg/kg in the contaminated areas (Niaz et al., 2013). It was also found that the Zn amounts of *C. spinosa* leaves increased from 44 – 68 in uncontaminated or low-contaminated areas to 155 mg/g in contaminated areas (Al Khateeb, 2018). These results are in agreement with the findings of the present study. The permissible limit of Zn in medicinal plants is 50 mg/kg, and the human requirement is 11 mg/day (WHO, 1998).

In the research conducted in this region, it was found that the concentration of trace elements (As, Co, Cr, Cu, Mo, Ni, Pb, V, and Zn) in Hashtgerd is more than the average soils of the world (Shakeri and Modabberi, 2014). These researchers believed that soil pollution is not serious in this region. However, due to the increasing industrialization, agricultural activities, and urbanization in Hashtgerd, monitoring of soil contamination is necessary in the future. Some researchers stated that the high concentration of heavy elements in the soil of Hashtgerd is often compatible with the geochemical features of the region, and industrial pollution has a local effect on a few places (Mahmoudi et al., 2016). Anyway, high concentrations of heavy metals in the soil, caused by geological structure or industrial pollutants, are dangerous for the growth of plants, especially medicinal plants.

In this region, the amount of unnecessary heavy metals in *C. spinosa* fruit is very high and toxic. For example, the Cd content of *C. spinosa* fruit in uncontaminated and contaminated sites was about 4 and 10 times higher than the accepted level, respectively. In addition, Pb content in

C. spinosa fruit in uncontaminated and contaminated sites was about 13 and 19 times higher than the allowed level, respectively. Of course, the content of essential elements (except Mn) is also considerable in *C. spinosa* fruit so that Ni, Fe, Cu, and Zn in the uncontaminated site were 20.6, 4.3, 2.1, and 2.1 times higher than the allowed content and 22, 4.7, 2.9, and 2.4 times higher than the allowed content in the contaminated site, respectively. Accordingly, the use of *C. spinosa* fruit in Hashtgerd is not recommended because it has irreversible side effects on the health of the native people. Heavy metals cause toxicity and negative pharmacological effects.

Conclusion

The plants absorb heavy metals by roots from the soil. These elements accumulate in some parts of medicinal plants and enter the biological cycle after consuming by animals and humans. Local people traditionally use medicinal plants to treat various diseases, and thus, they receive heavy metals. Thus, the toxicity of heavy metals in a human causes side effects instead of a cure for disease. The content of heavy metal accumulation varies in different parts of plants. It depends on the chemical composition of the soil and plant uptake. Therefore, it is essential to analyze any medicinal plants such as *C. spinosa* for toxicity of heavy metals before use by local people. The high content of heavy metals in *C. spinosa* in contaminated areas strengthens this idea that its potential to absorb heavy metals can be used for soil purification by planting in contaminated areas. Therefore, this species can be used to remediate contaminated areas and prepare the soil for the cultivation of subsequent crops; provided that the aerial and underground organs of these plants are moved from the contaminated area to the unusable area. These crops should not be used as medicine or food.

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Authors contributions
Authors have contributed equally in preparing and writing the manuscript.

Availability of data and materials
The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Conflict of interests
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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