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The usage of fulvic acid and zeolite on the absorption of cadmium in *Spinacia oleracea* var. inermis.

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ABSTRACT

Purpose: One of the problems in today's world is the pollution of the environment with heavy metals, which extraction of metals from mines, industrial and agricultural activities have caused significant pollution of soils and waters to metals. **Research method:** Fulvic acid at 15, 30, and 60 L ha-1 and zeolites at 1000, 2000, and 4000 kg ha-1 have formed the treatments, and the cadmium content was added to the region. The absorbance of cadmium, zinc, and nitrogen elements, as well as the dry weight of aerial organs and root of Spinacia oleracea var. inermis, was investigated. **Findings:** The application of fulvic acid and zeolite resulted in higher absorption of the elements and higher biomass weight compared to the control. In this case, fulvic acid at 30 L ha-1 and zeolite at 4000 kg ha-1had the best results own, and the accumulated amounts of cadmium and zinc in the root of the Spinach were higher than that of the aerial organs. Due to its acidic nature, fulvic acid caused higher mobility of elements than zeolite. Also, the application of the treatments caused better vegetative growth of the plant and moderation of the effects of stress. **Limitations:** There was no restriction on conducting this research. **Originality/Value**: Based on the results, the use of fulvic acid and zeolite can significantly increase the absorption of cadmium toxic metal from the organs of the Spinach plant and help the further release of this element from the soil.

INTRODUCTION

Today, environmental pollution of heavy metals is one of the significant environmental hazards increasing due to the uncontrolled entry of various types of waste and industrial wastes. Because of its high durability properties in living tissue, these heavy metals can contaminate the food chain in organisms and humans, and subsequently leave many harmful effects on human health (Wang & Chen, 2009). The existence of rich, heavy metal resources and deposits in nature does not only increase their concentration in the environment of those areas, but during the extraction, transportation, and processing of metal resources, some of these elements are dispersed and environmental pollution with them. Mercury, cadmium, chromium, and lead are the metals that pose the most significant risks to human health even in small amounts (Carolin et al., 2017). The toxicity of plants with cadmium is up to 20 times higher than other metals (Jafarnejadi et al., 2011). Cadmium can enter the air through various industrial activities such as mining, fossil fuels, and industrial waste, and then immediately into the soil and water over long distances. Also, excessive use of fertilizers containing phosphorus leads to a high accumulation of cadmium and soil toxicity with this substance (Lefèvre et al., 2009).

Cadmium metal is of particular importance due to the numerous environmental impacts on the one hand and high relative mobility in the soil and plant system. On the other hand and has received considerable attention in innumerable studies. The accumulation of this element in the soil, in addition to the harmful effects on plants and soil, can enter the human body through plant products and foodstuffs. Cadmium with semicircular osteogenesis of 20 years and accumulation in the liver and kidneys, causes insufficiency in this section, cardiovascular diseases, elevated blood pressure, bone diseases, swollen lice and so on (Mauskar, 2007). Other harms of cadmium metals in the human body include loss of sense of smell, cancer, and stroke (Lalor, 2008). The results of various researches show that cadmium metal is highly toxic to the plant and the permissible limit of this element in the plant is 0.05 to 0.2 mg kg^{-1} dry weight (Xioa et al., 2008). Ali et al. (2015) also stated in their research that exposing the plant to high cadmium concentrations can cause ultrastructure changes in the cell, such as damage to the nucleus and membranes, thereby reducing plant growth.

In this research, we tried to use the phytoremediation method and materials that are present in nature to extract and purify the soil from cadmium. So to this end, they were selected for this purpose by using fulvic acid and zeolite. Phytoremediation is one way purification of soil contamination has been widely considered in recent decades and is a sustainable, cheap, simple, and eco-friendly way compared to other refining methods. Phytoremediation is one of the valuable technologies in which the ability of plants and their various organs to absorb, transport, and accumulate soil contaminants, including heavy metals, is used (Nadeem et al., 2014). In this method, the soil is refined from heavy metals and semi-metals by hyper accumulator plants with high biomass (Zhao & McGrath, 2009), and toxic elements in the soil enter the roots, branches, leaves, and even vacuoles of these plants (Reza et al., 2020). Many researchers have investigated the role of heavy metals in generating various contaminations related to the toxicity of these elements, and the application of phytoremediation methods to counteract the adverse effects of these metals, and different views have been proposed (Jafarnejadi et al., 2011). The plants grown in these conditions should not be used by humans and livestock. These plants have high levels of these metals in their bodies and roots (Asadi Kapourchal et al., 2009). El-Mahrouk et al. (2019) reported that the use of *Salix mucronata* could have a positive effect on reducing the heavy metals in the soil. In another study, Ratnasari et al. (2020) investigated the ability of *Ipomoea* *reptans* Poir to absorb cadmium and stated that by planting this plant at the appropriate density, up to 57.7% of the amount of cadmium in the soil can be decreased.

Fulvic acid is a derivative of humus material. This organic acid is a natural absorbent with large amounts of carbon functional groups. Humic acids are one of the humus substances that are found in abundance in nature. Also these materials play an essential role in the formation of organic matter in soils. Humic acids can combine with metal ions to form stable complexes (KluIáková & Pavlíková, 2017). The use of humic materials has positive effects on the physical, chemical, and biological properties of soil and directly effects on the physiological and biochemical stages of plants (Sangeetha et al., 2006). Therefore, humic acid and fulvic acid, in addition to having a significant effect on increasing plant growth and yield, can also play an essential role in reducing the harmful effects of cadmium on plant growth (Farouk et al., 2011). KluIáková and Pavlíková (2017) examined the efficiency of lignitic humic acids in the absorption of cadmium, copper, lead and zinc and concluded that the absorption efficiency of humic acids for these elements is very high (more than 90%). In this regard, the results of research by Zhang et al. (2013) show that humic acid and fulvic acid have a stimulating effect on the accumulation of cadmium metal in plant tissues, and these substances can be an influential factor for refining and extracting heavy metals such as cadmium from soil.

On the other hand, one of the absorbents that have been studied in recent years is zeolites. The chemical formula of these minerals is Ma/n (AlO_2) x (SiO_2) yw H_2O. The zeolite belongs to the family of hydromorphone aluminosilicates of the alkaline and alkaline earth metals with a crystalline structure (Leung et al., 2007). Zeolite is an aluminosilicate with a three-dimensional structure that is joined together by oxygen atoms. This mineral has a high cation exchange capacity, large surface spaces, temperature resistance, and high heat capacity (Andronikashvili et al., 2009). Of course, environmental and climatic conditions, type, and size of zeolite particles are the most critical factors for the persistence and effectiveness of zeolite in soils. Besides, there are many differences in the cation exchange capacity of natural zeolites due to their different structure and ion adsorption abilities (Contin et al., 2019). Zeolites have tiny pores that act like a solid molecular sieve and cause some cations such as ammonium to be deposited on their network due to their high cation exchange capacity. Also, they can have a nutritional role for the plant and result in better plant growth and yield in areas with low cationic potential (Polat et al., 2004). In addition to the above, zeolite is a highly effective material for clearing the soil of heavy metals for reasons such as molecular sieving properties and high ion exchange capacity (Murtić et al., 2020). The basis of the removal of heavy metals from the soil by zeolite is the cationic exchange between heavy metals and cations or the precipitation of heavy metal hydrates on the structure of zeolite (Castaldi et al., 2008). Accordingly, the results of various experiments show that zeolites can be used as high-performance adsorbents for refining heavy metals from water and soil (Vhahangwele & Mugera, 2015). Murtić et al. (2020), by examining the ability of zeolite to reduce the mobility of heavy metals in the soil, concluded that the use of zeolite could prevent the availability and mobility of cadmium in the ground and is an excellent way to stabilize this metal in the ground. The results of research studies such as Esmaeili et al. (2019) also show the high efficiency of zeolite in reducing the mobility of heavy metals in contaminated soils.

According to the above, heavy metals have many negative effects in the environment and the life cycle of living organisms. So, their stabilization or clearance from the soil is essential and necessary. Besides, the use of fulvic acid and zeolite can have many beneficial effects on plants and soils and play a positive role in clearing the ground of heavy metals. Therefore, the present study was to apply these organic substances on the uptake of cadmium by *Spinacia oleracea* var. Inermis, which is one of the most widely used leafy vegetables in the world, was performed. The sub-target of this study was to determine the growth of this plant by the application of experimental treatments.

MATERIALS AND METHODS

This study was carried out in a farm field located in Shahriyar city, in Tehran Province, Iran, in 2017. The experiment was carried out in a completely randomized design with three replications. Fulvic acid from resource Fulvimax (SWISS GROW product, made in Switzerland) was used at three levels of 15, 30, and 60 L ha^{-1}. Zeolite from the clinoptilolite type was prepared from Semnan mine and used at three levels of 1000, 2000, and 4000 kg ha^{-1}. Also, the non-application of these materials formed part of the control field. The characteristics of zeolite and fulvic acid used in Tables 1 and 2 are summarized.

Table 1. Specifications of fulvic acid used*

N (%)	Organic Matter (%)	Fulvic Acid (%)	Humic Acid (%)	C/N Ratio	pH
3.4	40.8	44	13	15.7	4.5-4.7

*Fulvimax (SWISS GROW product).

Table 2. Specimen of zeolite used*

Al_2O_3 (mg l^{-1})	SiO_2 (mg l^{-1})	K_2O (mg l^{-1})	Particle Density (g cm^{-3})	Bulk Density (g cm^{-3})	CEC (Meq 100 g^{-1})	Organic Matter (%)	EC (dS cm^{-1})	pH
11.2	65.9	2.31	2.29	1.10	98	0.29	2.7	8.1

*Zeolite from the clinoptilolite type (Semnan mine, Iran).

Seeds of Spinach (*Spinacia oleracea* var. inermis) were first washed well and disinfected and sterilized. For this purpose, the seeds were disinfected in 70% ethanol for two minutes and then in 5% sodium hypochlorite for 5 minutes. Then, the seeds were washed five times with distilled water (Wang & Oyaizu, 2009) and soaked for 24 hours. In the next stage, 4 mg kg^{-1} of cadmium per kg of soil was added from the source of cadmium nitrate to the ground containing test treatments alike. It is necessary to mention that the soil was sandy loam, and the amount of cadmium and zinc in the tested ground was 0.36 and 1.68 mg kg^{-1}, respectively. In this study, four plots were used for sowing seeds; each plot had four planting lines with a length of two meters with a row spacing of one meter. A distance of two meters was considered between the main plots. Then, Fulvic acid and zeolite treatments were applied to the soil. Different amounts of zeolite were spread evenly on the soil surface using a fertilizer sprayer. Fulvic acid was mixed with water in specific concentrations and sprayed on the soil surface with the help of sprinkler irrigation. After these steps, the soil was plowed to a depth of 50 cm so that zeolite and fulvic acid were readily available to the plant roots, and 40 seeds were sown per square meter. Irrigation was carried out in such a way that no water stress was observed during the experiment. The plants were irrigated manually every other day between and before the drying of the soil surface layer. Because the growth period of this plant is 40 days, no poison was used to deal with various diseases and pests. After the growth period of Spinach, the plant samples were taken. Then, the aerial organs were removed from the top of the soil, washed with distilled water, and dried in an oven for 48 hours at 85 °C (Khodaverdiloo & Homaee, 2008). The fresh weight and dry weight of the aerial organs and the roots were measured by digital scales. For digestion of the plant sample, 2 g of dried samples were placed in a round balloon, and 4 ml of concentrated perchloric acid, 2 ml of concentrated sulfuric acid, and 20 ml of concentrated nitric acid were added. The above solution was boiled under a hood and on a heater to reduce its volume. The next step added 20 ml of water to dissolve the sediment and reheated to reduce the volume. Then, the solution was smooth, and the volume was measured to 250 ml (Radojevec & Baskin, 1999), and the amounts of cadmium and zinc were measured by atomic absorption. Measurement of total nitrogen in the aerial organs was done by the Kjeldahl method. In the end, SPSS software was used for statistical analysis of the data, and the comparison of the meanings was made using the Duncan Multi Range Test at the probability level of 1% or 5%.

RESULTS AND DISCUSSION

According to Table 3, the effect of the studied treatments on all the studied traits was significant at 1 and 5% probability levels.

Nitrogen content in aerial organs of Spinach

Tables 4 and 5 show that the use of fulvic acid and zeolite increased nitrogen absorption significantly in treated plants compared with control. Therefore, the use of fulvic acid at 30 L ha^{-1} and zeolite at 4000 kg ha^{-1} showed the best results. These treatments have caused the accumulation of nitrogen in the amounts of 6.46 and 4.66% in the aerial organs of the Spinach plant, respectively.

Table 3. Comparison of the effect of different levels of fulvic acid on the characteristics examined in Spinach

S.O.V	df	Nitrogen in the aerial organs (%)	Zinc in the aerial organs (mg kg^{-1})	Zinc in the root (mg kg^{-1})	Cadmium in the aerial organs (mg kg^{-1})	Cadmium in the root (mg kg^{-1})	Dry weight of the root (g)	Dry weight of the aerial organs (g)
Treatment	3	3.78*	11.98**	14.20**	0.95**	1.69**	0.66*	0.94**
Error	8	7.51	10.31	4.66	15.42	13.12	3.13	0.91
Total	11							

** and *: Significantly at the probability level of 1 and 5%, respectively

Table 4. Comparison of the effect of different levels of fulvic acid on the characteristics examined in Spinach

Level of Fulvic acid (L ha^{-1})	Nitrogen in aerial organs (%)	Zinc in aerial organs (mg kg^{-1})	Zinc in root (mg kg^{-1})	Cadmium in aerial organs (mg kg^{-1})	Cadmium in root (mg kg^{-1})	Dry weight of the plant root (g)	Dry weight of the plant aerial organs (g)
Control	3.53 c	41.22 d	56.32 d	5.44 c	7.17 c	1.53 c	27.25 c
15	4.61 b	65.44 b	77.51 b	8.11 b	8.86 b	2.36 b	32.96 b
30	6.46 a	73.66 a	93.66 a	9.19 a	12.06 a	2.88 a	36.57 a
60	5.61 b	56.15 c	76.64 b	8.26 b	11.95 a	2.31 b	33.09 b

In each column, averages that have at least one common letter are in statistically similar groups with Duncan's multiple range tests at 1% possibility.

Table 5. Comparison of the effect of different levels of zeolite on the characteristics examined in Spinach

Level of Zeolite (kg ha^{-1})	Nitrogen in aerial organs (%)	Zinc in aerial organs (mg kg^{-1})	Zinc in root (mg kg^{-1})	Cadmium in aerial organs (mg kg^{-1})	Cadmium in root (mg kg^{-1})	Dry weight of the plant root (g)	Dry weight of the plant aerial organs (g)
Control	3.59 c	40.26 c	54.56 c	5.11 b	6.32 c	1.67 b	26.91 b
1000	3.70 c	45.24 b	73.43 b	7.19 a	7.93 b	1.96 a	27.11 b
2000	4.17 b	70.61 a	89.69 a	7.53 a	9.19 a	2.01 a	29.69 a
4000	4.66 a	72.27 a	90.11 a	7.80 a	9.32 a	1.98 a	29.44 a

Humic materials such as humic acid and fulvic acid have a lot of beneficial effects on soil and plants. By clamping the essential elements required for the plant, they increase their absorption and improve soil fertility and plant yield. Humic materials increase the absorption of nutrients, including nitrogen, and increase the chlorophyll content and photosynthesis of the plant, thereby increasing growth (Khayyat et al., 2007). The results of Ayas and Gulser's (2005) research have shown that humic acid improves the growth and yield of carrot plants through an increase in the amount of nitrogen absorbed by the plant. Farouk et al. (2011), in their research, have pointed out the positive effect of humic acids on the absorption of essential plant elements such as nitrogen.

Zeolites are crystalline and porous minerals. In addition to modifying the soil structure, they can cause plant growth. This vegetative effect, especially on land with lower cationic and sandy areas, can be observed (Polat et al., 2004). On the other hand, zeolite has many voids and channels in its structure. It can trap various cations such as sodium, potassium, and calcium or large molecules and cationic groups such as nitrate ions, carbonate ions, and ammonia (Zeng et al., 2010). This unique property of zeolite can reduce the conversion of ammonium ions to nitrate and thus leach nitrogen into the soil and cause better absorption of this trace element by the roots. Rosalina et al. (2019) also reported that the use of zeolite could positively increase the content of soil nutrients, including nitrogen.

Zinc content in aerial organs and root of Spinach

Tables 3 and 4 show that fulvic acid and zeolite can accumulate more amounts of zinc element in the organs of the plants compared to the control. Increasing the consumption of fulvic acid to a concentration of 30 L ha^{-1} increased the amount of this element in the aerial organs and root of the Spinach plant. The higher concentration reduced the amount of this element compared to the two previous concentrations. The results of Asik et al. (2009) indicate that humic materials can increase the absorption of components such as copper and zinc in the soil, according to the ideal properties of these materials for soil improvement and plant growth, results not expecting any more. Humic acids have a high adsorption capacity for various metals due to their low molecular weight. This binding of heavy metals to these organic acids is also related to the type of metals, but in general humic acids have a high adsorption capacity for these elements (KluIáková & Pekar, 2006). By comparing the amounts of zinc absorbed in the treatments of fulvic acid and zeolite, we find that the use of fulvic acid has caused a higher uptake of this element in the aerial organs and root of the plant. The reason for this can be attributed to the lower pH of fulvic acid compared to zeolite. The strength and performance of humic-metal complexes are directly related to soil pH so that humic acids increase the uptake of heavy metals at low soil pH and decrease at high soil pH. This indicates different mechanisms of action of humic acid and fulvic acid in acidic and alkaline soil conditions (Boruvka & Dradek, 2004). Therefore, we must keep in mind that since fulvic acid has an acidic pH and zeolite has an alkaline pH, higher uptake of zinc in fulvic acid treatments is not unexpected and confirms the results of previous researchers. Zhang et al. (2013) reported in their research that the effect of humic acid and fulvic acid on the availability of cadmium and lead metals is directly related to the soil pH of the region so that with decreasing pH and acidic conditions, the absorption of this elements is increased by the plant.

In zeolite-containing treatments with increasing the amount of this substance, the amount of accumulated element in the tissues investigated increased compared to the control plants, which is 2000 and 4000 kg ha^{-1} was not statistically significant. The reason for this may be attributed to the presence of this metal in the soil. According to Tables 3 and 4, the amount of accumulated zinc in Spinach plant aerial organs is less than that got in the roots, which are consistent with the results obtained from some studies such as *Raphanus sativus* L. for absorption and purification of the element lead from soil (Asadi Kapourchal et al., 2009). Murtić et al. (2020) also reported that the corn plant has a greater tendency to store the heavy metal cadmium in the roots than in its shoots. Therefore, based on the above results, the Spinach plant has a greater tendency to absorb zinc in the roots than the aerial organs, and the use of fulvic acid and zeolite also help to better absorb this heavy metal in the organs of this plant.

Cadmium content in aerial organs and root of Spinach

According to Tables 3 and 4, all treatments containing fulvic acid and zeolite exhibited higher levels of cadmium uptake compared to control. Fulvic acid at 30 L ha-1 caused the highest accumulation of cadmium in the aerial organs and root of the Spinach with 9.19 and 12.06 mg kg^{-1}, respectively. This concentration of fulvic acid is more effective than other amounts of this organic matter. The behavior of heavy metals in the soil is related explicitly to the soil properties of the region, so the presence of organic matter and especially humic substances can directly affect the availability, mobility, and complexity of heavy metals in the soil (Barančíková & Makovníková, 2003). Fulvic acid is a natural absorbent of heavy metals with large amounts of carbon functional groups. The results of research by Zhang et al. (2013) show that humic acid and fulvic acid have a stimulating effect on the accumulation of cadmium metal in plant tissues. Also, these substances can be an influential factor for refining and extracting heavy metals such as cadmium from the soil. Therefore, humic substances can clean the ground from heavy and toxic metals (KluIáková & Pavlíková, 2017). Humic materials with their low pH, cause the mobility of heavy metals and their better absorption by the plant. Thus, fulvic acid and humic acid clean and improve the soil.

Treatment of 4000 kg ha^{-1} of zeolite, although not significantly different from other values of this mineral, showed higher uptake of cadmium compared with the control. Reducing the concentration of heavy metals by mineral clays such as zeolite in areas with soils contaminated is one of the valuable solutions in this field (Murtić et al., 2020). The high potential of zeolite in reducing the mobility and availability of heavy elements is directly related to the structure and its ion-exchange mechanism (Murtić et al., 2020). Zeolite has an aqueous aluminosilicate framework that can absorb positive cations such as sodium, potassium, calcium, and magnesium. These positive cations can be exchanged with some heavy metal cations, and thus zeolite can absorb these metals and reduce their mobility and availability (Golomeova & Zendelska, 2016). Accordingly, Esmaeili et al. (2019) also explained that zeolite could be a suitable and low-cost adsorbent to reduce the mobility of heavy metals in soil. However, the lower pH of fulvic acid has caused more mobility of this element and its better absorption compared to zeolite. Since cadmium is a metal that has high mobility in the soil, the mobility and availability of this element are more elevated in acidic soils. Therefore the risk of contamination of plants with this metal in this type of soil is more elevated than alkaline soils. Thus, the increased mobility of cadmium in the ground makes it easier for the plant to refine this element because it accumulates more in the roots and can be easily transferred to plant organs and removed from the soil (Di Toppi & Gabbrielli, 1999). However, the accumulation of cadmium in the plant is related to various factors such as soil pH, soil metal concentrations, and also the amount of organic matter (Zhang et al., 2013).

In addition to the material mentioned above, mention of a critical point can also affect the absorption of heavy metals in this experiment. It is the soil texture of the loamy, sandy region of the area. Sandy loamy and sandy loamy clay soils compared with clay loam soils, has a higher leakage of metals. These soils have a low cation exchange, less food, clay, and more sand than silt clay loamy silt. As the amount of organic matter in a soil is higher, heavy metal fixation in the soil also increases as organic metal complexes. This matter can lead to a decrease in the mobility of heavy metals in the ground (Foley, 2002), and this mobility of metals further affects the absorption of cadmium and zinc by fulvic acid and zeolite. On the other hand, as the percentage of sand in the soil is higher, there are more gaps in the ground, which can also contribute to the mobility of metals. Also, the presence of high levels of organic matter in the ground can reduce the absorption of metals (Ahlberg et al., 2006). Kaschl et al. (2002) reported that leakage of metals is different from the application of compost on soils with sandy and loamy soils. Accordingly, in loamy soils containing high clay content and higher organic matter content, compared with sandy soils, decrease the leakage of metals. These researchers have attributed this to more absorbent sites in loamy soils. Wong et al. (2007) also showed that loam, clay, and sandy loam with their pH being the same and have an alkaline state, but the amount of leakage of metals in loamy, sandy soils is higher than loamy clay. The researchers reported a low percentage of clay in nonabsorbent metals in loamy sand soils. Therefore, the physical properties of the ground and especially the portion of silt and clay have a more significant effect on chemical properties.

The dry weight of aerial organs and root of Spinach

According to Tables 4 and 5, all treatments in this experiment caused an increase in the dry weight of aerial parts and the root of the spinach plant compared to control plants. In the case of fulvic acid, the highest values of these two traits were related to a concentration of 30 L ha^{-1}. Cadmium poisoning causes damage to cell membranes, ion leakage, impaired nutrient uptake, and reduced relative leaf water content (Smiri et al., 2011). Also, high concentrations of this metal in the plant can cause severe damage to chlorophyll synthesis, protein complex formation, and the development of thylakoid membranes (Basa et al., 2014), all of which can severely affect plant growth. In this regard, Liu et al. (2020) reported that high levels of cadmium could significantly affect the dry weight and overall biomass of *Populus cathayana*. However, humic substances have many beneficial effects on the physical, chemical, and biological properties of soil (Farouk et al., 2011). These organic materials can significantly reduce stress-induced damage to plant tissues. Farouk et al. (2011) reported that increasing the concentration of cadmium in the plant strongly affects the morphological and physiological characteristics of the radish plant and reduces its growth and yield; however, the use of humic substances positively reduces the adverse effects of cadmium and helps the plant to grow better. In another study, Zhang et al. (2013) pointed to the positive impact of humic acid and fulvic acid in plant poisoning with cadmium and lead metals.

Zeolite also had higher dry weight in both the aerial organs and root at 2000 kg ha^{-1}. However, this was superior in dry weight of aerial organs with 1000 and 4000 kg ha^{-1} treatments and root dry weight with 4000 kg ha^{-1} of this material was not significant. Cadmium can reduce the growth of aerial organs of the plant by reducing the amount of chlorophyll and the activity of photosystem I (Shah et al., 2008). Oxidative stresses directly affect the rate of plant photosynthesis and subsequently cause a significant reduction in plant growth indices and overall biomass production. The results of research by Alyemeni et al. (2018) also indicate the negative effect of cadmium on the growth and yield of *Solanum lycopersicum*. But in these conditions, the use of natural zeolite can have many benefits for the plant. Clinoptilolite is the most abundant zeolite species in Iran. By maintaining some cations in its structure and then releasing them in the medium, zeolite increases the growth and production of plants and improves the soil moisture balance. Besides, control the growth environment more efficiently and lowering the cost of needed nutrients. Li et al. (2009) reported that adding natural zeolite to soil improves the physical and chemical properties of the soil, including cation exchange capacity and pH. This mineral material prevents the deterioration of soil organic matter. It also contains the movement of lead in the ground by increasing the pH. In another study, Contin et al. (2019) reported that the addition of zeolite to soils contaminated with heavy metals increases the dry weight and overall biomass of the plant. Also, the use of this substance by improving the physical and chemical properties of the soil causes its fertility and thus improves plant nutrition. In the present study, it is observed that more accumulation of cadmium in the roots of Spinach has provided more growth opportunities for the plant under stress conditions. The result is visible in increasing the total biomass weight of the plant. Based on the results, fulvic acid and natural zeolite help to remove cadmium metal from the soil. Also, they moderate the adverse effects of stress on the plant and provide better plant growth.

Considering the ability of Spinach plants to absorb cadmium, high plant yield, and ability to transfer and accumulate cadmium in removable organs, the Spinach plant is a suitable plant for green refining of soils contaminated with cadmium. Also, the Spinach plant can be harvested at least four times in a year. Hence this plant is suitable for the purification of polluted soils and, unlike most plants that have small biomass, can save significant biomass in the year. Earlier studies in this area also showed that the different species of Chenopodiaceae have an excellent ability to absorb heavy metals in contaminated soils by producing high biomass. For example, the results of research by Rangnekar et al. (2013) showed that Spinach has an increased tendency to absorb cadmium in soils contaminated with this heavy metal. But the point is that given that the Spinach plant is one of the most commonly used edible vegetables in the world, it should be used immediately after harvest as if it were used for plant extraction purposes. Burning and burying it does not threaten the environment or the food cycle of living creatures. Therefore, cadmium is one of the heavy elements that leaves very high toxicity effects on the tissues of living organisms. By using the fulvic acid and zeolite, large amounts of the mobile metal cadmium can be extracted from the soil with the *Spinacia oleracea* var. inermis.

CONCLUSION

The results of this study indicate that the use of fulvic acid and zeolite results in the higher absorption of heavy elements from the soil. These materials can increase the absorption, increase in efficiency, and time to clear ground from heavy metal cadmium and zinc. The findings of this study showed that the fulvic acid, due to its acidic nature, causes higher mobility and availability of cadmium and zinc elements in the soil and result, more absorption of these metals by the plant. In contrast, the higher pH of zeolite somewhat reduces the availability of these metals to plant roots. Still, in general, the application of fulvic acid and zeolite in all concentrations can effectively reduce the accumulation of cadmium and zinc in the soil. Also, due to the positive role of these organic and mineral substances in plant nutrition, they can reduce the adverse effects of heavy metals stress on the plant and provide better vegetative growth for the plant. Therefore the use of natural materials such as fulvic acid and zeolite, can in addition to soil refinement of heavy elements has a significant positive effect on soil texture regeneration and improvement of its physical and chemical properties. Also, they provide better growth and development of plants to accelerate the process of treatment plants.

Conflict of interest
The authors have no conflict of interest to report.

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