The Effect of Some Soil Amendments (Manure and Biochar) on the Bioaccumulation Capacity of Hexavalent Chromium by Two Species of (Salicornia Persica) and (Salicornia Perspolitana) From Contaminated Soil

Fahime Ashrafi
Ferdowsi University of Mashhad
Ava heidari (ava.heidari@yahoo.com)
Ferdowsi University of Mashhad
Mohammad Farzam
Ferdowsi University of Mashhad
Alireza Karimi
Ferdowsi University of Mashhad
Malihe Amini
University of Jiroft

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The effect of some soil Amendments (manure and biochar) on the Bioaccumulation capacity of Hexavalent Chromium by two species of *(Salicornia persica)* and *(Salicornia perspolitana)* from Contaminated soil

Fahime Ashrafi[1], Ava Heidari[1*], Mohammad Farzam[2], Alireza Karimi[3], Malihe Amini[4†]

[1]Department of Environmental Science, Faculty of Natural Resources and Environment, Ferdowsi University of Mashhad, Mashhad, Iran

[2]Department of Range and Watershed Management, Faculty of Natural Resources and Environment, Ferdowsi University of Mashhad, Mashhad, Iran

[3]Department of Soil Sciences, Faculty of Agriculture, Ferdowsi University of Mashhad, Iran

[4]Department of Environmental Science and Engineering, Faculty of Natural Resources, University of Jiroft, P.O. Box: 8767161167, Jiroft, kerman, Iran

***Abstract*:** Heavy metals are among the most dangerous contaminants in the environment. Application of organic compounds and plant species with the ability to accumulate and stabilize heavy metal in their organs is the best option for remediation of these elements in the soil. Therefore, this study aimed to investigate the effects of manure and biochar on the accumulation of heavy metals by *Salicornia* species. Two species of *Salicornia* ,including *S. persica* and *S. perspolitana,* were cultivated outdoor in experimental pots. The effects of experimental treatments ,including hexavalent chromium concentrations, manure ,and biochar on the two studied species, were investigated. The results indicated a significant effect ($P < 0.05$) of biochar on the accumulation of heavy metals by two species ,*S. persica*and *S. perspolitana*, so that chromium concentrations in the roots and shoots were 258 and 5.41 mg/kg, respectively. Also, chromium accumulations under manure treatments in the roots and shoots were 334.34 and 9.79 mg/kg, respectively. Plant dry weight and height for both species in manure treatment were higher than control and biochar treatments. S. *persica* showed higher growth than S. *perspolitana* species. The content of photosynthetic pigments in both S.persica and *S. perspolitana* species

[*] Corresponding author. heidari@um.ac.ir, ava.heidari@yahoo.com, Phone number: +9838805471

[†] Co- Corresponding author: m.amini@ujiroft.ac.ir

under biochar treatment was higher than control and manure treatments. In general, one can conclude that the accumulation of chromium in *S. perspolitana* was higher than in *S. persica* ,and the application of biochar and manure amendments could stabilize chromium in soil and reduce chromium accumulation in both *S. persica* and *S. perspolitana* species.

Keywords: Soil contamination, *Salicornia*, phytoremediation, chromium.

1. Introduction

Increased agricultural activities , as well as industrialization in recent decades, have significantly resulted in the accumulation of various contaminants in the environment, especially in soil and water. These contaminants enter into the environment through mining operations, discharge of industrial effluents, pesticide-based agriculture, fertilizers, etc. , and are challenging and problematic due to harmful effects on soil biological systems (Edao 2017). Removal of heavy metals from soil could be a severe issue as heavy metals are special contaminants, which in many cases, could remain in the soil for hundreds (even thousands) of years. Several methods have been documented for removing heavy metals from contaminated soils. Despite being efficient, conventional technologies are costly, time-consuming , and environmentally destructive. Phytoremediation is defined as applying of various plants to reduce the concentration of heavy metals in the soil. Generally, bioremediation has been considered as a cost-effective and environmentally friendly method (Yao et al. 2012). Species store and accumulate high concentrations of heavy metals in their tissues without being poisoned by that metals. During the phytoremediation process, metals are effectively removed from the soil. The efficiency of the phytoremediation process is dependent on the plant functioning as well as the effective transfer of metals from the plant roots to the shoots. Plants that accumulate considerable amounts of metal usually have small shoots, so the critical point for increasing the

efficiency of the phytoremediation process is how to change plant biomass to enhance metal uptake (Evangelou et al. 2007). Biochar is a porous carbon product obtained from the pyrolysis of plant-derived organic matter (tree bark, rice husk, pinewood, etc.) or non-plant-derived biomass (cattle manure, poultry manure, etc.) with high cation exchangeable capacity and alkaline properties that improve soil structure. The addition of biochar to soil leads to increased soil biological activities , thereby improved crop yield(Bashir et al. 2018). In addition to biochar, applying of some other materials such as animal manure in soils could also increase the phytoremediation efficiency. Organic fertilizers, especially animal manures, contain large amounts of organic matter compared to chemical ones , which can provide nutrient sources such as nitrogen, phosphorus , and potassium overtime for the plant and improve soil physical, chemical , and biological properties. Increased organic matter in soils leads to the formation of aggregates and improves soil moisture-holding capacity, hydraulic conductivity, bulk density, degree of compaction, fertility , and soil resistance to water and wind erosion (Zebarth et al. 1999). A study by Al-Wabe et al.(Al-Wabel et al. 2015) showed that the application of biochar improved the growth of maize (*Zea mays* L.) and increased the soil water holding capacity of the soil. Biochar also leads to alkaline properties in the soil , and this effect varies depending on biochar characteristics, production temperature , and its raw materials.

Few plant species, including *Sutera fodina*, *Dicoma niccolifera*, and *Leptospermum scoparium*, have been reported to have the ability to accumulate high concentrations of chromium in their tissues. Also, mustard (*Brassica juncea*) and sunflower (*Helianthus annus* L.) have been reported to accumulate high concentrations of chromium in their tissues(Shahandeh &Hossner 2000). Coupe et al.(Coupe et al. 2013) compared the ability of three plant species , namely *Eucalyptus camaldeulensis*, *Brassica juncea* , and *Medicago sativum* , to uptake copper, zinc , and

chromium from soil and stated that *Eucalyptus camaldeulensis* had the highest capability to uptake these contaminants from the soil. *Salicornia*, a halophyte and salt-tolerant plant, serves as a suitable plant for phytoremediation of heavy metals due to its characteristic roots and the ability to stabilize metals (Van Oosten et al. 2015).

Several studies have been carried out on the phytoremediation ability of *Salicornia* plant species for removal of heavy metals other than chromium, but for the first time, we aimed to investigate the ability of *Salicornia persica* and *Salicornia perspolitana* in phytoremediation of chromium-contaminated soils. To do this, we studied the effect of manure and biochar on the capacity of the two mentioned species to accumulate chromium in their tissues from contaminated soil. In this regard, experimental treatments including hexavalent chromium concentrations, manure , and biochar.

2. Materials and methods:

2.1 Experiments

All steps of this research were performed in 2019-2020 in the laboratory and growth chamber of Faculty of Natural Resources and Environment, the Ferdowsi University of Mashhad, Iran, located at 25 km apart from northeast of Mashhad city (59° 31' 16" E and 36° 29' 92" N). The soil samples were taken from a 0-40 cm and passed through a 2-mm sieve after air-drying.

2.2 Determination of soil physical and chemical properties

Soil texture was characterized by the hydrometric method using a soil texture triangle. Soil pH and EC (electrical conductivity) were measured using pH-meter (20+, Crison, Spain) and EC-meter (4510, Jenway, England) devices, respectively. Soil organic carbon was determined by the dry combustion method (Park et al. 2017). Total nitrogen was measured using a Kajeldal (V50) device. Soil available phosphorus was measured according to the Olsen method (1954) using a

spectrophotometer (model DR 5000) at the wavelength of 660 nm. Available potassium was determined using the ammonium acetate method and photoelectric flame photometer (Jenway PFP7).

2.3 Determination of physical and chemical properties of manure and biochar

The completely rotten manure used in this study was prepared from the greenhouse of Ferdowsi University of Mashhad, Iran. The applied biochar was a mixture of the wood obtained from eucalyptus, poplar , and ironwood prepared at 500° C at the laboratory of Tarbiat Modares University, Iran. Before performing the experimental treatments, manure and biochar samples were analyzed using conventional laboratory methods previously applied to measure the soil samples. Calcium and sodium contents were determined by inductively coupled plasma spectroscopy (ICP-OES) (model ICP-OES, SPECTRO ARCOS-76004555) and the percentages (%) of carbon, nitrogen, hydrogen, oxygen , and sulfur in the biochar using elemental analysis (FLASH EA 1112 SERIES, Themo Finnigan).

2.4 Preparation of soil samples

The soil in the natural habitat was characterized by clay-sandy loam texture and an EC of 1800 μs/cm , and we prepared the soil samples containing sand, NaCl , and hexavalent chromium (concentrations of 0-150 mg/kg) in order to simulate the soil of the natural site in terms of texture and salinity. After implementing treatments, the soil samples were completely homogenized and mixed , and stored in plastic bags. The treated soil samples were then moistened to the field capacity and kept at this constant moisture level for 15 days in order to simulate the soil contamination with heavy elements somewhat similar to the natural conditions in the contaminated fields. Then, biochar and manure were added (10 g of amendment per 1 kg

of soil) to each sample of the contaminated soils with three replicates. Soil samples were mixed well for each pot to make soil conditions homogeneous in all parts.

2.5 Germination of Salicornia seeds

Metal chromium was added to the seeds of *S. persica* , and *S. perspolitana* species as a factorial design in 3 replicates and seven concentration levels (0, 5, 10, 15, 50, 100 , and 150 mg/l). Inside each Petri dish, there were 10 ml of hexavalent chromium solution at different concentrations as well as 25 seeds. Then, all the petri dishes were incubated in a growth chamber at a temperature of 25-30 °C with a photoperiod of 16 h of light and eight h of darkness. The seeds with a radicle of 2 mm were considered as a germinated seed , and all the germinated seeds were counted on a daily basis. Because no germination was observed from the 14th to the 16th day, the counting was stopped on the 16th day. Also, the length of root and stem , as well as the length of seedling (the sum of root length and stem length) was measured on the 16th day by an mm-sized ruler. Germination percentage, germination rate, seedling length, seed vigor index , and allometric index were calculated based on the equations 2-1 to 2-5, respectively(Saberi et al. 2010).

$$GP = \frac{G}{N} \times 100 \quad (2\text{-}1)$$

$$GS = \Sigma \frac{ni}{Di} \quad (2\text{-}2)$$

$$TL = SL + RL \quad (2\text{-}3)$$

$$SVI = \frac{GR \times Mean(SI + RL)}{100} \quad (2\text{-}4)$$

$$Allometry = \frac{root\ length}{shoot\ lrngth} \quad (2\text{-}5)$$

Where, GP: germination percentage, G: the final number of germinated seeds N: number of seeds sown (25 seeds in this study), GS: germination rate, ni: number of germinated seeds on

counting days and Di: number of days in the experiment, TL: total seedling length, SVI: seed vigor index, SL: stem length, RL: root length

2.6 Cultivation of seeds

When the soil contamination period ended up , and after adding manure and biochar to the contaminated soil, 12 seeds of Salicornia were planted at a depth of 0.5 cm for each pot. After one month, due to lack of seed germination observed in the contaminated soil, the seedlings of *S. persica* and *S. perspolitana* were planted in a mixture of coco-peat (Coir) and perlite (1: 1) in the growth chamber at a temperature of 25-30 °C and a photoperiod characterized by 16 h of light and eight h of darkness. In the early stages of the seedlings' growth, Hoagland nutrient solution was used every two weeks. After five months, seedlings of the same size were selected and transferred to the contaminated soil in the pots. The pots were incubated in contaminated soil from June to November. The seedlings were died at 50, 100 , and 150 mg/kg, but survived at 0, 5, 10 , and 15 mg/kg concentrations. The seedlings of *S. persica* and *S. perspolitana* were harvested after six months of growth in the contaminated soil. After harvesting, the roots and shoots were washed first with tap water and then with distilled water.

2.7 Measurement of plant growth and morphological traits

Root and shoot weight were calculated in g per kg pot. The content of photosynthetic pigments , including chlorophyll *a* and *b* , and carotenoids in *S. persica* and *S. perspolitana* were read by UV-Visible spectrophotometer (HACH, DR 5000, America). The total phenolic content of the samples was calculated as gallic acid equivalent (mg of gallic acid per g of dry matter). The concentration of hexavalent chromium was determined by a UV-Visible spectrophotometer (HACH, DR 5000, America).

2.8 Analyzes

Scanning electronic microscopy (SEM) (Model VP 1450, LEO - Germany) was applied for determining the morphology of the materials used in this study. Experimental treatments included plant species (2 levels), the addition of metal hexavalent chromium in 7 levels (zero, 5, 10 and 15, 50, 100 and 150 mg/kg) , and soil amendments in 3 levels (manure, biochar , and control). The total number of experimental units (number of pots) was 126. A factorial experiment in the form of a completely randomized design with three replicates was used to evaluate the results of this study. Statistical analysis of data including ANOVA was performed in Minitab v.16 software , and Tukey test was applied for comparison of means (both the main effects and interactions) at the significance level of 0.05.

3. Results

3.1 Physical and chemical properties of soil, cattle manure , and biochar

The soil used in this study was characterized by heavy texture, low salinity (EC of 1.8) , and approximately alkaline pH (8.14). The soil texture was clayey-sandy loam with the organic carbon content of 2.56%. The percentages of organic carbon, total nitrogen, phosphorus, potassium in the manure were 56%, 2.95%, 0.62% , and 0.94%, respectively. The values for carbon, hydrogen, nitrogen, oxygen and sulfur in biochar were 73.41%, 2.71%, 0.37%, 23.51% and 0%, respectively. The results of heavy metal analysis tests showed that the concentration of hexavalent chromium in the soil was 2.91 mg/kg, while there was no hexavalent chromium in manure and biochar.

3.2 Scanning electron microscopy (SEM)

Figure 1 shows the Scanning Electron Microscopy images (SEM) of the biochar surface. As shown, biochar is characterized by a large surface area and a relatively regular network of

honeycomb-shaped pores on its surface, which leads to the absorption of heavy metals. Also, this honeycomb network represents a carbon skeleton in the biochar structure(Ghani et al. 2013).

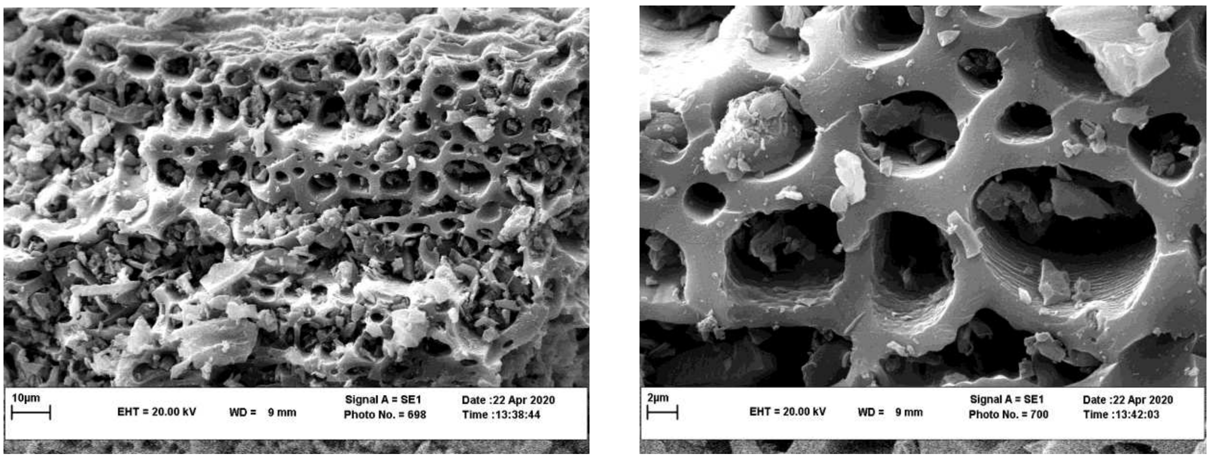

Figure 1. Scanning electron microscopy (SEM) of biochar surface

3.3 Effect of different concentrations of hexavalent chromium on germination traits of S. persica and S. perspolitana

The results of the effect of different concentrations of chromium on the germination percentage (%) and rate, and root length, shoot length , and seedling length in the two studied species shown in Table 1 indicated a higher resistance of *S. persica* compared to *S. perspolitana* against metal hexavalent chromium stress so that all germination traits declined with increasing the concentration of chromium from the control (0 mg/l) to 150 mg/l, and eventually led to plant dying.

Table 1. Results of comparison of the means related to simple effect of different concentrations of chromium on germination traits of *S. persica* and *S. perspolitana*

Treatment	**Germination percentage**	**Germination rate**	**Stem length**	**Root length**	**Seedling length**
species					
S. persica	58. 89^{a}	1.05 ^{a}	10. 11^{a}	1. 088 ^{a}	21. 11a
S. perspolitana	50. 44 ^{b}	0. 900 ^{b}	8. 16 ^{b}	1. 080^{a}	9. 24^{b}
Different concentrations of chromium					
Cr_{0}	67.68 ^{a}	1.22 ^{a}	11.38 ^{a}	1. 91^{a}	13.3 ^{a}
Cr_{5}	64.67 $^{b\ a}$	1.15 ^{ab}	11. 29^{a}	1. 50^{b}	12.83 ^{a}
Cr_{15}	60.67 ^{b}	1.08 ^{b}	9. 60^{b}	0.91 ^{c}	10.51^{b}
Cr_{50}	52.67 ^{c}	0.94 ^{c}	8.89 ^{bc}	0.85 ^{c}	9.75^{bc}
Cr_{100}	45.33 ^{d}	0.80 ^{d}	8.03 ^{c}	0.73 ^{c}	8.76 ^{c}
Cr_{150}	36.00 ^{e}	0.64 ^{e}	5.62 ^{d}	0.57 ^{c}	6.2 ^{d}

Different letters indicate statistically significant differences at $P > 0.05$.

3.4 Effect of different concentrations of hexavalent chromium, manure , and biochar on the plant height of two species S. persica and S. perspolitana

As seen in table 2, the effect of different concentrations of chromium on the plant height of the two studied species was statistically significant ($P < 0.05$). The highest and lowest heights were related to Cr_{0} (no chromium) and Cr_{15} (chromium concentration of 15 mg/g) with corresponding heights of 45.33 and 27 cm, respectively. The height reported for *S. persica* was 6.42% higher than *S. perspolitana*, which can be attributed lower accumulation of chromium in *S. persica* tissues. As shown in the table, the effect of different amendments on the height of the two studied plant species was also significant. Manure and biochar treatments indicated an increased height by 22.42% and 12.51%, respectively, compared to the control treatment.

Table 3. Results of mean comparison of interaction effects between different concentrations of chromium, manure and biochar on the height of *S. persica* and *S. perspolitana*

treatments	**Plant height (cm)**	**Shoot dry weight (g per pot)**	**Root dry weight (g per pot)**
amendments			
control	31.79 [c]	0.53 [c]	0.06 [c]
biochar	31.79 [c]	0. 68[b]	0.07 [b]
manure	35. 26[b]	0.79 [a]	0.098 [a]
species			
S. persica	92.38 [a]	0.97 [a]	0.11 [a]
S. perspolitana	36.60 [a]	36.0 [b]	0.039 [b]
Different concentrations of Cr			
Cr_0	34.39 [b]	1.12 [a]	0.17 [a]
Cr_5	45.33 [a]	0.81 [b]	0.067 [b]
Cr_{10}	37.28 [b]	0.45 [c]	0.046 [c]
Cr_{15}	32.36 [c]	0.29 [d]	0.02 [d]

Different letters indicate statistically significant differences at $P > 0.05$.

3.5 The effect of different concentrations of hexavalent chromium, manure , and biochar on the root and shoot dry weight of S. persica and S. perspolitana

Table 3 shows the results of the mean comparison of the interaction effects of different concentrations of chromium, manure , and biochar on the root and shoot dry weight of *S. persica* and *S. perspolitana*. According to these results, different concentrations of chromium negatively influenced on the dry shoot weight of the two studied species. Concentrations of chromium as 5, 10 , and 15 mg/kg resulted in decreased shoot dry weight of *S. persica* species by 52.59%, 66.88% , and 88.31%, while the decreases reported for *S. perspolitana* species were found to be 17.07%, 36.58% , and 48.78%, respectively, compared with controls.

According to the results, the organic compounds (manure and biochar) applied in this study showed a positive effect on the dry shoot weight of the two studied species. When applying biochar treatment at concentrations of 5, 10 , and 15 mg/kg chromium, shoot dry weight increased by 84.9%, 9.80% and 111.1%, in *S. persica* species, and by 82.8%, 7.69% and 19.04%

in *S. perspolitana* species compared to the control (without amendment), respectively. The increases in shoot dry weight for the manure treatment at concentrations of and 5, 10 and 15 mg/kg chromium were 115.06%, 49.01% , and 100% for *S. persica* species and 50%, 26.9% , and 76.1% for *S. perspolitana* compared to the controls, respectively.

The results concerning the mean comparison of the interaction effects of different concentrations of chromium, manure , and biochar on the root dry weight of *S. persica* and *S. perspolitana* in Table 3 indicated the negative effect of different concentrations of chromium on the root dry weight of the two studied species , so that root dry weight decreased with increasing the concentration of this element in the soil. The decreases reported at concentrations of 5, 10 , and 15 mg/kg chromium were 69.23%, 89.61% , and 94.61% for *S. persica*, and 30%, 63.6% , and 63.4% for *S. perspolitana* species compared to the controls, respectively. Again, the organic compounds used in this study , including manure and biochar , positively influenced on the root dry weight of the two species. By applying of biochar into the soil at chromium concentrations of 5, 10 , and 15 mg/kg, the root dry weight increased by 8.75%, 137.0% , and 28.5% in *S. persica* and 8.5%, 26.37% , and 1.09% in *S. perspolitana* compared to the controls (without amendment), respectively. The increases for the manure treatment at chromium concentrations of 5, 10 , and 15 mg/kg were 50%, 200% , and 321.4% for *S. persica* and 8.5%, 251.6% , and 156.8% in *S. perspolitana*, compared to the controls, respectively. For biochar treatment at chromium concentrations of 5, 10 , and 15 mg/kg, the root dry weight decreased by 8.75%, 137.0% , and 28.5% in *S. persica* and by 8.5%, 26.37% , and 1.09% in *S. perspolitana* compared to the controls (without amendment), respectively.

Table 3. Mean comparison of the interaction effects of different concentrations of chromium, manure and biochar on shoot and root dry weight in *S. persica* and *S. perspolitana*

different concentrations of chromium	Species	amendments	Shoot dry weight (g per pot)	Root dry weight (g per pot)
Cr_0	***S. persica***	control	1.54^{c}	0.26^{b}
		biochar	1.82^{ab}	0.27^{b}
		manure	1.93^{a}	0.3^{a}
	S.perspolitana	control	0.41^{fghi}	0.05^{efgh}
		biochar	0.49^{efgh}	0.06^{defg}
		manure	0.52^{defg}	0.062^{defg}
Cr_5	***S. persica***	control	0.73^{de}	0.0 8^{de}
		biochar	1.35^{c}	0.087^{d}
		manure	1.57_{bc}	0.12_{c}
	S.perspolitana	control	0.34_{fghi}	0.035_{fghi}
		biochar	0.37_{fghi}	0.038_{fghi}
		manure	0.51_{defg}	0.038_{fghi}
Cr_{10}	***S. persica***	control	0.51_{fghi}	0.027_{ghi}
		biochar	0.56_{def}	0.064_{def}
		manure	0.76_{d}	0.081_{de}
	S.perspolitana	control	0.26_{ghi}	0.0182^{hi}
		biochar	0.28_{ghi}	0.023_{hi}
		manure	0.33_{fghi}	0.064_{def}
Cr_{15}	***S. persica***	control	0.18_{i}	0.0183_{hi}
		biochar	0.38_{fghi}	0.0185_{hi}
		manure	0.36^{fghi}	0.047^{efghi}
	S.perspolitana	**control**	0.21^{i}	0.014^{i}
		biochar	0.25^{hi}	0.018^{hi}
		manure	0.37^{fghi}	0.059^{defg}

3.6 Effect of different concentrations of chromium, manure , and biochar on chlorophyll a and b of S. persica and S. perspolitana

The results of the mean comparison of the interaction effects of different concentrations of chromium, manure , and biochar on the content of photosynthetic pigments in S. *persica* and *S. perspolitana* are shown in Tables 4. According to these results, different concentrations of chromium negatively influenced on the content of chlorophyll *a* and *b* in the two studied species so that the content of chlorophyll a and b decreased with increasing chromium in the soil. At chromium concentration of 5, 10 and 15 mg/kg in *S. persica*, the reported decreases were

31.37%, 28.23% and 27.45% for chlorophyll *a* and 39.54%, 38.04% and 35.03% for chlorophyll *b*, respectively. In *S. perspolitana*, the contents of chlorophyll decreased by 28.01%, 28.8% , and 39.22% , and of chlorophyll *b* by 15.6%, 18.7% and 27.6% compared to controls, respectively. Applying of hexavalent chromium caused a significant reduction in chlorophyll contents. There was a significant difference between different levels of chromium concentrations as 5, 10 , and 15 mg/kg in terms of decreases in chlorophyll *a* and *b* (Table 4). The organic compounds (manure and biochar) used in this study also indicated a positive effect on the content of photosynthetic pigments in the two studied species. By applying the biochar treatment on *S. persica* species at chromium concentrations of 5, 10 , and 15 mg/kg, chlorophyll content increased by 49.71%, 19.6% , and 11.35% and the values for chlorophyll *b* by 61.4%, 25.4% , and 0.3%. For *S. perspolitana*, applying of biochar resulted in increases in chlorophyll *a* by 29.9%, 35.75 , and 52.48% and in chlorophyll *b* by 26.85%, 36.52% , and 40.66% compared to the controls (without amendment), respectively. Also, in the manure treatment at chromium concentrations of 5, 10 , and 15 mg/kg, the content of chlorophyll *a* decreased by 3.4%, 5.4% and 6.4% , and of chlorophyll *b* by 10.74%, 10.19%, and 15.3% in *S. persica* species, respectively. For *S. perspolitana* species, the decreases in chlorophyll *a* were as 29.3%, 24.24% , and 47.5% , and in chlorophyll *b* as 24.21%, 9.6% , and 29.9%, respectively, compared to the control (without amendment). In general, the highest content of chlorophyll *a* (2.73 mg/g fresh weight) was found in *S. persica* species at chromium concentration of 0 mg/kg in biochar treatment , and the lowest content (1.41 mg/g fresh weight) was found for *S. perspolitana* species at chromium concentration of 15 mg/kg in the control treatment (without amendment). Also, the highest content of chlorophyll *b* (0.669 mg/g fresh weight) was found in *S. persica* species at chromium concentration of 0 mg/kg in Biochar treatment , and the lowest content (0.36 mg/g

fresh weight) has belonged to *S. perspolitana* species at chromium concentration of 15 mg/kg in the control treatment (without amendment)

Table 4. Mean comparison of the interaction effects of different concentrations of chromium, manure and biochar on the content of photosynthetic pigments in *S. persica* and *S. perspolitana*

different concentrations of chromium	**Species**	**amendments**	**Chlorophyll *a* (mg. fresh weight)**	**Chlorophyll *b* (mg. fresh weight)**	**Carotenoid (mg. fresh weight)**
Cr_0	***S. persica***	control	2.55 ^{bc}	0.665 ^{a}	160.5 ^{c}
		biochar	2.73 ^{a}	0.669 ^{a}	160.5 ^{c}
		manure	2.63 ^{ab}	0.661 ^{a}	160.1 ^{c}
	S.perspolitana	control	2.32 ^{ef}	0.4989 ^{h}	147.4 ^{k}
		biochar	2.47 ^{cd}	0.5100 ^{fg}	163.3 ^{b}
		manure	2.42 ^{de}	0.5043 ^{gh}	156.1 ^{d}
Cr_5	***S. persica***	control	1.75 ^{mn}	0.402 ^{o}	115.3 ^{s}
		biochar	2.62 ^{b}	0.649 ^{b}	167.2 ^{a}
		manure	1.81 ^{m}	0.4471 ^{jk}	124.9 ^{o}
	S.perspolitana	control	1.67 ^{n}	0.4208 ^{m}	131.3 ^{o}
		biochar	2.17 ^{gh}	0.5338 ^{d}	149. 2^{e}
		manure	2.16 ^{ghi}	0.5227 ^{e}	148.4 ^{f}
Cr_{10}	***S. persica***	control	1.83 ^{lm}	0.412 ^{mn}	119. 7^{q}
		biochar	2.19 ^{g}	0.517 ^{ef}	137.9 ^{i}
		manure	1.93 ^{kl}	0.454 ^{j}	128.6 ^{m}
	S.perspolitana	control	1.65 ^{n}	0.4052 ^{no}	122.1 ^{p}
		biochar	2.24 ^{fg}	0.5532 ^{c}	163.6 ^{b}
		manure	2.05 ^{ij}	0.4442 ^{k}	134.3 ^{j}
Cr_{15}	***S. persica***	control	1.85 ^{lm}	0.432 ^{l}	125.2 ^{o}
		biochar	2.06 ^{hij}	0.445 ^{k}	129 ^{lm}
		manure	1.97 ^{jk}	0.4983 ^{h}	126.3 ^{n}
	S.perspolitana	control	1.41 ^{o}	0.361 ^{p}	118.4 ^{r}
		biochar	2.15 ^{ghi}	0.5078^{g}	144.3 ^{h}
		manure	2.08 ^{hig}	0.4691 ^{i}	129 ^{l}

different letters indicate statistically significant differences at $P > 0.05$.

3.7 Effect of different concentrations of hexavalent chromium, manure , and biochar on carotenoid content in S. persica and S. perspolitana

Table 4 shows the results of the mean comparison of the interaction effects of different concentrations of chromium, manure , and biochar on the carotenoid content of *S .persica* and *S.*

perspolitana. According to these results, the different concentrations of chromium negatively influenced on the carotenoid content in the two species so that the carotenoid content decreased with increasing the concentration of this heavy metal in the soil. At three chromium concentrations of 5, 10 , and 15 mg/kg, the carotenoid content decreased by 28.16%, 25.42% , and 21.99% in *S. persica*, and by 10.92%, 17.16% , and 19.67% in *S. perspolitana* compared to the controls, respectively. Thus, the organic compounds used in this study indicated a positive effect on the carotenoid content of the two species. In biochar treatment at chromium concentrations of 5, 10 , and 15 mg/kg, the carotenoid content in *S. persica* increased by 45.01%, 15.20% , and 3.03% and in *S. perspolitana* by 13.6%, 33.9% , and 21.8% compared to the controls, respectively. Also, in manure treatment at chromium concentrations of 5, 10 , and 15 mg/kg, the increases in carotenoid content were 8.32%, 7.43% , and 0.87% in *S. persica*, and 13.02%, 9.99% , and 8.95% in *S. perspolitana* compared to the controls, respectively.

3.8 The effect of different concentrations of chromium, manure , and biochar on the total phenolic content in S. persica and S. perspolitana

Table 5 shows the results of the mean comparison of the interaction effects of different concentrations of chromium, manure , and biochar on the total phenolic content in *S. persica* and *S. perspolitana*. According to these results, different concentrations of chromium negatively affected on the total phenolic content of the two studied species , and so that the total phenolic content in the plant decreased with increasing the concentration of chromium in the soil. At three chromium concentrations of 5, 10 , and 15 mg/kg , the total phenolic content in *S. persica* species decreased by 39.30%, 39.86% , and 49.73% , and in *S. perspolitana* by 34.75%, 42.44% , and 60.35% compared to the controls, respectively. Again, the organic compounds used in this study showed a positive effect on the total phenolic content of the two species. In biochar

treatment at chromium concentrations of 5, 10 , and 15 mg/kg, the total phenolic content increased by 62.95%, 67.30% , and 111.16% in *S. persica* species, and by 24.26%, 11.31% , and 36.40% in *S. perspolitana* compared to the controls, respectively. Also, in manure treatment at concentrations of 5, 10 , and 15 mg/kg, the increases in total phenolic content were 39.14%, 32.87% , and 21.22% for *S. persica*, and 3.54%, 5.26% , and 22.35% for *S. perspolitana* compared to the controls, respectively.

Table 5. Mean comparison of the interaction effects of different concentrations of chromium, manure and biochar on the total phenolic content in *S. persica* and *S. perspolitana*.

different concentrations of chromium	**Species**	**amendments**	**total phenolic content (mg gallic acid per g fresh weight)**
Cr_0	***S. persica***	control	37.58 ^{bc}
		biochar	44.15 ^{a}
		manure	38.57 ^{b}
	S.perspolitana	control	30.7 ^{de}
		biochar	34.39 ^{cd}
		manure	34 .13^{cd}
Cr_5	***S. persica***	control	22.81 ^{fg}
		biochar	37.17 ^{bc}
		manure	31.74 ^{de}
	S.perspolitana	control	20.03 ^{ghij}
		biochar	24.89 ^{f}
		manure	20. 74^{ghi}
Cr_{10}	***S. persica***	control	22.6 ^{fgh}
		biochar	37.81 ^{bc}
		manure	30.03 ^{e}
	S.perspolitana	control	17.67 ^{ijk}
		biochar	19.67 ^{ghij}
		manure	18.6 ^{hijk}
Cr_{15}	***S. persica***	control	81.89 ^{ghijk}
		biochar	39.89 ^{b}
		manure	22.9 ^{fg}
	S.perspolitana	control	12.17 ^{l}
		biochar	61.60 ^{jk}
		manure	14.89 ^{kl}

different letters indicate statistically significant differences at $P > 0.05$.

3.9 The effect of different concentrations of chromium, manure , and biochar on the values of accumulated chromium in roots and shoots of S. persica and S. perspolitana

Table 6 shows the mean comparison of the interaction effects of different concentrations of chromium, manure , and biochar on the accumulation of total chromium, Cr (VI) , and Cr (III) in the roots of S.*persica* and S.*perspolitana* after six months of growing in the contaminated soil. Generally, the accumulation of chromium in the roots of both species was significantly affected by the chromium content in the soil so that the accumulation of chromium in the roots of both species increased by increasing the chromium concentration from 0 to 15 mg/kg in the soil. In *S. persica,* at chromium concentration of 0 mg/kg, accumulation of total, hexavalent , and trivalent chromium in the roots of plants grown in contaminated soil (without amendment) were 17.77, 5.38 , and 16.86 mg/kg. At chromium concentration of 15 mg/kg, the corresponding values for accumulation of total, hexavalent , and trivalent chromium were 357.61, 6.41 , and 351.19 mg/kg for this species. In *S. perspolitana*, at chromium concentration of 0 mg/kg, accumulation of total, hexavalent , and trivalent chromium in the roots of plants grown in contaminated soil (without amendment) were 21.58, 7.73 , and 13.85 mg/kg. At chromium concentration of 15 mg/kg, the values for accumulation of total, hexavalent , and trivalent chromium were 624.91, 46.08 , and 578.83 mg/kg for this species. In total, the accumulation of total, hexavalent and trivalent chromium in the roots of *S. perspolitana* was higher than *S. persica*. The organic compounds used in this study showed a negative effect on the accumulation of total, hexavalent and trivalent chromium in the roots of the two studied species. In biochar treatment, the accumulation of total, hexavalent and trivalent chromium in *S.persica* species decreased by 33.89%, 50.39% and 33.60% at chromium concentration of 5 mg/kg, 76.47%, 73.09% and 76.5% at chromium concentration of 10 mg/kg and 25.01%, 81.7% and 23.39% at chromium concentration of 15

mg/kg. In S. perspolitana species under biochar treatment, the accumulation of total, hexavalent and trivalent chromium decreased by 53.60%, 19.96% and 54.4% at chromium concentration of 5 mg/kg, 23.72%, 14.54% and 24.0% at chromium concentration of 10 mg/kg and 11.39%, 32.53% and 9.50% at chromium concentration of 15 mg/kg. In the manure treatment, the accumulation of total, hexavalent and trivalent chromium in *S.persica* species decreased by 23.82%, 36.24% and 23.61% at chromium concentration of 5 mg/kg, 22.73%, 51.85% and 22.13% at chromium concentration of 10 mg/kg and 1.75%, 52.32% and 0.22% at chromium concentration of 15 mg/kg. In S. perspolitana species under biochar treatment, the accumulation of total, hexavalent and trivalent chromium decreased by 26.36%, 8.11% and 22.96% at chromium concentration of 5 mg/kg, 21.55%, 2.02% and 22.22% at chromium concentration of 10 mg/kg and 9.48%, 16.96% and 8.81% at chromium concentration of 15 mg/kg.

Table 6. Mean comparison of the interaction effects of different concentrations of chromium, manure and biochar on chromium accumulation in the roots of *S. persica* and *S. perspolitana*

different concentrations of chromium	**Species**	**amendments**	**Hexavalent chromium (mg.kg)**	**Total chromium (mg.kg)**	**Trivalent chromium (mg.kg)**
Cr_0	***S. persica***	control	1.05^{p}	20.39^{n}	19.34^{o}
		biochar	0.8^{p}	16.27^{n}	15.47^{o}
		manure	0.85^{p}	16.63^{n}	15.78^{o}
	S.perspolitana	control	9.39^{j}	24.54^{n}	15.14^{o}
		biochar	6^{l}	19.4^{n}	13.4^{o}
		manure	7.8^{k}	20.8^{n}	13^{o}
Cr_5	***S. persica***	control	7.56^{k}	440.99^{fg}	433.42^{fg}
		biochar	3.75^{n}	291.53^{k}	287.78^{l}
		manure	4.82^{m}	335.91^{j}	331.08^{k}
	S.perspolitana	control	15.03^{f}	589.67^{c}	574.63^{b}
		biochar	12.03^{h}	273.59^{l}	261.53^{m}
		manure	13.81^{g}	434.18^{g}	420.36^{h}
Cr_{10}	***S. persica***	control	10.78^{i}	532.27^{d}	521.48^{e}
		biochar	9.2^{no}	$125. 21^{m}$	$122. 31^{n}$
		manure	5.19^{lm}	411.27^{h}	406.07^{i}
	S.perspolitana	control	$19. 8^{d}$	$588. 99^{c}$	569.19^{bc}
		biochar	16.92^{e}	449.27^{f}	432.34^{g}

		manure	19.4 ^{d}	462.06 ^{e}	442.66 ^{f}
Cr_{15}	***S. persica***	control	11. 6^{hi}	392.81 ^{i}	381.21 ^{j}
		biochar	2.12 ^{o}	294.53 ^{k}	292.01 ^{l}
		manure	5.53 ^{lm}	385.9 ^{i}	380.36^{j}
	S.perspolitana	control	55.18^{a}	671.66^{a}	616.48^{a}
		biochar	37.23 ^{c}	595.13 ^{c}	557.89 ^{d}
		manure	45.82 ^{b}	607.94 ^{b}	562.11 ^{cd}

different letters indicate statistically significant differences at $P > 0.05$.

Table 7 indicates the mean comparison of the interaction effects of different concentrations of chromium, manure , and biochar on the accumulation of total, hexavalent , and trivalent chromium in the shoots of *S. persica* and *S. perspolitana* after six months of growing in the contaminated soil. Like the results obtained for the roots, the accumulation of chromium in the shoots of both species was significantly affected by the chromium content in the soil. In other words, chromium accumulation in the shoot showed an increasing trend with increasing chromium concentration from 0 to 15 mg/kg in the soil for both species. In *S. persica,* at chromium concentration of 0 mg/kg, accumulation of total, hexavalent , and trivalent chromium in the shoots of plants grown in contaminated soil (without amendment) were 2.07, 0.56 , and 1.51 mg/kg. At chromium concentration of 15 mg/kg, the corresponding values for accumulation of total, hexavalent , and trivalent chromium were 33.54, 1.12 , and 32.42 mg/kg for this species. In *S. perspolitana*, at chromium concentration of 0 mg/kg, accumulation of total, hexavalent , and trivalent chromium in the shoots of plants grown in contaminated soil (without amendment) were 1.03, 0.33 , and 0.7 mg/kg. At chromium concentration of 15 mg/kg, the values for accumulation of total, hexavalent , and trivalent chromium were 28.41, 1.25 , and 27.16 mg/kg for this species.

Unlike roots, accumulation of total, hexavalent , and trivalent chromium in shoots of *S. persica* was higher than *S. perspolitana*. As shown in table 7, the organic compounds used in this study

hurt the accumulation of total, hexavalent , and trivalent chromium in the shoots of the two species.

In biochar treatment, the accumulation of total, hexavalent and trivalent chromium in *S.persica* species decreased by 91.83%, 39.66% and 93.61% at chromium concentration of 5 mg/kg, 93.22%, 72.53% and 93.78% at chromium concentration of 10 mg/kg and 93.60%, 24.42% and 94.66% at chromium concentration of 15 mg/kg. In *S. perspolitana* species under biochar treatment, the accumulation of total, hexavalent and trivalent chromium decreased by 73.06%, 29.68% and 75.07% at chromium concentration of 5 mg/kg, 60.05%, 26.08% and 61.29% at chromium concentration of 10 mg/kg and 70.96%, 61.11% and 71.36% at chromium concentration of 15 mg/kg. In the manure treatment, the accumulation of total, hexavalent and trivalent chromium in *S.persica* species decreased by 51.53%, 14.04% and 52.77% at chromium concentration of 5 mg/kg, 88.50%, 55.44% and 89.40% at chromium concentration of 10 mg/kg and 90.62%, 19.08% and 91.71% at chromium concentration of 15 mg/kg. In *S. perspolitana* species under biochar treatment, the accumulation of total, hexavalent and trivalent chromium decreased by 48.89%, 21.87% and 50.14% at chromium concentration of 5 mg/kg, 33.23%, 21.73% and 33.65% at chromium concentration of 10 mg/kg and 60.90%, 50% and 61.36% at chromium concentration of 15 mg/kg.

Table 7. Mean comparison of the interaction effects of different concentrations of chromium, manure and biochar on chromium accumulation in shoots of *S. persica* and *S. p*

different concentrations of chromium	**Species**	**amendments**	**Hexavalent chromium (mg.kg)**	**Total chromium (mg.kg)**	**Trivalent chromium (mg.kg)**
Cr_0	***S. persica***	control	0.65^{fghi}	2.06^{lm}	1.41^{kl}
		biochar	0.5^{hijk}	2.14^{lm}	1.64^{kl}
		manure	0.54^{ghij}	2.03^{lm}	1.49^{kl}
	S.perspolitana	control	0.37^{jk}	1.05^{m}	0.68^{l}
		biochar	0.28^{k}	0.99^{m}	0.7^{l}

		manure	0.33 ^{jk}	1.04 ^{m}	0.71 ^{l}
Cr_5	***S. persica***	control	1.21 ^{bc}	37.24 ^{d}	36.02 ^{d}
		biochar	0.73 ^{fgh}	3.04 ^{kl}	2.3 ^{jk}
		manure	1.04 ^{cd}	18.05 ^{f}	17.01 ^{f}
	S.perspolitana	control	0.64 ^{fghi}	14.48 ^{g}	13.84 ^{g}
		biochar	0.45 ^{ijk}	3.9 ^{jk}	3.45 ^{ij}
		manure	0.5 ^{hijk}	7.4 ^{h}	6.9 ^{h}
Cr_{10}	***S. persica***	control	1.93 ^{a}	72.73 ^{b}	70.8 ^{b}
		biochar	0.53 ^{ghij}	4.93 ^{ij}	4.4 ^{i}
		manure	0.86 ^{def}	8.36 ^{h}	7.5 ^{h}
	S.perspolitana	control	0.69 ^{fgh}	20.13 ^{e}	19.43 ^{e}
		biochar	0.51 ^{hijk}	8.04 ^{h}	7.52 ^{h}
		manure	0.54 ^{ghij}	13.44 ^{g}	12.89 ^{g}
Cr_{15}	***S. persica***	control	1.31 ^{b}	86. 93^{a}	85.62^{a}
		biochar	0.99 ^{cde}	5.56 ^{i}	4.57 ^{i}
		manure	1.06 ^{cd}	8.15 ^{h}	7.09 ^{h}
	S.perspolitana	control	1.98 ^{a}	50.7 ^{c}	48.71 ^{c}
		biochar	0.77 ^{efg}	14.72 ^{g}	13.95 ^{g}
		manure	0.99 ^{cde}	19.82 ^{e}	18.82 ^{e}

different letters indicate statistically significant differences at $P > 0.05$.

3.10 Determination of TF[1], BCF[2], BAC[3] indices to evaluate the phytoremediation ability of S. persica and S. perspolitana species

In order to evaluate the phytoremediation potential of plants after determining the concentration of heavy metals in plant and soil samples, it should be necessary to calculated indices including TF (Translocation Factor; a ratio of metal concentrations in shoots to roots), BCF (Bio Concentration Factor; ratio of metal concentrations in roots to soil), BAC (Biological Accumulation Coefficient; the a ratio of metal concentration in shoots to soil) because the phytoremediation potential of a species is calculated using these indices. The values of TF greater than 1 indicate that the plant is suitable for extracting the contaminants. Also, plants with values of TF and BAC greater than one are suitable for phytoremediation of the contaminants. Plants with TF value lower than 1 and BCF values higher than one are suitable for phytostabilization(Yoon et al. 2006).

The results concerning the effect of the studied treatments on the accumulation of metals in Table 8 showed that the value of TF for heavy metals was lower than 1 in all treatments. Therefore, both *S. persica* and *S. perspolitana* would serve as suitable species for phytostabilization.

Table 8. Mean values of TF, BCF and BAC related to total chromium in *S. persica* and *S. perspolitana*.

different concentrations of chromium	**Species**	**amendments**	**TF**	**BAC**	**BCF**
Cr_0	***S. persica***	control	0.010	0.028	0.280
		biochar	0.13	0.029	0.223
		manure	0.12	0.027	0.228
	S.perspolitana	control	0.043	0.014	0.33
		biochar	0.051	0.013	0.26
		manure	0.050	0.014	0.28
Cr_5	***S. persica***	control	0.084	0.47	5.67
		biochar	0.010	0.039	3.75
		manure	0.053	0.915	4.32
	S.perspolitana	control	0.02	0.18	7.59
		biochar	0.0142	0.050	3.52
		manure	0.017	0.095	5.59
Cr_{10}	***S. persica***	control	0.13	0.87	6.43
		biochar	0.039	0.059	1.51
		manure	0.020	0.102	4.91
	S.perspolitana	control	0.034	0.24	7.12
		biochar	0.017	0.097	5.43
		manure	0.029	0.16	5.58
Cr_{15}	***S. persica***	control	0.22	0.99	4.480
		biochar	0.01	0.063	3.35
		manure	0.021	0.092	4.40
	S.perspolitana	control	0.075	0.57	7.66
		biochar	0.024	0.167	6.78
		manure	0.032	0.22	6.93

Cr_0: without chromium, Cr_5: chromium concentration of 5 mg/kg, Cr_{10}: chromium concentration of 10 mg/kg, Cr_{15}: chromium concentration of 15 mg/kg

4. Discussion

4.1 The effect of hexavalent chromium on germination traits of S. persica and S. perspolitana

Heavy elements are limiting factors in the germination and growth stages of the seedling. Response to environmental stresses is a complex and undeniable phenomenon in higher plants(Díaz et al. 2001). In this regard, both *S. persica* and *S. perspolitana* species showed a similar response of reduced germination under increased chromium concentration. Seed germination is dependent on protein activity, which decreased under chromium treatment. Both Salicornia species indicated decreased growth with increasing hexavalent chromium concentration , and it was also found that chromium negatively influenced on root and stem length. Decreased growth in a plant may be due to reduced growth in the roots and consequently deficient transfer of nutrients to the upper parts of the plant. Also, the transfer of chromium to the plant shoot could directly affect its cellular metabolism, which leads to a decreased seedling length(Shanker et al. 2005).

The study of the effect of heavy metals on plant characteristics seems to be necessary to identify suitable plants for phytoremediation of contaminated soils on the one hand, and to identify resistant plant genotypes on the other hand. Bhardwaj et al.(Bhardwaj et al. 2009). demonstrated that the germination (%) of bean plant was not affected at low cadmium concentrations than the control, but prevented germination was observed at higher cadmium concentrations of 3 g/kg soil. Peralta et al. (Peralta et al. 2001). reported a decrease in seed germination, root , and shoot length with increasing concentration of cadmium in soil under alfalfa plant (*Medicago satival*). Seed germination was the same as the control at a cadmium concentration of 5 mg/l, although the stem length reduced by about 17% and the root length increased by about 22% compared to the control treatment, but decreases in seed germination and seedling length was significant at a

concentration of 10 mg/l. Also, a cadmium concentration of 40 mg/l led to the death of most alfalfa seedlings. Authors have reported delayed seedling emergence as a sign of cadmium toxicity. Therefore, the results of the mentioned studies are consistent with the findings of this investigation so that a decrease in the traits related to seed germination rate was observed with increasing the concentration of heavy metals in both *S. persica* and *S. perspolitana*.

Heavy metals cause serious damage to the cell by inducing a lack of active oxygen production. As vital stages in the plant life cycle, germination and establishment of seedlings can be affected by high levels of heavy metals. Chromium toxicity at concentrations of 5, 25, 100 , and 150 mg/kg had a significant effect on seedling growth. Chromium treatment at 100 mg/kg negatively influenced on germination indices of okra (*Hibiscus esculentus*)(Amin et al. 2013).

Also, in a study, it was found that the *Vigna radiata* did not tolerate chromium concentrations higher than 50 mg/l (Murtaza et al. 2018). In the present study, the results regarding the seedling growth showed a similar trend, so that stem length also decreased with decreasing root length in both *S. persica* and *S. perspolitana* species, which may be due to reduced nutrients. Therefore, the present study confirmed that seed germination and seedling growth are affected by different concentrations of chromium. Results with different concentrations of chromium in comparison with the control treatment demonstrated a decrease in germination parameters for both *S. persica* and *S. perspolitana* species confirming the findings obtained by other researchers.

4.2 Effect of experimental treatments on plant physiological parameters (plant height, root and shoot length)

This study showed that different concentrations of chromium in the soil negatively influenced on growth indices in both species so that the plant height decreased with increasing the concentration of chromium in the soil, which is consistent with the results of other researches. In

the case of *Eruca sativa* species, the presence of hexavalent chromium at a concentration of 500 mg/kg in the soil(Kamran et al. 2017). Caused a decrease in plant height. By studying a number of 32 plants, Lukina et al.(Lukina et al. 2016) reported that the toxicity of hexavalent chromium (1000 mg/kg) indicated an adverse effect on 94% of the studied species. Decreased growth and shoot height in plants are due to reduced root growth and development under the presence of chromium, which can result in the decreased transfer of water and nutrients to the aerial parts of the plant. Also, increased chromium transport to the shoot can directly affect sensitive plant tissues (leaf), photosynthesis, and cellular metabolism of the shoot, thereby reducing plant height. The effect of soil chromium on plant growth and yield appears in various forms , including decreased water and nutrient uptake, impaired cell division, imbalance in critical nutrient uptake and transport, plant inefficiency in selective uptake of inorganic nutrients, increased production of free radicals , and oxidative damage to sensitive plant tissues such as mitochondria, pigment content, RNA, DNA, lipids, etc.(Shahid et al. 2017).

All of these factors, individually or in combination, can influence on plant growth and development as well as plant functioning at cellular and molecular levels.

The negative effect of chromium on root dry weight as a result of damage to root cells has also been reported. Sundaramoorthy et al.(Sundaramoorthy et al. 2010).

found that chromium concentrations higher than 20 and 40 mg/l led to an inhibitory effect on plant growth. Hexavalent chromium at a concentration of 200 mg/l also resulted in reduced root growth in Asian rice (*Oriza sativa* L). In another study, the roots of *Zea maize* under hexavalent chromium treatment were characterized by shorter roots, lower dry weight , and brown color (Bhalerao &Sharma 2015). In the present study, different concentrations of chromium caused a

decrease in the dry weight of the roots in both studied species, confirming the results of other studies.

Our results also showed that the applied amendments had a positive effect on the growth indices for both studied species. addition, addition of organic matter to the culture medium serves as one of the factors improving soil conditions for plant growth. Organic matter can increase soil aeration and improve soil physical structure and cation exchange capacity , which varies depending on the type of organic matter (Moameri et al. 2017).

Atiyeh and Lee (2002) in an experiment investigated the effect of organic fertilizers on tomato and cucumber seedlings They indicated that application of these fertilizers had a positive effect on dry shoot weight, which can be attributed to the positive effect of organic matter on the physical properties and nutrient status of the soil.

Due to its nutrients and porous structure, specific surface , and strong functional groups, biochar is anion that can increase crop yield by improving soil nutrients and physical and chemical propertiesv(Xiang et al. 2017). The application of manure and biochar provided suitable conditions in terms of nutritional balance for plants , so that plant growth and yield increased significantly under these treatments, which is consistent with the results of other studies. Ahmad et al. (Ahmad et al. 2014). Investigated the effect of biochar application on the biomass of maize (*Zea mays* L.) and reported a significant increase in plant biomass compared to the control sample. Also, Lehmann and Joseph (Lehmann et al. 2009). reported argued adding biochar to the soil would preserve nutrients and thus increase plant growth, which is consistent with the results of the present study so that we observed that amending compounds led to increased growth of the two species of *S. persica* and *S. perspolitana*.

4.3 Accumulation of chromium in the roots and shoots of *S. persica* and *S. perspolitana*

This study showed that the accumulation of chromium in the roots and shoots of both species increased with increasing the chromium concentration in the soil. Most of the chromium accumulation occurred in the roots , which is consistent with the findings of Shahid et al.(Shahid et al. 2017). who stated that in *Pisum sativum*, the chromium concentration in different parts of the plant was as following: root > stem > leaf > seed. Liu et al.(Liu et al. 2009). observed the highest concentration of chromium in the root cell wall and intercellular spaces of the rhizome. Chromium fixation in plant roots is probably due to the formation of insoluble chromium compounds in the plant. Some authors have reported that chromium storage in root cells may be increased due to its storage in vacuoles of root cells, which may be a plant reaction to heavy metal toxicity to limit the toxic potential of the heavy metals(Shahid et al. 2017). In the present study, the effect of different chromium concentrations on the chromium accumulation in the two studied species at different concentrations of 0, 5, 10, 15 was significantly different , so that the accumulation of chromium in the shoots for both species increased with increasing chromium concentration in the soil. Also, chromium accumulations in the shoots were significantly different among the experimental treatments. During plant growth and development, higher chromium accumulation was observed for the roots. Higher accumulation of chromium in the roots compared to the shoots is a characteristic phenomenon observed in crops. Brunetti et al.(Brunetti et al. 2011). stated that higher accumulation of chromium, copper, lead , and zinc in the roots than the shoots represents the mechanism of plant tolerance and adaptation to high concentrations of metals in the soil. Singh et al.(Singh et al. 2004) attributed the higher accumulation of heavy metals in the roots than that of the shoot to the complexation of these metals with sulfhydryl groups, which prevented the transfer of these metals to the shoot. This

prevents the transfer of heavy metals to the food chain. Although the transfer of chromium from the plant roots to the shoots is minimal, the transfer of chromium within the plant tissue depends on its chemical form. The decreased chromium transport to plant shoots may be due to the conversion of hexavalent to trivalent chromium within plants due to the tendency of trivalent chromium to attach to cell walls. As shown in table 7, trivalent chromium has a higher tendency to be absorbed into the plant than hexavalent form, which may indicate the ability of Salicornia to convert hexavalent chromium to trivalent form in its tissues. In terms of growth, the plant needs to absorb trivalent chromium.

Also, the results obtained from this study showed that the amendments negatively influenced on the accumulation of chromium in the roots and shoots in both species. The highest decrease in chromium uptake was observed in biochar treatment. Biochar can be applied as an effective amendment for stabilizing heavy metals in contaminated soils. Namgay et al.(Namgay et al. 2010). showed that adding biochar as an amendment to soils contaminated with heavy metals could reduce the availability and absorption of these metals in plants. Zheng et al.(Zheng et al. 2012) also demonstrated that cadmium concentration in rice roots (*Oryza sativa* L.) decreased after the application of biochar.

The addition of manure to the soil also resulted in decreased uptake of chromium compared to soil without amendment. By increasing the cation exchange capacity of the soil, manure's application can be effective in reducing the availability of heavy metals in soil and plants. Containing a large amount of humus, manure's addition to the soil can alter the availability of heavy metals by changing soil physical and chemical properties(Pinto et al. 2004). Antoniadis et al.(Antoniadis et al. 2017) proved that the addition of manure would lead to reduced bioavailability of chromium in the chicory (*Cichorium spinosum*) and serves as the best way for

minimizing the toxic effect of chromium on the plant. Therefore, adding manure may be the most appropriate option for the stabilization of hexavalent chromium to inhibit the absorption of this heavy metal by the plant. As a result, manure's addition leads to decreased bioavailability of hexavalent chromium and slows down the phytoremediation process.

4.4 The effect of chromium toxicity on total phenolic content and photosynthetic pigments

The results of the present study showed that total phenolic content and photosynthetic pigments significantly decreased as a result of heavy metal toxicity. This decrease was greater in *S. perspolitana* than in *S. persica* and probably due to the higher accumulation of the metal in S. *perspolitana*. Also, the organic compounds (biochar and manure) caused an increase in the number of photosynthetic pigments with the higher contribution for biochar than manure, which can be explained by biochar's effect on increasing growth and reducing the accumulation of heavy metals in the plant. The non-enzymatic defense system of plants produces phenolic compounds , and these antioxidant compounds react with free radicals and convert them to stable radicals by giving up electrons to free radicals (Falleh et al. 2012).

In the present study, different chromium concentrations strongly affected the phenolic content of the plant , so that the accumulation of chromium at the concentration of 15 mg/kg completely suppressed the antioxidant properties of the plant , and the weakened defense system resulted in decreased plant growth. Levizou et al.(Levizou et al. 2019) showed that the total phenolic content in the marjoram plant (*Origanum vulgare*) decreased with the increasing chromium in the soil. Islam et al.(Islam et al. 2016).

Attributed the decreased phenolic content to the malfunctioning of critical enzymes in the biosynthesis of these compounds, which was consistent with the results of the present study regarding the effect of chromium stress on reducing phenolic content.

In addition to inhibiting chlorophyll biosynthesis due to the presence of heavy metals, Dhir et al.(Dhir et al. 2008) reported a decrease in iron and magnesium in the chlorophyll structure as a possible reason for the decrease in chlorophyll efficiency. In the present study, the contents of chlorophyll a and b significantly decreased under the effect of metal chromium compared to control plants, which is consistent with the findings of other studies.

Carotenoids are lipophilic secondary metabolites and the second most abundant pigment in nature. While chlorophylls are classified as optical pigments, carotenoids in stressful situations mainly contribute to protect the chlorophyll photosynthetic system. To quench the photodynamic reactions that can lead to chlorophyll loss, carotenoids replace peroxides to prevent the chloroplast membrane from collapsing (Duarte et al. 2012). The results of our study showed that chlorophyll *a*, *b* , and carotenoids contents in the shoots of both *S. persica* and *S. perspolitana* decreased with increasing the chromium concentration in the soil, confirming the results reported by many authors regarding the effect of chromium on plant pigments such as maize (*Zea mays* cv 704)(Rahmaty &Khara 2011) and wheat (*Triticum aestivum* L) (Subrahmanyam 2008).

4.5 The effect of Salicornia on the phytoremediation process

The results of phytoremediation in the present study showed that *S. persica* and *S. perspolitana* were able to absorb heavy metals from the soil with higher uptake of metals in the roots than shoots for both species. Considering that the TF index was lower than 1 for all treatments, one can conclude that *S. persica* and *S. perspolitana* were not suitable for phytoextraction of metal chromium. Also, the BCF factor for metal chromium was higher than 1, indicating that both *S. persica* and *S. perspolitana* stabilize the metal in the soil during the stabilization process. Both species mostly absorb trivalent form of chromium , indicating the high efficiency for converting hexavalent to trivalent form.

Yoon et al. (Yoon et al. 2006) found that plants with high accumulation and low transfer factors can stabilize heavy metals. Plant species that have been used to stabilize heavy metals so far include *Festuca rubra* (lead and zinc) and *Brassica juncea* (cadmium) (Ghosh &Singh 2005) , which is consistent with our findings in this research.

5. Conclusions

This study aimed to investigate the accumulation capacity of chromium metal in shoots and roots of *S. persica* and *S. perspolitana*. In this study, manure and biochar amendments were used to immobilize and stabilize metal chromium in contaminated soils. Chromium concentration in shoots and roots in *S. persica* and *S. perspolitana* were significantly affected by the concentration of this metal in the soil. In all observations, chromium accumulation in shoots and roots of the two studied species increased with increasing soil metal concentration. Both *S. persica* and *S. perspolitana* can be used for phytoremediation of soils contaminated with low concentrations of chromium as these two species could not tolerate the accumulation of chromium at high concentrations. The results also indicated higher chromium accumulations in the roots than the shoots for both *S. persica* and *S. perspolitana* species. Therefore, these two species can change the rhizosphere environment of the root and influence on concentration and bioavailability of metal chromium. The results of the present study showed a characteristic feature of converting hexavalent to trivalent chromium for both studied species. Accumulation of total chromium, trivalent chromium , and hexavalent chromium in the shoots was higher in *S. persica* than *S. perspolitana*. The photosynthetic pigments and total phenolic content in both studied species also decreased with increasing chromium concentration.

Our findings also showed that the use of organic compounds could improve the growth and yield of *S. persica* and *S. perspolitana* and , at the same time , decrease accumulation of chromium in

roots and shoots. Application of biochar and manure in *S. persica* and *S. perspolitana* species in the reclamation of soils contaminated with heavy metals caused increases of 49.05% and 28.30% in dry shoot weight, 53.12% and 14.06% in root dry weight, 23.93% , and 13.29% in chlorophyll *a*, 22.72% and 13.63% in chlorophyll *b* and 16.93% and 6.92% in carotenoids content compared to the controls (without amendment). Biochar and manure treatments also reduced the total phenolic content by 11.83% and 13.12% in *S. persica* and *S. perspolitana*, respectively. Biochar and manure treatments caused decreases by 36.69% and 17.98% in accumulation of metal chromium in the roots, and 84.83% and 72.55% in the shoots, respectively, compared to controls (without amendment). In general, the application of biochar treatment had a greater effect on reducing the accumulation of heavy metals than manure treatment because biochar has a higher ability to absorb metals due to its porous structure. As a result, it reduces the bioavailability of metals in both species. Therefore, using organic amendments in soils contaminated with heavy metals can stabilize these metals in the soil and prevent entering them into the food chain.

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Authors' contributions:

Fahime Ashrafi: Investigation, Project administration, Resources, Validation Writing – original draft, Software, Formal analysis, funding acquisition.

Ava Heidari: Supervision, Visualization, Writing – review & editing, Conceptualization, Data curation, Methodology, Project administration, Funding acquisition.

Mohammad Farzam: Supervision, Data curation, Validation.

Alireza Karimi: Supervision, Data curation, Validation.

Malihe Amini: Writing – review & editing, Formal analysis, Validation

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Figures

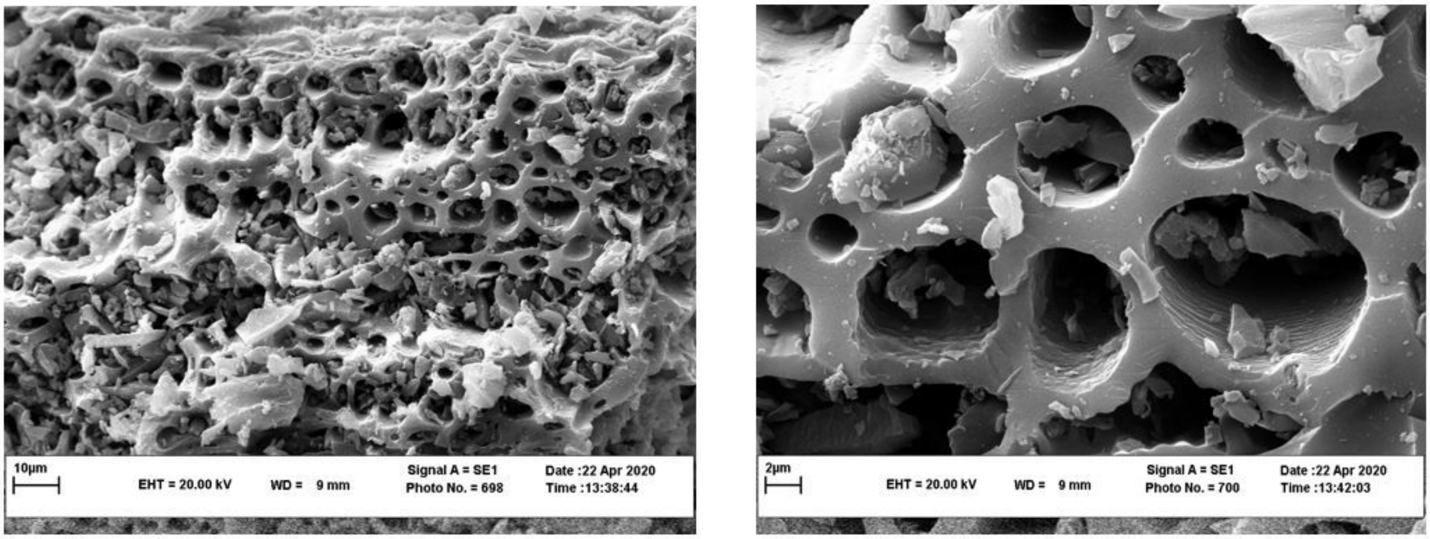

Figure 1

Scanning electron microscopy (SEM) of biochar surface