# Bioaugmentation and Bioaugmentation – Assisted Phytoremediation of Heavy Metals Contaminated Soil By a Synergistic Effect of Cyanobacteria Inoculation, Biochar, and Purtolaca Oleracea

**Fahimeh Zanganeh**
Ferdowsi University of Mashhad
**Ava heidari** (ava.heidari@yahoo.com)
Ferdowsi University of Mashhad
**Adel Sepehr**
Ferdowsi University of Mashhad
**Abbas Rohani**
Ferdowsi University of Mashhad

---

**Research Article**

**Keywords:** Oscillatoria sp, Co-contamination, Biochar-Alga-plant association, Metal Uptake, Sequential extraction

**Posted Date:** June 2nd, 2021

**DOI:** https://doi.org/10.21203/rs.3.rs-439162/v1



---

# Abstract

In recent decades, soil contamination with heavy metals has become an environmental crisis due to their long-term stability and adverse biological effects. Therefore, bioremediation is an eco-friendly technology to remediate contaminated soil, that its efficiency requires further research. This study was conducted to comparatively investigate two strategies, including bioaugmentation by using *Oscillatoria sp* and bioaugmentation assisted phytoremediation by using *Oscillatoria sp -portulaca oleracea* for the bioremediation of heavy metal (Cr (III), Cr (VI), Fe, Al, and Zn) contaminated soil at 180 days. To facilitate the remediation process, various quantities of biochar (0, 0.5, 2, and 5% (w/w)) were used in the experiments. The results of the bioaugmentation showed a significant improvement in chlorophyll *a*, nitrogen, organic carbon contents of soil and decrease all heavy metal bioavailability and EC of soil. The remediation efficiency test using plants proved the success of remediation treatments. Moreover, the findings of bioaugmentation-assisted phytoremediation displayed an improvement in soil fertility and a substantial reduction in the bioavailable fraction of heavy metals, especially in soil amended with 5% biochar. Cyanobacteria inoculation and biochar amendment dramatically enhanced the root lengths and shoot heights of *portulaca oleracea* while it significantly decreased their heavy metal accumulation compared to the control. For all heavy metals, TF and BAC (except Zn) values were found to be less than 1.0 at all treatments, illustrated the successful phytoextraction by the *P.* In conclusion, cyanobacteria inoculation along with biochar addition enhanced the TI quantities while diminished BAC and BCF values, suggesting the feasibility their applying in heavy metal contaminated soil for the facilitation of phytoremediation and their ability in pollutant immobilization.


# Highlights

- Bioaugmentation method displayed an improvement in contaminated soil fertility.
- Bioaugmentation method decreased the bioavailability of heavy metals in soil.
- Cyanobacteria and biochar dramatically enhanced the growth of *portulaca oleracea.*
- Cyanobacteria and biochar diminished BAC and BCF factors of *portulaca oleracea* .

# 1. Introduction

Contamination with heavy metals is a dangerous environmental issue as the mining processing, and use of these elements has enhanced in different world regions (Dhal et al. 2013). Heavy metals are one of the primary pollutants, which exist in the soil for many years. These pollutants are toxic and pose a severe threat to food safety, human and environmental health due to their accumulation in the food chain (Song et al. 2017, Tao et al. 2017). Several physicals, chemical, and biological methods including surface capping, encapsulation, electrokinetic extraction, soil flushing, chemical immobilization, landfilling, soil washing, phytoremediation, bioremediation, solidification, and vitrification have been developed for cleanup and remediation of metal contaminated soils (Liu et al. 2018a).

The biological or bioremediation methods displays numerous advantages, e.g. cost-effective (Liu et al. 2018a, Shah &Daverey 2020), environment friendly (Shah &Daverey 2020), high public acceptance (Liu et al. 2018a), simple to implement(Liu et al. 2018a), sustainable(Megharaj &Naidu 2017, Pandey et al. 2016), without disturbing the soil fertility, and biodiversity (Ahmad et al. 2016, Xiao et al. 2019), compared to others. Bioremediation can be classified into phytoremediation, microbial remediation, and animal remediation, based on the organism utilized (Luo et al. 2017). Although the phytoremediation technique is an appropriate option for remediation of heavy metal contaminated soil, it faced some challenges, including the lengthy period for cleanup, the slow growth rate of the plant, hard to mobilize some metals, an application for the site with low pollution, transfer accumulated metals into the food chain (Mahar et al. 2016). Recently, many researchers focused on improving its process efficiency by using amendment materials and microorganisms (Girolkar et al. 2021, Gong et al. 2021, Sharma et al. 2021). For example, several studies proved that microbe-assisted phytoremediation (e.g., *pseudomonas, burkholderia, bacillus, rhizobium, enterobacter, bradyrhizobium, paenibacillus, thiobacillus, Arbuscular mycorrhizal fungi*, etc.) showed a positive effect on the phytoremediation by enhancement of the growth, health, and establishment of the plant, and sequestration of heavy metal in soil (Harindintwali et al. 2020, Khalid et al. 2021, Lebeau et al. 2008). On the contrary, few research applied soil algae (e.g., cyanobacteria) for soil remediation (Biglari Quchan Atigh et al. 2020b). However, cyanobacteria inoculation experience demonstrated that their species such as *Nostoc*, *Anabaena*, etc have outstanding capabilities for the restoration of degraded soil in the arid area (Chamizo et al. 2018b, Li et al. 2019).

Cyanobacteria are known as blue-green bacteria, belong to the kingdom prokaryotes, which are found in various habitats, even in the desert, salty soil (Li et al. 2019, Rossi et al. 2017). They are considered as ecosystem engineers (Jones et al., 1996), due to their ability to sequestration of carbon, fixation of nitrogen, secretion nutritious, therefore improvement of soil conditions (fertility, microbial community, and productivity) and as results provide an appropriate environment for the existence of micro and macro-organisms (Kheirfam &Roohi 2020, Kheirfam et al. 2017, Mugnai et al. 2018). The previous studies proved that cyanobacteria have the potential to be developed as a bioremediating agent for plant growth promotion and salt-affected soil remediation through most effective mechanisms such as nitrogen fixation, production of extracellular polysaccharides, and growing the organic carbon contents (Li et al. 2019, Muñoz-Rojas et al. 2018). Therefore, cyanobacteria inoculation can be considered a novel technique for remediation of contaminated soil (Biglari Quchan Atigh et al. 2020a).

The combined utilization of microorganisms, plant, and amendment materials was suggested as an innovative, effective method for remediation of heavy metal contaminated soil. Among various amendments, biochar “a carbon-rich material” was considered as an exciting remediation option for contaminated soil. According to literature (Chen et al., 2018; Lahori et al., 2017; Siedt et al., 2020), it is most effective in increasing the soil water holding capacity, alterations chemical soil properties (e.g., pH, cation exchange capacity, and buffering capability), retaining plant available water, increment nutrient concentration, decreasing the bioavailability of heavy metal, reduce plant uptake, and microbial communities. Furthermore, some studies indicated that the combination of biochar with animal-

 robial-remediation (such as bacteria,

*Pseudomonas sp., Enterobacter sp.* ) enhanced the soil fertility and metal uptake by the plant due to its synergistic effects (Chen et al. 2019, Sanchez-Hernandez et al. 2019, Tu et al. 2020, Wu et al. 2019, Xiao et al. 2020). However, the combination mechanism between microorganisms and biochar for the transformation or stabilization of heavy metal in the soil is not well understood. There is also a research gap in the effect of biochar on microbial assisted phytoremediation, especially from the aspect of plant growth and heavy metal bioavailability.

In this study, Oscillatoria sp, a filamentous cyanobacteria species, *purtolaca oleracea*, and biochar were employed as microbial, plant, and material agents to remediate soil contaminated with heavy metals (Cr (III), Cr (VI), Fe, Al, and Zn), respectively. However, to the best of our knowledge, interactions between biochar and cyanobacteria on heavy metal uptake by *purtolaca oleracea* grown on contaminated soil have not been reported. Hence, the specific aim of this study is to: (1) evaluate the effect of cyanobacteria inoculation accompany with biochar amendment on the properties of soil and heavy metal bioremediation efficiency, (2) explore the influence of *Oscillatoria sp* - *purtolaca oleracea* - biochar partnerships on soil properties and heavy metal bioremediation efficiency, (3) assess the heavy metal accumulation in parts of *purtolaca oleracea* under metal stress and assisted remediated materials.

## 2. Material And Method

# 2.1 Chemicals

Chemicals with analytical grade including potassium dichromate ($K_2Cr_2O_7$), magnesium carbonate ($MgCO_3$), ethanol 99.8% ($C_2H_5OH$), acetic acid ($CH_3COOH$), hydroxylamine hydrochloride ($NH_2OH.HCl$), hydrogen peroxide 35% ($H_2O_2$), ammonium acetate ($C_2H_7NO_2$), nitric acid 65% ($HNO_3$), hydrochloric acid 37% (HCl), acetone ($CH_3O_6$), BG 11 Broth, and diphenylcarbazide ($C_{13}H_{14}N_4O$) were purchased from Merck Company.

# 2.2 Contaminated soil description and characterization

The soil was collected from a contaminated industrial area in Mashhad, Iran (36° 29' 92" N, 59° 31'16" E). The sample was gathered from 0 to 40 cm of soil depth and air-dried at room temperature. After homogenizing and sieving (> 2 mm mesh), the soil texture was determined using a hydrometer method (Bouyoucos 1962), which was classified as loam (sand 50%, silt 28%, clay 22%). Soil pH and EC were characterized using pH meter (basic 20, Crison, Spain) and EC meter (4510, Jenway, England) with soil to distilled water ratio of 1: 25. The organic carbon (OC) and total nitrogen (N) contents of soil samples were determined by loss of ignition (Park et al. 2017) and the Kjeldahl method (Kirk 1950), respectively. Some of the important soil properties were as follows: pH; 9.05, EC; 121.8 $dsm^{-1}$, OC; 0.022 g $kg^{-1}$, N; 1120 mg $kg^{-1}$, K; 101.7 mg $kg^{-1}$, and P; 155 mg $kg^{-1}$.

The concentration of heavy metals in soil was determined by the aqua regia digestion method (Alghanmi et al. 2015). 1 g of air-dried soil was mixed with 5 ml of HNO3 (65%) and 15 ml of HCl (37%) and stirred at room temperature for 12 h. After this, the obtained mixture was digested at 130°C for three h and then filtered. The heavy metal content of filtrate was measured by inductively coupled plasma-optical emission spectrometry (76004555, Spectro Arcos, Germany). The concentration of Cr (VI) was determined by using UV-VIS Spectrophotometer (DR5000- Hach, USA). The following results were acquired: Cr (III) ; 65.45 mg $kg^{-1}$, Cr (VI) ; 12.71 mg $kg^{-1}$, Zn; 104.07 mg $kg^{-1}$, Al; 11287 mg $kg^{-1}$, and Fe; 27405 mg $kg^{-1}$.

Metal distribution in the soil samples was determined using the European Bureau of References (BCR) (Peña-Icart et al. 2011, Wuana et al. 2010). Three sequential extraction phases, including extractable (step1), reducible (step 2), organic-bound (step3), and residual (R), were conducted to attain various fractions of heavy metals in soil. According to this method, 1g of dried soil (105 ˚C for three h) was digested using sequential extractions (4 steps), which its detail as illustrated in Table 1. The heavy metal ions content of each fraction in soil was measured with the ICP instrument.

Table 1

BCR sequential extraction procedure utilized for Cr, Zn, Al, and Fe speciation.

| Step | Soil phase | Reagents | Stirring time and temperature | Separation and storage |
|---|---|---|---|---|
| S1 | Water- and acid-soluble, exchangeable<br>(extractable) | 40 mL of 0.11 M acetic acid | 16 h at room temperature | Centrifuging at 1500 rpm for 15 min<br>store into a polyethylene bottle at 4 ºC |
| S2 | Bound to Fe–Mn oxides (reducible) | 40 mL of 0.5M hydroxylamine hydrochloride | 16 h at room temperature | |
| S3 | Bound to organic<br>matter and sulfides (oxidizable) | 10 mL of 8.8 M hydrogen peroxide | 1h at room temperature, then 85°C for 1h | |
| | | Then 10mL of 8.8M $H_2O_2$ | 85°C for 1h | |
| | | 50 mL of 1.0 M ammonium acetate | 16h at room temperature | |
| R | Residual | 37.5 ml of 12 M HCl and 12.5 ml of 17 M $HNO_3$<br>(3:1) | digested for 24h at room temperature<br>then, digested at 130°C for 15 min | Filter with filter paper |

## 2.3 Production and characterization of biochar

The biochar used in this study was produced from the pyrolysis of three wood wastes, including Eucalyptus, Populus, and Parrotia, at a temperature of 400 ˚C. The physicochemical properties of the
 n the following. It had a pH of 7.73 and EC of

935 ds $m^{-1}$ measured in 1:5 suspensions of biochar to distilled water using pH and EC meters, respectively. The total contents of C, N, O, H, and S were measured by the elemental analyzer (FLASH EA 1112 SERIES, Thermo Finnigan, USA). The quantities of C, O, H, N, and S of biochar were 73.41, 23.51, 2.71, 0.37, 0 %, respectively. Besides, the surface functional groups of the bio-char were determined by Fourier to transform infrared spectroscopy (FTIR) (AVATAR 370, Thermo Nicolet, USA). Also, the surface morphology of biochar was analyzed by scanning electron microscopy (SEM) (VP 1450, LEO, Germany).

## 2.4 Experimental design setup

All experiments were conducted in a greenhouse at the Ferdowsi University of Mashhad, Mashhad, Iran, from July to September 2019. Generally, two experiment series were designated: (1) bioaugmentation by cyanobacteria and (2) bioaugmentation-assisted phytoremediation by cyanobacteria and *purtolaca oleracea*. Biochar was used as an amendment in both series. Table 2 illustrates the experimental design, a 4× 2 factorial design, with three republications with four quantities of biochar (0, 1, 2, 5 % w/w) and two types of treatments (*Osillatoria sp* as microorganism and *purtolaca oleracea* as a plant).

For bioaugmentation testing, the Petri dishes with a diameter of 8 cm and a height of 1 cm, were filled with 80 gr of sterilized contaminated soil, which previously mixed with various amounts (0, 1, 2, 5 % w/w) of biochar. Then, 1.4 g (wet weight) of cyanobacteria separated from the culture medium by centrifugation at 1500 rpm for 15 min, mixed and homogenized with 20 ml of distilled water at room temperature for 24 h, was added to the soil surface using a sterile syringe. The inoculated plates were placed in the greenhouse at 2 ± 27°C under the light intensity of 1600 lux and light to a dark ratio of 16 h: 8 h. They were irrigated with 24 mL of distilled water every 24 hours.

For bioaugmentation-assisted phytoremediation examination, 1 kg of sterilized contaminated soil mixed with different ratios (0, 1, 2, 5 % w/w) of biochar and placed into several polyethylene pots with a total volume of 2 L (area of 133 $cm^2$ and depth of 10 cm). The drainage holes were embedded in the bottom of the pots to guarantee the health of the roots. The plant seeds were disinfected with ethanol 99.8 % and planted directly in the soils. 0.8 g (wet weight) of the cyanobacteria were added to the surface soil in the pots when the seeds germinated. The pots were placed in the greenhouse at 30 ± 5°C with a light/dark ratio of 16 h: 8 h and irrigated with 50 ml of distilled water every 24 h. After four weeks, the surface soil of each pot was also inoculated with 0.8 g of cyanobacteria mat uniformly. Twelve weeks after planting, the plants were harvested. After washing thoroughly with deionized water, they were dried at 70°C for 24 h and store in a polyethylene bag for future analysis.

Table 2

Design of experiment

| Description | Abbreviation | Treatment |
|---|---|---|
| Control Soil | S | Bioaugmentation |
| Control Soil + Cyanobacteria | SC | |
| Control Soil + Cyanobacteria + Bio-char 0.5 | SCB0.5 | |
| Control Soil + Cyanobacteria + Bio-char 2 | SCB2 | |
| Control Soil + Cyanobacteria + Bio-char 5 | SCB5 | |
| Control Soil + Plant | SP | Bioaugmentation - Phytoremediation |
| Control Soil + Cyanobacteria + Plant | SCP | |
| Control Soil + Cyanobacteria + Plant + Bio-char 0.5 | SCPB0.5 | |
| Control Soil + Cyanobacteria + Plant + Bio-char 2 | SCPB2 | |
| Control Soil + Cyanobacteria + Plant + Bio-char 5 | SCPB5 | |

## 2.5 Bioremediation efficiency

Lettuce and radish were applied as indicators to evaluate the bioremediation success of tests recommended in prior works (Chen et al. 2018b, Mahbub et al. 2017). 25g of a bio-remediated soil sample by cyanobacteria were placed into small pots (length ×width ×height: 5 ×5 ×10 cm) for planting. Five seeds of each plant (lettuce and radish) were planted in the soil of pots. Plants were irrigated with distilled water every day. After 14 days, the germination, root, hypocotyl, and shoot lengths of the lettuces and radish were measured.

## 2.6 Plant analysis

After three months of planting, first, the *purtolaca oleracea* plant was cut, and its stem heights and root lengths were measured. The plant parts were washed thoroughly with deionized water and then dried at 70°C for 24 h. The concentration of heavy metals (Cr (III), Cr (VI), Zn, Al, and Fe) in the roots and shoots of *purtolaca oleracea* were determined using $HNO_3$ - $H_2O_2$ digestion procedure according to Ashrafı et al. [5]. 0.2 g of the sample was digested with 5 mL $HNO_3$ (65%) at 110°C for two h. Then, after cooling, 1 mL of $H_2O_2$ (30%) was added and boiled for 1 hour. Finally, the obtained solution was passed through filter paper, and its heavy metal contents were analyzed using the ICP instrument. The capability of the plant to tolerate and accumulate heavy metals was determined by bioconcentration factor (BCF) (Mani et al. 2016), Translocation Factor (TF) (Meeinkuirt et al. 2016), and bioaccumulation coefficient (BAC) (Hamzah et al. 2015), and Tolerance Index (TI) (Ali et al. 2002) which were estimated using the following formula:

$$BCF = \frac{Concentration of heavy metals in root}{Concentration of heavy metals in soil} \quad (1)$$

$$BAC = \frac{Concentration of heavy metals in the shoot}{Concentration of heavy metals in soil} \quad (2)$$

$$TF = \frac{Concentration of heavy metals in the shoot}{Concentration of heavy metals in root} \quad (3)$$

$$TI = \frac{Root length in contaminated soil}{Root length in the control soil} \quad (4)$$

## 2.7 Statistical analysis

The Kolmogorov–Smirnov test was utilized to test data normality and homogeneity of variance. The statistically significant difference between data among control and treatments was determined using one-way analysis of variance (ANOVA) followed by Tukey's and Duncan's test. The significant difference ($p$-value < 0.05) among various treatments was displayed by using different letters. Data analysis was performed using IBM SPSS Statistics version 20.

## 3. Results And Discussion

## 3.1 Characterization of Biochar

The surface morphology of biochar was observed by SEM analysis (Fig. 1a). From this image, regular pores similar to honeycombs can be seen on its surface, which indicates its ability the adsorption of heavy metals. This honeycomb structure was related to the carbon skeleton of biochar (Ghani et al. 2013). The surface functional groups were determined by FTIR analysis (Fig. 1b). The peak at 3425 $cm^{-1}$ was attributed to the –OH stretching or phenolic groups (Liu et al. 2018b). The bands located at 3033 and 1591 $cm^{-1}$ were related to aliphatic $-CH_2$ (Bandara et al. 2017) and –COO – antisymmetric stretching or aromatic C = C (Yang et al. 2016a), respectively. The peak at about 1431 $cm^{-1}$ corresponds to the stretching vibrations of conjugated C = O bonds in aromatic rings (Meier et al. 2017). The bands presented at about 1376, 751, and 874 $cm^{-1}$ were associated with -COO (Dong et al. 2017) and C-H aromatic groups (Song et al. 2012), respectively. Besides, the peaks at 1230 $cm^{-1}$ were belonged to -CO and OH aromatic groups (Liu et al. 2018b).

## 3.2 Bioaugmentation

### 3.2.1 Cyanobacteria inoculation

Figure 2 showed the application *Oscillatoria sp.* in the heavy metal contaminated soil amended with various biochar. After three months of inoculation, cyanobacteria were progressively distributed and covered on the soil's surface (Fig. 2 (b)). The SEM image, taken from the surface of inoculated soil, displays mineral particles, and soil fragments were coated by exopolysaccharides (EPS) assumed to have been secreted by the cyanobacteria strain. Similar results were reported by Maqubela et al.

(Maqubela et al. 2010), Issa et al. (Issa et al. 2007), Chamizo et al. (Chamizo et al. 2018a) after cyanobacteria inoculation in soil.

## 3.2.2 Soil physicochemical properties

Table 3 summarized the influence of bioaugmentation on soil physicochemical properties under different treatments. The pH and EC contents of soil were decreased, while the OC, N, and Chlorophyll-a amounts were enhanced in various treatments. Compared with the control soil (S), there were higher soil EC, but lower OC, N, and Chlorophyll-a among treatments with cyanobacteria inoculation and biochar amendment ($p < 0.05$). Previous studies (Biglari Quchan Atigh et al. 2020a, Kheirfam &Roohi 2020, Kheirfam et al. 2017, Nisha et al. 2018, Peng &Bruns 2019) found that the inoculated cyanobacteria into soil reduced the pH and EC, while elevated OC, N, and Chlorophyll a. It implies that *Oscillatoria sp*. used in this study as a genus of cyanobacteria, with carbon and nitrogen fixation process, which convert atmospheric carbon dioxide and nitrogen to organic compounds and ammonium or nitrate, results in improvements in OC and N. Besides, cyanobacteria reduce pH and EC by producing organic acids and absorbing sodium ions from the soil (Kheirfam &Roohi 2020, Kheirfam et al. 2017, Peng &Bruns 2019). The biochar treatments (SCB0.5, SCB2, and SCB5) had the highest OC, N, and chlorophyll a. The biochar was found to considerably increase the availability of nutrients, water holding capacity, and heavy metals sorption (Siedt et al. 2021, Wang et al. 2021). It also affects the soil microorganisms directly or indirectly by providing C and other nutrients, and space (macro-, meso- and micro-pores) for colonization and changing soil conditions (Siedt et al. 2021). The SCB5 represented the highest OC, N, and chlorophyll contents, mainly attributed to the most significant growth of inoculated cyanobacteria in soil.

Table 3

Effect of bioaugmentation on soil physicochemical properties

| Treatment | pH | EC(ds $m^{-1}$) | OC(g $kg^{-1}$) | N(mg $kg^{-1}$) | Chlo a(mg $m^{-2}$) |
|---|---|---|---|---|---|
| S | $9.05 \pm 0.07^{a}$ | $121.8 \pm 2.88^{a}$ | $0.0221 \pm 0.007^{b}$ | $1120 \pm 12.7^{c}$ | 0 ± 0 |
| SC | $9.04 \pm 0.02^{a}$ | $50.56 \pm 2.22^{b}$ | $0.059 \pm 0.012^{b}$ | $1610 \pm 62.35^{b}$ | $3.5478 \pm 0.24^{b}$ |
| SCB0.5 | $9.01 \pm 0.03^{a}$ | $50.1 \pm 3.95^{b}$ | $0.0601 \pm 0.019^{c}$ | $1652 \pm 30.02^{b}$ | $4.3076 \pm 0.84^{ab}$ |
| SCB2 | $8.99 \pm 0.04^{a}$ | $48.03 \pm 0.88^{b}$ | $0.07495 \pm 0.003^{ab}$ | $1708 \pm 12.12^{ab}$ | $5.1094 \pm 0.47^{ab}$ |
| SCB5 | $8.89 \pm 0.06^{a}$ | $44.56 \pm 4.29^{b}$ | $0.0955 \pm 0.001^{a}$ | $1792 \pm 17.32^{a}$ | $5.7994 \pm 0.82^{a}$ |

## 3.2.3 Metal speciation in the contaminated soil

Figure 3 displays the speciation of heavy metals including Cr (III), Cr (VI), Al, Zn, and Fe during various treatments. As shown in Fig. 3, with cyanobacteria inoculation and biochar addition into the soil, the S1 fractions of Cr (III), Cr (VI), Zn, Al, and Fe declined by 15–38 %, 1.7–87 %, 45–59 %, 5–47 %, and 1.6–58

%, relative to control, respectively. In other words, the fractions of heavy metals in soil were noticeably influenced by the treatments. A significant decrease in the S2 form (Fe- and Mn-bound) of Cr (VI) was observed by 49–72 % following the treatments. In contrast, a sharp increment in the S2 fraction of Cr (III), Fe, Al, and Zn was obtained after treatments. The fraction bound to organic matters and sulfides (S3) of Cr (III) and Fe increased by 8.9–324 and 133–219 while that of Al, Zn, and Cr (VI) decreased by 83–91 %, 18–66 % and 13–67 %, respectively. The previous researches pointed that the application of biochar in soil notably enhanced the proportion of organic matter bond various metals (Park et al. 2011, Rinklebe &Shaheen 2015). Similarly, the cyanobacteria inoculation and the addition of biochar also grew the residual forms (S4) of Cr (III), Al, and Fe by 291–439 %, 82–207 %, 133–219 %, respectively. The Cr (VI) and Zn indicated the reduction trend in the S4 forms. The exchangeable bound form displays the labile (easily bioavailable) fraction, while the Fe- and Mn, organic matter bound, and residual fractions are related to the recalcitrant (less bioavailable) portion. The effectiveness of immobilization assesses by both the labile and recalcitrant fractions. Nie et al. (Nie et al. 2018) and Tu et al. (Tu et al. 2020) found that the easily bioavailable fraction of Cd and Cu in soil reduced biochar addition. In the present study, a similar trend was observed.

### 3.2.4 Evaluation of the efficacy of soil bioaugmentation

Prior works (Aparicio et al. 2015, Chen et al. 2018a, Wang et al. 2014) proved that radish and lettuce could be used as a biomarker for assessing bioremediation efficiency. Figure 4 displays the root and hypocotyls lengths and the vigour index (VI) of the radish and lettuce among various treatments. As shown in this figure, the root and hypocotyls lengths and VI of lettuces and radish in control soil were significantly smaller ($p < 0.05$) than those in soil with treatments, indicating that the bioremediation treatments improved plant growth by the diminution of heavy metals stress in soil. Furthermore, the soil inoculation with cyanobacteria and 5 % biochar (SCB5) supported the most development of longer root and hypocotyls lengths and higher VI. Similar results were observed by Mahbub et al. (Mahbub et al. 2017), who confirmed that the root lengths of lettuce were a good indicator for evaluating the remediation effectiveness of mercury-contaminated soil. This also supported the findings of Aparicio et al.(Aparicio et al. 2015). They utilized the roots and hypocotyls lengths and the VI of lettuce as a biomarker for bioremediation efficiency of soil contaminated with Cr (VI) and lindane. In general, our finding confirms that the bioremediation effectiveness of cyanobacteria and biochar amendment.

## 3.3 Bioaugmentation-assisted phytoremediation

### 3.3.1 Influence of treatments on Portulaca oleracea growth

The effect of cyanobacteria inoculation and biochar amendment on *Portulaca oleracea* growth was measured (Table 4). By cyanobacteria inoculation in soils, the shoot and root lengths increased from 12.73 and 1.67 cm to 18.87 and 3.07 cm, respectively. This was attributed to the enhancement of nitrogen and organic carbon content of the soil in the presence of cyanobacteria, which promote plant growth (Rossi et al. 2017). Besides, with an increment of biochar dose from 0 to 5 percent in the soils, the shoot and root lengths grew 4.6 and 3 fold, respectively. This may be due to the decreased availability of heavy metals and improvement in nitrogen and organic carbon content (Table 3) in the soil by adding biochar (Puga et al. 2015). The weight of dry plant biomass after all treatments increased significantly by 25 fold as compared to control. This is in good agreement with Puga et al. (Puga et al. 2015), Kiran and Prasad (Kiran &Prasad 2019), and Qi et al. (Qi et al. 2020). They observed that a significant enhancement in plant growth factors in soil applied with biochar.

Table 4

The effect of various treatments on growth performances of Portulaca oleracea cultivated in heavy metal contaminated soil

| Treatments | The dry weight of shoot (g) | The dry weight of root (g) | The dry weight of the whole plant (g) | Root length(cm) | shoot height(cm) |
|---|---|---|---|---|---|
| SP | $0.0211 \pm 0.013^{b}$ | $0.0012 \pm 0.001^{b}$ | $0.0223 \pm 0.014^{b}$ | $1.67 \pm 0.08^{b}$ | $12.73 \pm 5.79^{b}$ |
| SCP | $0.0644 \pm 0.014^{ab}$ | $0.0040 \pm 0.001^{ab}$ | $0.0685 \pm 0.015^{ab}$ | $3.07 \pm 0.63^{ab}$ | $18.87 \pm 5.55^{b}$ |
| SCPB0.5 | $0.0928 \pm 0.019^{ab}$ | $0.0051 \pm 0.002^{ab}$ | $0.079 \pm 0.021^{ab}$ | $3.33 \pm 1.16^{ab}$ | $22.67 \pm 8.20^{b}$ |
| SCPB2 | $0.3820 \pm 0.142^{a}$ | $0.0138 \pm 0.007^{a}$ | $0.3958 \pm 0.149^{a}$ | $4.47 \pm 0.95^{a}$ | $49.77 \pm 5.21^{a}$ |
| SCPB5 | $0.4657 \pm 0.093^{a}$ | $0.0343 \pm 0.10^{a}$ | $0.5 \pm 0.193^{a}$ | $5 \pm 0.28^{a}$ | $58.5 \pm 7.94^{a}$ |

## 3.3.2 Metal speciation in the contaminated soil

To explore the influence of the cyanobacteria inoculation and biochar percentage on heavy metal speciation, the BCR method was carried out after bioremediation experiments. Their results for Cr (III), Cr (VI), Fe, Zn, and Al illustrates in Fig. 5. As shown in Fig. 5 (a), the concentration of Cr (III) in S1 fraction (water- and acid-soluble and exchangeable) reduced from 3.5 % in the sample with cyanobacteria inoculation (SCP) and to 90 % in the sample with the highest value of biochar (SCP5). Besides, the S1 fraction of Cr (VI), Zn, Al, and Fe in contaminated soil decreased by 22 – 13, 2–68, 9–38 %, and 43–93 %, with the addition of biochar and cyanobacteria. The largest decrease in solubility of heavy metal was obtained for the samples were treated with 5 % biochar. The metals with the ionic form or bond to carbonate mainly refer to the acid-soluble and are recognized as the exchangeable fraction. This soil phase can be easily bioaccumulated in living organisms (Gong et al. 2019). Lu et al (Lu et al. 2017) observed a similar reduction in the acid-soluble fraction of Cd, Cu, Pb, and Zn using bamboo and rice straw biochars amendments in soil. Méndez et al. (Méndez et al. 2012) showed that the sewage sludge-derived biochar significantly decreases the extractable fraction of Cd, Pb, and Zn in agricultural soil. Gong

 and Bashir et al. (Bashir et al. 2018a) also reported similar declining trends for Cd in contaminated soil with various biochar. Cao et al. (Cao et al. 2011) reported that the reduction of the extractable Pb in the soil was increased with the increasing biochar rates. This phenomenon was ascribed to the immobilization of metal by biochar (Gong et al. 2019, Lu et al. 2017). The specific properties of some biochars, including micro-porous structure, high pH, surface oxygenated-containing functional groups, and large surface area causes, the solubility of heavy metal reduced through adsorption, complexation, and precipitation (Chan &Xu 2009, Gong et al. 2019, Yuan et al. 2011, Zhang et al. 2013).

The S2 fraction (reducible) indicates the metal bound to Fe–Mn oxides or hydroxides. From Fig. 5 (b and c), an increase in S2 fraction of Al and Zn by 24–75 % and 10–49 % can be observed, while Fe displayed a declining trend from 47–83 %. The reducible portion of Cr (III) was dropped by 14–46 %, while it increased for Cr (VI) by 7–64 %, except for SCP5. Jiang et al. (Jiang et al. 2012) found that the reducible fraction of Cu, Cd, and Pb rose in contaminated soil with biochar. Bashir et al. (Bashir et al. 2018a) reported that the reducible form of Cd grew in the soil with an increment application rate of biochar. The increase in reduction portions of metals might be due to (1) the precipitation of metal with iron, oxygen, hydrogen, and manganese presents on the biochar surface (Bashir et al. 2018a) and (2) surface complexation creation because of high organic matter the presence of iron and manganese oxides in the soil (Bashir et al. 2018b).

The incorporation of biochar and cyanobacteria increased the S3 fraction (bound to organic matter and sulfides) of all heavy metals. S3 portion of Cr (III), Cr (VI), Zn, Al, and Fe climbed sharply by 290–566 %, 1.5–33 %, 73–188 %, 294–326 %, and 323–1137 %, with cyanobacteria inoculation and biochar amendment. The elevated S3 fraction might be primarily ascribed to high organic matter value in the contaminated soil due to cyanobacteria growth and biochar amendment, which inhibit the bioavailability of heavy metals. This is consistent with Liang et al. 's results (Liang et al. 2017). They found that the organic portion of Cu rose by utilizing biochar. Chen et al. (Chen et al. 2020) observed that the application of biochars along with ferrous sulfate and pig manure S3 form of Cd in the contaminated soil. Su et al. (Su et al. 2016) reported that biochar-supported zero-valent iron nanoparticles increased the conversion of more easily available Cr ( exchangeable) to the less available forms ( organic matter- bound). Bashir et al. (Bashir et al. 2018a) found that the S3 forms of Cd enhanced in the contaminated soil with an increment application rate of various biochar types.

S4 fraction (Residual) indicates the main primary and secondary minerals that block the metals within their crystal structures (Tokalioğlu et al. 2006). The S2 portion of Cr (VI), Al, and Fe fell by 20 %, 18 %, and 5% with the application of biochar and cyanobacteria. The Cr (III) and Zn display an increasing trend. Similar results were reported by Liang et al. (Liang et al. 2017). They reported that the residual portion of Zn increased while that of Cu was reduced, with the addition of biochar into contaminated soil. Besides, our results have some similarities with Chen et al. 's (Chen et al. 2020), Bashir et al. 's (Bashir et al. 2018a), and Chen et al. 's (Chen et al. 2020) findings.

## 3.3.3 Heavy metal concentration in plant

Figure 6 shows the concentrations of Cr (VI), Cr (III), Zn, Al, and Fe in shoots and roots of *Portulaca oleracea* depending on the treatment under different treatments. As shown in Fig. 6, the heavy metals concentrations in shoots and roots of *Portulaca oleracea* growing in the remediated soil were significantly ($p < 0.05$) decreased relative to the control. The metals accumulation in the root of *Portulaca oleracea* was higher than the shoot. In other words, the application of biochar and cyanobacteria inoculation significantly reduced the above-mentioned heavy metals concentrations in *portulaca oleracea*. The minimum concentrations of the shoot and the root metals were obtained when the 5 % biochar accompany with cyanobacteria (SCPB5) were used in the pots. Moreover, the highest decrease of -88.7 % and − 94.6 % for Cr (III), -93.15 % and − 97 % for Cr (VI), -96.6 % and − 96.5 % for Zn, -92.7 % and − 96 % for Fe, and − 93.7 % and − 90 % for Al (III) were obtained in shoot and root, respectively. The results showed that the addition of biochar and cyanobacteria to the soil led to a decrease in the heavy metal concentration of *portulaca oleracea*. These findings are consistent with literature results concerning the influences of biochar on the accumulation of Zn (II)(Al-Wabel et al. 2015, Martins et al. 2018), Cr (VI) (Lyu et al. 2018), Fe (Al-Wabel et al. 2015), and Al (Qian et al. 2018) in the plants. The biochar, due to its characteristics (e.g., large surface area and various functional groups), immobilized the heavy metals in the soils through two approaches: (1) direct adsorption of metals via physical adsorption, electrostatic attraction, ion exchange, and complexation; (2) improving indirect adsorption via soil particles due to altering its physicochemical properties such as cation exchange capacity and organic matter content (He et al. 2019a, He et al. 2019b, Wang et al. 2020). Therefore, the immobilization of metals in the soil reduces their phytoavailabilty and phytotoxicity.

Besides, the parts of *portulaca oleracea* grown in the soils inoculated with *Oscillatoria sp.* (SCP) have also indicated a decreased levels of above heavy metals. Although the concentration of all desired metals in roots and shoots decreased, the highest percentages of reduction were obtained for Zn (II, > 85 %) and Cr (III, > 76 %). The prior studies reported that cyanobacteria have great potential for salt-affected soil remediation (Chamizo et al. 2018b, Li et al. 2019). Cyanobacteria can produce organic carbon and extracellular polymeric materials, including exopolysaccharides (EPS) and extracellular proteins in the soil (Joshi et al. 2020, Li et al. 2019). By biosorption via exocellular polysaccharides and organic matters, they reduce heavy metal mobility and toxicity (Joshi et al. 2020).

### 3.3.4 Phytoremediation potential

The BCF, BAC, and TF were estimated for various treatments and were shown in Fig. 7. BCF indicates a plant's ability to accumulate heavy metals from soil, and TF shows a plant's ability to translate heavy metal from the roots to the shoots. The translocation of heavy metals from soil into the above-ground parts of the plant, such as the shoot, is called the phytoextraction process (Garbisu &Alkorta 2001). This process occurred in the plant when the BCF and TF > 1 (Nazir et al. 2011). In this study, for all heavy metals, TF values were found to be less than 1.0 at all treatments (Fig. 7). It has been proved that plants with TF more than 1 accumulate the metals in the aerial part while that with TF lower than 1 shows the lower ability for transferring the heavy metal from root to shoot (Arán et al. 2017, Chen et al. 2015, Prasad et al. 2001). This demonstrated that *Portulaca oleracea* removed the heavy metals from contaminated soil and had a lower capability for Translocation them from root to shoot. The highest TF quantities for Cr (III), Cr (VI), and Fe were obtained in planted amendment with 5 % biochar. For Al, the TF values reduced with increasing biochar percentage into the soil. There was no significant difference between the SP, SCP, and SCP 0.5. Besides, it was not observed any significant difference between control and SCP5. This result is also confirmed by the work of Chuaphasuk and Prapagdee (Chuaphasuk &Prapagdee 2019), who reported that the addition of biochar into cadmium contaminated soil did not significantly change the TF of *Chlorophytum laxum*. Also, similar findings were observed by Chen et al. (Chen et al. 2020), which reported that the influence of biochar on TF of cadmium using wheat.

The stabilization of heavy metal within the plant 's root decreases their mobility or bioavailability called phytostabilization (Bolan et al. 2011). It was determined in a plant when the BCF value > 1 and TF value < 1 (Sudmoon et al. 2015, Yoon et al. 2006). As shown in Fig. 7, *Portulaca oleracea* has the potential for phytostabilization of Cr (III), Cr (VI), and Zn (II). The order of TF was Fe > Al (III) > Zn (II) > Cr (VI) > Cr (III). The highest BCF (122.7) was obtained for Zn (II), and the lowest BCF (0.07) occurred for Fe. The intensity of plant roots for metal absorption was classified into five groups: (1) intensive 100 > BCF > 10; (2) strong 10 > BCF > 1; (3); intermediate 1 > BCF > 0.1; (4) weak 0.1 > BCF > 0.01; and (5) 0.01 > BAC > 0.001 (Nagaraju &Karimulla 2002). Regarding the above classification for BCF, the *Portulaca oleracea* showed the intensive tendency for Zn (II) and Cr (III) uptake, a strong tendency for Cr (VI) absorption, and a weak tendency for Al (III) and Fe.

Tolerant plants impede soil to root and root to shoot transfers, while hyperaccumulators translocate metals into aerial parts of the plant. BAC factor, which indicates the absorption and accumulation of the metals in the shoot, is another effective tool to assess the phytoremediation ability in polluted soil (Hamzah et al. 2015). It was ranked as (1) excluder BAC < 1; (2) accumulator 1 < BAC < 10; and (3) hyperaccumulator BAC > 10 (Baker 1981). For Zn, the BAC was higher than 1 illustrates that the *Portulaca oleracea* has the potential to be an accumulator. For other metals, it was excluder due to having a BAC of less than 1.

Another factor was the tolerance index (TI), which analyzed the difference between the plant roots at the control and various treatments (Fig. 7). As shown in this figure, for all heavy metals, TI values were increased significantly in treatments compared to control soil. Besides, in the higher dose of biochar, the roots were more developed and distributed. Previous research (Brennan et al. 2014, Lu et al. 2015, Puga et al. 2015) confirmed that the biochar addition to the contaminated soil enhanced the plant roots.

According to Fig. 7, for all heavy metal uptake by *portulaca oleracea*, the mean values of BCF, BAC, and TI in control were higher than those of remediated soil. In other words, the inoculation of cyanobacteria into soil and biochar amendment significantly reduced the phytostabilization potential of this plant. For example, the treatments were reduced the BCF of Portulaca oleracea by more than 77 %, 8 %, 85 %, 33 %, and 69 % for Cr (III), Cr (VI), Zn (II), Al (III), and Fe, respectively. Besides, the quantities of BAC in the remediated samples were decreased by more than 80 %, 38 %, 85 %, 44 %, and 71 % for Cr (III), Cr (VI), Zn (II), Al (III), and Fe, respectively. It demonstrated that biochar and cyanobacteria reduced the migration ability of heavy metals from soil to plant roots and shoots. It proved the effectiveness of biochar amendment and cyanobacteria inoculation for the remediation of heavy metal contaminated soil. This result was in agreement with the research of Puga et al. (Yang et al. 2016b). They found that biochar amendment effectively decreases the accumulation of Pb (II) in cabbage mustard. Also, similar findings were observed by Chen et al. (Chen et al. 2020). They found that the bio-concentration factor including BCF and BAC values of wheat, was reduced by biochar amendments.

Our findings indicated that inoculation of cyanobacteria, although promoted plant growth but it significantly decreased the bioconcentration and bioaccumulation factors (BAC and BCF). It is good to mention that inoculation of some microorganisms, including Arthrobacter sp. (Rojjanateeranaj et al. 2017), Micrococcus sp.(Chuaphasuk &Prapagdee 2019, Rojjanateeranaj et al. 2017), *Pseudomonas koreensis* (Babu et al. 2015), and Pseudomonas sp. (Rojjanateeranaj et al. 2017), and into soil enhanced the heavy metal accumulation in the shoots and roots. The bioavailability and phytoavailability of heavy metals in the contaminated soil have a crucial effect on absorption or uptake by microorganisms and plants. However, soil chemistry (i.e., pH and organic matters) and plant-microbe interaction are played an essential role in bioavailability and phytoavailability (Shah &Daverey 2020).

# 4. Conclusion

In this study, two approaches, including bioaugmentation and bioaugmentation-assisted phytoremediation, were utilized for remediation of heavy metals (Fe, Cr (III), Cr (VI), Al, and Zn) contaminated soil. In bioaugmentation experiments, cyanobacteria inoculation accompany with an increment of biochar dose ( from 0 to 5 %) resulted in enhancement of the soil chlorophyll a, nitrogen, and organic carbon contents, reduction of the pH and EC, and also a significant decrease in the bioavailability of all heavy metals. Besides, evaluation of remediation efficiency with the help of lettuce and radish plants, after three months of treatment, confirmed the improvement of soil conditions. The results of bioaugmentation-assisted phytoremediation using biochar- cyanobacteria - *Portulaca oleracea* showed a substantial improvement in the above – mentioned properties of soil and a notable reduction in the bioavailability of all heavy metals, especially at a 5 % dose of biochar. Moreover, the *Portulaca oleracea* displays low translocation (TF < 1) for all heavy metals, immobilized, and accumulates the metals in root than shoot, which show their phytostabilization potential. According to the BCF values, the *Portulaca oleracea* showed an intensive tendency for Zn and Cr (III) uptake, a strong tendency for Cr (VI) absorption, and a weak tendency for Al and Fe. Although inoculation of cyanobacteria and the addition of biochar into contaminated soil promoted plant growth, it significantly reduced the phytostabilization potential of this plant. It can be concluded that the combined use of biochar and bioaugmentation with cyanobacteria is a successful technique for immobilization of heavy metal in soil and mitigate their toxic impacts on the plant.

# Declarations

The authors would like to thank the Ferdowsi University of Mashhad for its financial support ( Grant number: 47728) for this research.

**Ethics approval and consent to participate:** “Not applicable”

**Consent for publication:** “Not applicable”

**Availability of data and materials:** The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Competing interests:** "The authors declare that they have no competing interests".

**Funding:** This study was funded by the Ferdowsi University of Mashhad (47728) of Iran.

**Authors' contributions:**

Fahimeh Zanganeh: 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.

Adel Sepehr: Funding acquisition, Supervision.

Abbas Rohani: Software, Writing – review & editing.

# References

1. Ahmad R, Tehsin Z, Malik ST, Asad SA, Shahzad M, Bilal M, Shah MM, Khan SA (2016) Phytoremediation potential of hemp (Cannabis sativa L.): identification and characterization of heavy metals responsive genes. CLEAN–Soil, Air, Water 44, 195–201
2. Al-Wabel MI, Usman ARA, El-Naggar AH, Aly AA, Ibrahim HM, Elmaghraby S, Al-Omran A (2015) Conocarpus biochar as a soil amendment for reducing heavy metal availability and uptake by maize plants. Saudi Journal of Biological Sciences 22:503–511
3. Alghanmi SI, Al Sulami AF, El-Zayat TA, Alhogbi BG, Abdel Salam M (2015) Acid leaching of heavy metals from contaminated soil collected from Jeddah, Saudi Arabia: kinetic and thermodynamics studies. International Soil Water Conservation Research 3:196–208
4. Ali NA, Bernal MP, Ater M (2002) Tolerance and bioaccumulation of copper in Phragmites australis and Zea mays. Plant Soil 239:103–111
5. Aparicio J, Solá MZS, Benimeli CS, Amoroso MJ, Polti MA (2015) Versatility of Streptomyces sp. M7 to bioremediate soils co-contaminated with Cr (VI) and lindane. Ecotoxicol Environ Saf 116:34–39
6. Arán DS, Harguinteguy CA, Fernandez-Cirelli A, Pignata ML (2017) Phytoextraction of Pb, Cr, Ni, and
Loading [MathJax]/jax/output/CommonHTML/fonts/TeX/fontdata.js nd its potential use in the treatment of

wastewater. Environ Sci Pollut Res 24:18295–18308

7. Babu AG, Shea PJ, Sudhakar D, Jung I-B, Oh B-T (2015) Potential use of Pseudomonas koreensis AGB-1 in association with Miscanthus sinensis to remediate heavy metal (loid)-contaminated mining site soil. Journal of environmental management 151:160–166
8. Baker AJ (1981) Accumulators and excluders-strategies in the response of plants to heavy metals. Journal of plant nutrition 3:643–654
9. Bandara T, Herath I, Kumarathilaka P, Hseu Z-Y, Ok YS, Vithanage, MJEg, health (2017) Efficacy of woody biomass and biochar for alleviating heavy metal bioavailability in serpentine soil. 39, 391–401
10. Bashir S, Shaaban M, Mehmood S, Zhu J, Fu Q, Hu H (2018a) Efficiency of C3 and C4 plant derived-biochar for Cd mobility, nutrient cycling and microbial biomass in contaminated soil. Bull Environ Contam Toxicol 100:834–838
11. Bashir S, Zhu J, Fu Q, Hu H (2018b) Cadmium mobility, uptake and anti-oxidative response of water spinach (Ipomoea aquatic) under rice straw biochar, zeolite and rock phosphate as amendments. Chemosphere 194:579–587
12. Beesley L, Moreno-Jiménez E, Gomez-Eyles JL (2010) Effects of biochar and greenwaste compost amendments on mobility, bioavailability and toxicity of inorganic and organic contaminants in a multi-element polluted soil. Environmental pollution 158:2282–2287
13. Biglari Quchan Atigh Z, Heidari A, Sepehr A, Bahreini M, Mahbub K (2020a) Bioremediation of Heavy Metal Contaminated Soils Originated from Iron Ore Mine by Bio-augmentation with Native Cyanobacteria. Iranian (Iranica. Journal of Energy Environment 11:89–96
14. Biglari Quchan Atigh Z, Heidari A, Sepehr A, Bahreini M, Mahbub KR (2020b) Bioremediation of Heavy Metal Contaminated Soils Originated from Iron Ore Mine by Bio-augmentation with Native Cyanobacteria. Iranian (Iranica. Journal of Energy Environment 11:89–96
15. Bolan NS, Park JH, Robinson B, Naidu R, Huh KY (2011) Chapter four - Phytostabilization: A Green Approach to Contaminant Containment. In: Sparks DL (ed) Advances in Agronomy. Academic Press, pp 145–204
16. Bouyoucos GJ (1962) Hydrometer method improved for making particle size analyses of soils 1. Agron J 54:464–465
17. Brennan A, Jiménez EM, Puschenreiter M, Alburquerque JA, Switzer C (2014) Effects of biochar amendment on root traits and contaminant availability of maize plants in a copper and arsenic impacted soil. Plant Soil 379:351–360
18. Cao X, Ma L, Liang Y, Gao B, Harris W (2011) Simultaneous immobilization of lead and atrazine in contaminated soils using dairy-manure biochar. Environ Sci Technol 45:4884–4889
19. Chamizo S, Mugnai G, Rossi F, Certini G, De Philippis R (2018a) Cyanobacteria inoculation improves soil stability and fertility on different textured soils: gaining insights for applicability in soil restoration. Frontiers in Environmental Science 6:49

20. Chamizo S, Mugnai G, Rossi F, Certini G, De Philippis R (2018b) Cyanobacteria Inoculation Improves Soil Stability and Fertility on Different Textured Soils: Gaining Insights for Applicability in Soil Restoration. Frontiers in Environmental Science 6
21. Chan KY, Xu Z (2009) Biochar: nutrient properties and their enhancement. Biochar for environmental management: science and technology 1, 67–84
22. Chen H, Zhang J, Tang L, Su M, Tian D, Zhang L, Li Z, Hu S (2019) Enhanced Pb immobilization via the combination of biochar and phosphate solubilizing bacteria. Environment international 127:395–401
23. Chen J, Dong J, Chang J, Guo T, Yang Q, Jia W, Shen S (2018a) Characterization of an Hg (II)-volatilizing Pseudomonas sp. strain, DC-B1, and its potential for soil remediation when combined with biochar amendment. Ecotoxicol Environ Saf 163:172–179
24. Chen J, Dong J, Chang J, Guo T, Yang Q, Jia W, Shen S (2018b) Characterization of an Hg(II)-volatilizing Pseudomonas sp. strain, DC-B1, and its potential for soil remediation when combined with biochar amendment. Ecotoxicol Environ Saf 163:172–179
25. Chen Z, Zhao Y, Fan L, Xing L, Yang Y (2015) Cadmium (Cd) localization in tissues of cotton (Gossypium hirsutum L.), and its phytoremediation potential for Cd-contaminated soils. Bull Environ Contam Toxicol 95:784–789
26. Chen Z, Lu Z, Zhang Y, Li B, Chen C, Shen K (2020) Effects of biochars combined with ferrous sulfate and pig manure on the bioavailability of Cd and potential phytotoxicity for wheat in an alkaline contaminated soil. Science of The Total Environment 753:141832
27. Chuaphasuk C, Prapagdee B (2019) Effects of biochar-immobilized bacteria on phytoremediation of cadmium-polluted soil. Environ Sci Pollut Res 26:23679–23688
28. Dhal B, Thatoi H, Das N, Pandey B (2013) Chemical and microbial remediation of hexavalent chromium from contaminated soil and mining/metallurgical solid waste: a review. J Hazard Mater 250:272–291
29. Dong H, Deng J, Xie Y, Zhang C, Jiang Z, Cheng Y, Hou K, Zeng GJJohm (2017) Stabilization of nanoscale zero-valent iron (nZVI) with modified biochar for Cr (VI) removal from aqueous solution. 332, 79–86
30. Garbisu C, Alkorta I (2001) Phytoextraction: a cost-effective plant-based technology for the removal of metals from the environment. Biores Technol 77:229–236
31. Ghani WAWAK, Mohd A, da Silva G, Bachmann RT, Taufiq-Yap YH, Rashid U, Al-Muhtaseb AaH (2013) Biochar production from waste rubber-wood-sawdust and its potential use in C sequestration: Chemical and physical characterization. Ind Crops Prod 44:18–24
32. Girolkar S, Thawale P, Juwarkar A (2021) Chap. 12 - Bacteria-assisted phytoremediation of heavy metals and organic pollutants: challenges and future prospects. In: Kumar V, Saxena G, Shah MP (eds) Bioremediation for Environmental Sustainability. Elsevier, pp 247–267
33. Gong X, Huang D, Liu Y, Zeng G, Chen S, Wang R, Xu P, Cheng M, Zhang C, Xue W (2019) Biochar

inated sediments: Metal behavior, plant toxicity, and microbial activity. Sci Total Environ 666:1126–1133

34. Gong X, Huang D, Liu Y, Zou D, Hu X, Zhou L, Wu Z, Yang Y, Xiao Z (2021) Nanoscale zerovalent iron, carbon nanotubes and biochar facilitated the phytoremediation of cadmium contaminated sediments by changing cadmium fractions, sediments properties and bacterial community structure. Ecotoxicol Environ Saf 208:111510
35. Hamzah A, Sarmani S, Yatim N (2015) Phytoremediation of Pb and Hg by using Scirpus mucronatus with addition of bacterial inoculums. J Radioanal Nucl Chem 304:151–155
36. Harindintwali JD, Zhou J, Yang W, Gu Q, Yu X (2020) Biochar-bacteria-plant partnerships: Eco-solutions for tackling heavy metal pollution. Ecotoxicol Environ Saf 204:111020
37. He E, Yang Y, Xu Z, Qiu H, Yang F, Peijnenburg WJ, Zhang W, Qiu R, Wang S (2019a) Two years of aging influences the distribution and lability of metal (loid) s in a contaminated soil amended with different biochars. Science of The Total Environment 673:245–253
38. He L, Zhong H, Liu G, Dai Z, Brookes PC, Xu J (2019b) Remediation of heavy metal contaminated soils by biochar: Mechanisms, potential risks and applications in China. Environmental pollution 252:846–855
39. Issa OM, Défarge C, Le Bissonnais Y, Marin B, Duval O, Bruand A, d'Acqui LP, Nordenberg S, Annerman M (2007) Effects of the inoculation of cyanobacteria on the microstructure and the structural stability of a tropical soil. Plant soil 290:209–219
40. Jiang J, Xu R-k, Jiang T-y, Li Z (2012) Immobilization of Cu (II), Pb (II) and Cd (II) by the addition of rice straw derived biochar to a simulated polluted Ultisol. J Hazard Mater 229:145–150
41. Joshi H, Shourie A, Singh A (2020) Cyanobacteria as a source of biofertilizers for sustainable agriculture, Advances in Cyanobacterial Biology. Elsevier, pp 385–396
42. Khalid M, Ur-Rahman S, Hassani D, Hayat K, Zhou P, Hui N (2021) Advances in fungal-assisted phytoremediation of heavy metals: A review. Pedosphere 31:475–495
43. Kheirfam H, Sadeghi SH, Homaee M, Darki BZ (2017) Quality improvement of an erosion-prone soil through microbial enrichment. Soil Tillage Res 165:230–238
44. Kheirfam H, Roohi M (2020) Accelerating the formation of biological soil crusts in the newly dried-up lakebeds using the inoculation-based technique. Science of The Total Environment 706:136036
45. Kiran BR, Prasad M (2019) Biochar and rice husk ash assisted phytoremediation potentials of Ricinus communis L. for lead-spiked soils. Ecotoxicol Environ Saf 183:109574
46. Kirk PL (1950) Kjeldahl Method for Total Nitrogen. Anal Chem 22:354–358
47. Lebeau T, Braud A, Jézéquel K (2008) Performance of bioaugmentation-assisted phytoextraction applied to metal contaminated soils: a review. Environ Pollut 153:497–522
48. Li H, Zhao Q, Huang H (2019) Current states and challenges of salt-affected soil remediation by cyanobacteria. Science of The Total Environment 669:258–272
49. Liang J, Yang Z, Tang L, Zeng G, Yu M, Li X, Wu H, Qian Y, Li X, Luo Y (2017) Changes in heavy metal mobility and availability from contaminated wetland soil remediated with combined biochar-

compost. Chemosphere 181:281–288

50. Liu L, Li W, Song W, Guo M (2018a) Remediation techniques for heavy metal-contaminated soils: principles and applicability. Sci Total Environ 633:206–219
51. Liu S-j, Liu Y-g, Tan X, Zeng G-m, Zhou Y-h, Liu S-b, Yin Z-h, Jiang L-h, Li M-f, Wen J (2018b) The effect of several activated biochars on Cd immobilization and microbial community composition during in-situ remediation of heavy metal contaminated sediment. Chemosphere 208
52. Lu H, Li Z, Fu S, Méndez A, Gascó G, Paz-Ferreiro J (2015) Combining phytoextraction and biochar addition improves soil biochemical properties in a soil contaminated with Cd. Chemosphere 119:209–216
53. Lu K, Yang X, Gielen G, Bolan N, Ok YS, Niazi NK, Xu S, Yuan G, Chen X, Zhang X (2017) Effect of bamboo and rice straw biochars on the mobility and redistribution of heavy metals (Cd, Cu, Pb and Zn) in contaminated soil. Journal of environmental management 186:285–292
54. Luo R, Li J, Zhao Y, Fan X, Zhao P, Chai L (2017) A critical review on the research topic system of soil heavy metal pollution bioremediation based on dynamic co-words network measures. Geoderma 305:281–292
55. Lyu H, Zhao H, Tang J, Gong Y, Huang Y, Wu Q, Gao B (2018) Immobilization of hexavalent chromium in contaminated soils using biochar supported nanoscale iron sulfide composite. Chemosphere 194:360–369
56. Mahar A, Wang P, Ali A, Awasthi MK, Lahori AH, Wang Q, Li R, Zhang Z (2016) Challenges and opportunities in the phytoremediation of heavy metals contaminated soils: a review. Ecotoxicol Environ Saf 126:111–121
57. Mahbub KR, Krishnan K, Andrews S, Venter H, Naidu R, Megharaj M (2017) Bio-augmentation and nutrient amendment decrease concentration of mercury in contaminated soil. Science of The Total Environment 576:303–309
58. Mani D, Kumar C, Patel NK (2016) Integrated micro-biochemical approach for phytoremediation of cadmium and lead contaminated soils using Gladiolus grandiflorus L cut flower. Ecotoxicol Environ Saf 124:435–446
59. Maqubela MP, Mnkeni PN, Muchaonyerwa P, D'Acqui LP, Pardo MT (2010) Effects of cyanobacteria strains selected for their bioconditioning and biofertilization potential on maize dry matter and soil nitrogen status in a South African soil. Soil Science Plant Nutrition 56:552–559
60. Martins GC, Penido ES, Alvarenga IFS, Teodoro JC, Bianchi ML, Guilherme LRG (2018) Amending potential of organic and industrial by-products applied to heavy metal-rich mining soils. Ecotoxicol Environ Saf 162:581–590
61. Meeinkuirt W, Kruatrachue M, Pichtel J, Phusantisampan T, Saengwilai P (2016) Influence of organic amendments on phytostabilization of Cd-contaminated soil by Eucalyptus camaldulensis. ScienceAsia 42:83–91
62. Megharaj M, Naidu R (2017) Soil and brownfield bioremediation. Microbial biotechnology 10:1244–

63. Meier S, Curaqueo G, Khan N, Bolan N, Cea M, Eugenia GM, Cornejo P, Ok YS, Borie FJJoS, Sediments (2017) Chicken-manure-derived biochar reduced bioavailability of copper in a contaminated soil. 17, 741–750
64. Méndez A, Gómez A, Paz-Ferreiro J, Gascó G (2012) Effects of sewage sludge biochar on plant metal availability after application to a Mediterranean soil. Chemosphere 89:1354–1359
65. Mugnai G, Rossi F, Felde VJMNL, Colesie C, Büdel B, Peth S, Kaplan A, De Philippis R (2018) Development of the polysaccharidic matrix in biocrusts induced by a cyanobacterium inoculated in sand microcosms. Biol Fertil Soils 54:27–40
66. Muñoz-Rojas M, Román J, Roncero-Ramos B, Erickson T, Merritt D, Aguila-Carricondo P, Cantón Y (2018) Cyanobacteria inoculation enhances carbon sequestration in soil substrates used in dryland restoration. Sci Total Environ 636:1149–1154
67. Nagaraju A, Karimulla S (2002) Accumulation of elements in plants and soils in and around Nellore mica belt, Andhra Pradesh, India – a biogeochemical study. Environ Geol 41:852–860
68. Nazir A, Malik RN, Ajaib M, Khan N, Siddiqui MF (2011) Hyperaccumulators of heavy metals of industrial areas of Islamabad and Rawalpindi. Pak J Bot 43:1925–1933
69. Nie C, Yang X, Niazi NK, Xu X, Wen Y, Rinklebe J, Ok YS, Xu S, Wang H (2018) Impact of sugarcane bagasse-derived biochar on heavy metal availability and microbial activity: a field study. Chemosphere 200:274–282
70. Nisha R, Kiran B, Kaushik A, Kaushik C (2018) Bioremediation of salt affected soils using cyanobacteria in terms of physical structure, nutrient status and microbial activity. International journal of environmental science technology 15:571–580
71. Pandey VC, Bajpai O, Singh N (2016) Energy crops in sustainable phytoremediation. Renew Sustain Energy Rev 54:58–73
72. Park C-H, Li XR, Zhao Y, Jia RL, Hur J-S (2017) Rapid development of cyanobacterial crust in the field for combating desertification. PloS one 12
73. Park JH, Choppala GK, Bolan NS, Chung JW, Chuasavathi T (2011) Biochar reduces the bioavailability and phytotoxicity of heavy metals. Plant soil 348:439
74. Peña-Icart M, Villanueva Tagle ME, Alonso-Hernández C, Rodríguez Hernández J, Behar M, Pomares Alfonso MS (2011) Comparative study of digestion methods EPA 3050B (HNO3–H2O2–HCl) and ISO 11466.3 (aqua regia) for Cu, Ni and Pb contamination assessment in marine sediments. Marine Environmental Research 72:60–66
75. Peng X, Bruns MA (2019) Development of a nitrogen-fixing cyanobacterial consortium for surface stabilization of agricultural soils. J Appl Phycol 31:1047–1056
76. Prasad M, Greger M, Landberg T (2001) Acacia nilotica L. bark removes toxic elements from solution: corroboration from toxicity bioassay using Salix viminalis L. in hydroponic system. Int J Phytorem 3:289–300
77. Puga A, Abreu C, Melo L, Beesley L (2015) Biochar application to a contaminated soil reduces the

um. Journal of environmental management

159:86–93

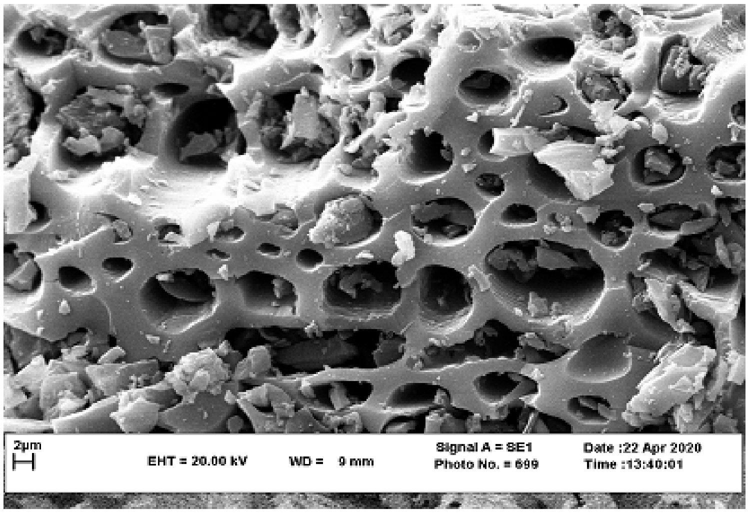
78. Qi X, Gou J, Chen X, Xiao S, Ali I, Shang R, Wang D, Wu Y, Han M, Luo X (2020) Application of mixed bacteria-loaded biochar to enhance uranium and cadmium immobilization in a co-contaminated soil. J Hazard Mater 401:123823
79. Qian L, Li Q, Sun J, Feng Y (2018) Effect of biochar on plant growth and aluminium form of soil under aluminium stress, IOP conference series: earth and environmental science, pp. 042123
80. Rinklebe J, Shaheen SM (2015) Miscellaneous additives can enhance plant uptake and affect geochemical fractions of copper in a heavily polluted riparian grassland soil. Ecotoxicol Environ Saf 119:58–65
81. Rojjanateeranaj P, Sangthong C, Prapagdee B (2017) Enhanced cadmium phytoremediation of Glycine max L. through bioaugmentation of cadmium-resistant bacteria assisted by biostimulation. Chemosphere 185:764–771
82. Rossi F, Li H, Liu Y, De Philippis R (2017) Cyanobacterial inoculation (cyanobacterisation): perspectives for the development of a standardized multifunctional technology for soil fertilization and desertification reversal. Earth Sci Rev 171:28–43
83. Sanchez-Hernandez JC, Ro KS, Díaz FJ (2019) Biochar and earthworms working in tandem: Research opportunities for soil bioremediation. Science of the total environment 688:574–583
84. Shah V, Daverey A (2020) Phytoremediation: A multidisciplinary approach to clean up heavy metal contaminated soil. Environmental Technology Innovation 18:100774
85. Sharma P, Tripathi S, Chaturvedi P, Chaurasia D, Chandra R (2021) Newly isolated Bacillus sp. PS-6 assisted phytoremediation of heavy metals using Phragmites communis: Potential application in wastewater treatment. Biores Technol 320:124353
86. Siedt M, Schäffer A, Smith KEC, Nabel M, Roß-Nickoll M, van Dongen JT (2021) Comparing straw, compost, and biochar regarding their suitability as agricultural soil amendments to affect soil structure, nutrient leaching, microbial communities, and the fate of pesticides. Science of The Total Environment 751:141607
87. Song B, Zeng G, Gong J, Liang J, Xu P, Liu Z, Zhang Y, Zhang C, Cheng M, Liu Y (2017) Evaluation methods for assessing effectiveness of in situ remediation of soil and sediment contaminated with organic pollutants and heavy metals. Environment international 105:43–55
88. Song Y, Wang F, Bian Y, Kengara FO, Jia M, Xie Z, Jiang XJJohm (2012) Bioavailability assessment of hexachlorobenzene in soil as affected by wheat straw biochar. 217, 391–397
89. Su H, Fang Z, Tsang PE, Zheng L, Cheng W, Fang J, Zhao D (2016) Remediation of hexavalent chromium contaminated soil by biochar-supported zero-valent iron nanoparticles. J Hazard Mater 318:533–540
90. Sudmoon R, Neeratanaphan L, Thamsenanupap P, Tanee T (2015) Hyperaccumulation of Cadmium and DNA Changes in Popular Vegetable, Brassica chinensis L. International Journal of Environmental Research 9

91. Tao X, Li A, Yang H (2017) Immobilization of metals in contaminated soils using natural polymer-based stabilizers. Environ Pollut 222:348–355
92. Tokalioğlu Ş, Kartal Ş, Gültekın A (2006) Investigation of heavy-metal uptake by vegetables growing in contaminated soils using the modified BCR sequential extraction method. Intern J Environ Anal Chem 86:417–430
93. Tu C, Wei J, Guan F, Liu Y, Sun Y, Luo Y (2020) Biochar and bacteria inoculated biochar enhanced Cd and Cu immobilization and enzymatic activity in a polluted soil. Environ Int 137:105576
94. Wang J, Shi L, Zhai L, Zhang H, Wang S, Zou J, Shen Z, Lian C, Chen Y (2020) Analysis of the long-term effectiveness of biochar immobilization remediation on heavy metal contaminated soil and the potential environmental factors weakening the remediation effect: A review. Ecotoxicol Environ Saf 207:111261
95. Wang J, Shi L, Zhai L, Zhang H, Wang S, Zou J, Shen Z, Lian C, Chen Y (2021) Analysis of the long-term effectiveness of biochar immobilization remediation on heavy metal contaminated soil and the potential environmental factors weakening the remediation effect: A review. Ecotoxicol Environ Saf 207:111261
96. Wang T, Sun H, Mao H, Zhang Y, Wang C, Zhang Z, Wang B, Sun L (2014) The immobilization of heavy metals in soil by bioaugmentation of a UV-mutant Bacillus subtilis 38 assisted by NovoGro biostimulation and changes of soil microbial community. J Hazard Mater 278:483–490
97. Wu B, Wang Z, Zhao Y, Gu Y, Wang Y, Yu J, Xu H (2019) The performance of biochar-microbe multiple biochemical material on bioremediation and soil micro-ecology in the cadmium aged soil. Science of the total environment 686:719–728
98. Wuana RA, Okieimen FE, Imborvungu JAJIJoES T (2010) Removal of heavy metals from a contaminated soil using organic chelating acids. 7, 485–496
99. Xiao R, Ali A, Wang P, Li R, Tian X, Zhang Z (2019) Comparison of the feasibility of different washing solutions for combined soil washing and phytoremediation for the detoxification of cadmium (Cd) and zinc (Zn) in contaminated soil. Chemosphere 230:510–518
100. Xiao R, Liu X, Ali A, Chen A, Zhang M, Li R, Chang H, Zhang Z (2020) Bioremediation of Cd-spiked soil using earthworms (Eisenia fetida): Enhancement with biochar and Bacillus megatherium application. Chemosphere 264:128517
101. Yang X, Liu J, McGrouther K, Huang H, Lu K, Guo X, He L, Lin X, Che L, Ye ZJES, Research P (2016a) Effect of biochar on the extractability of heavy metals (Cd, Cu, Pb, and Zn) and enzyme activity in soil. 23, 974–984
102. Yang Z, Fang Z, Zheng L, Cheng W, Tsang PE, Fang J, Zhao D (2016b) Remediation of lead contaminated soil by biochar-supported nano-hydroxyapatite. Ecotoxicol Environ Saf 132:224–230
103. Yoon J, Cao X, Zhou Q, Ma LQ (2006) Accumulation of Pb, Cu, and Zn in native plants growing on a contaminated Florida site. Science of the total environment 368:456–464
104. Yuan J-H, Xu R-K, Zhang H (2011) The forms of alkalis in the biochar produced from crop residues at

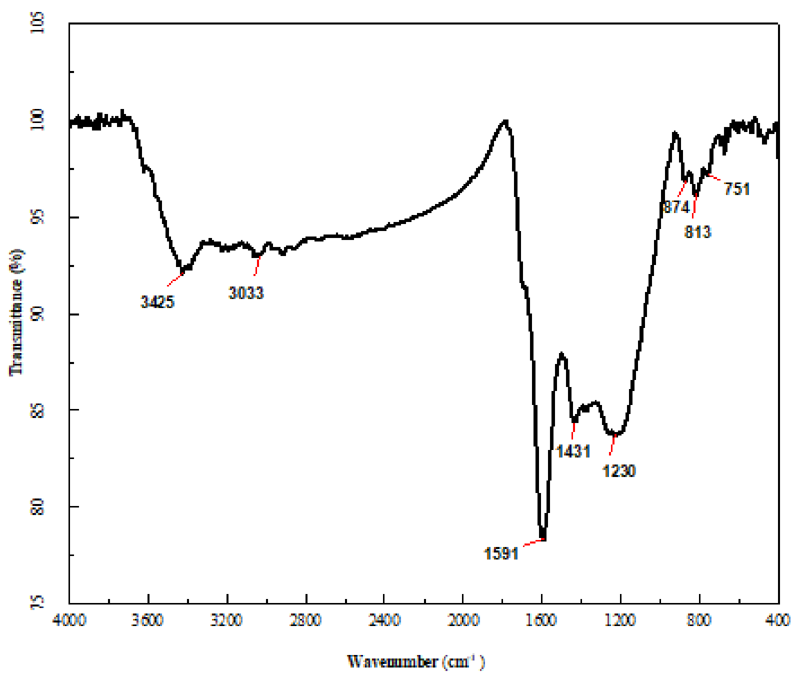
3497

105. Zhang X, Wang H, He L, Lu K, Sarmah A, Li J, Bolan NS, Pei J, Huang H (2013) Using biochar for remediation of soils contaminated with heavy metals and organic pollutants. Environ Sci Pollut Res 20:8472–8483

# Figures



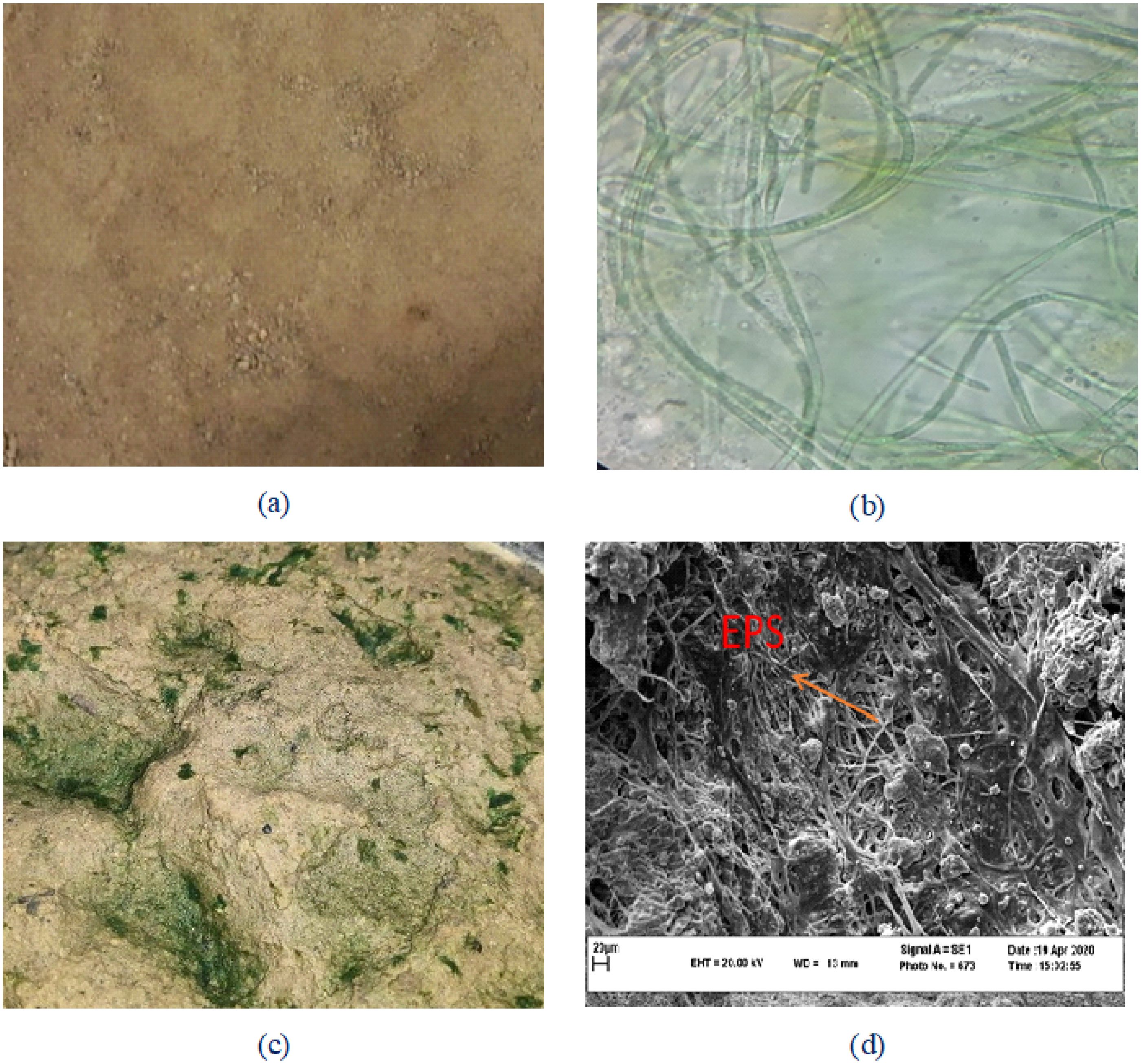

(a)




(b)

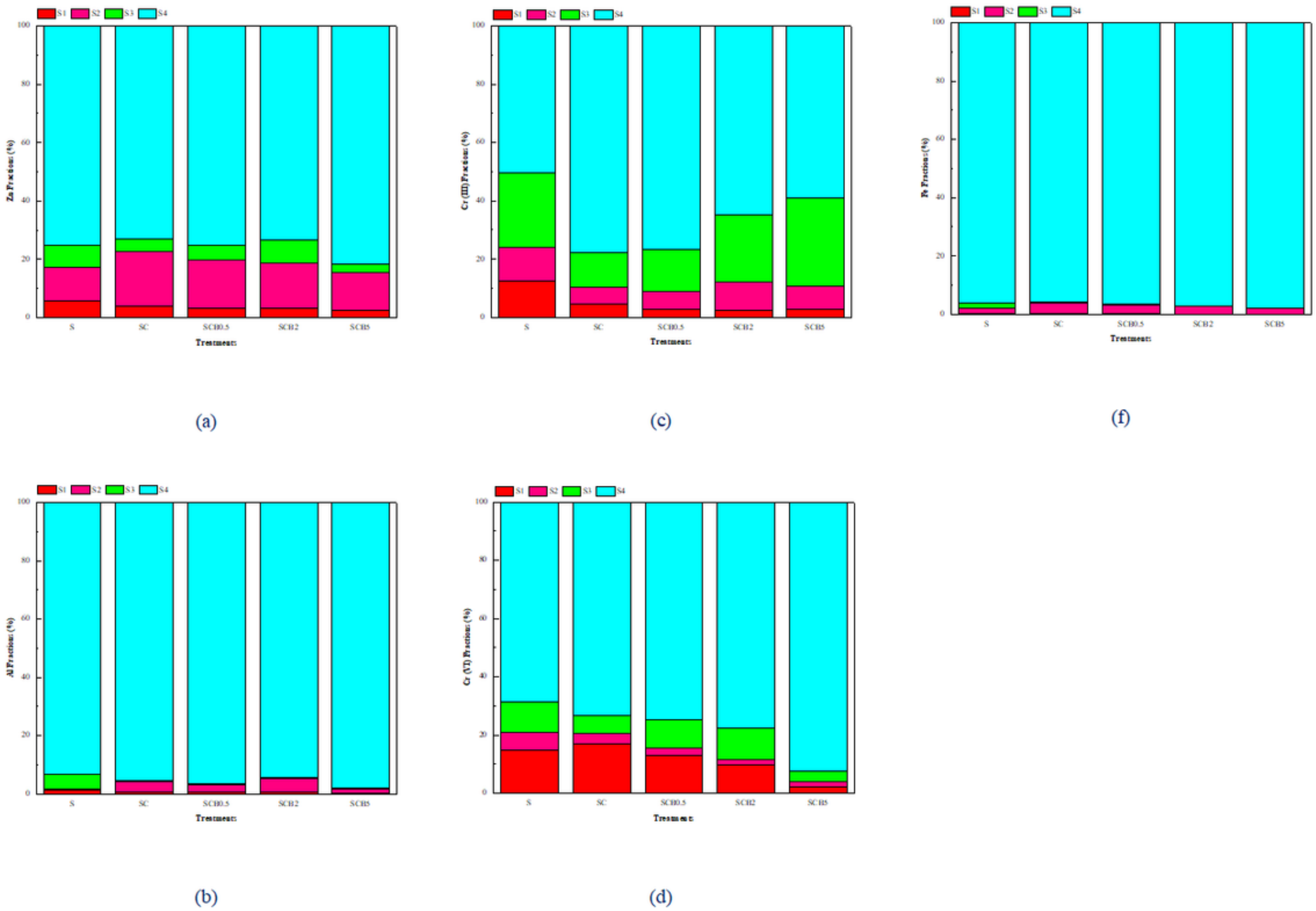
Loading [MathJax]/jax/output/CommonHTML/fonts/TeX/fontdata.js

The SEM image (a) and FTIR spectra (b) of biochar.




## Figure 2

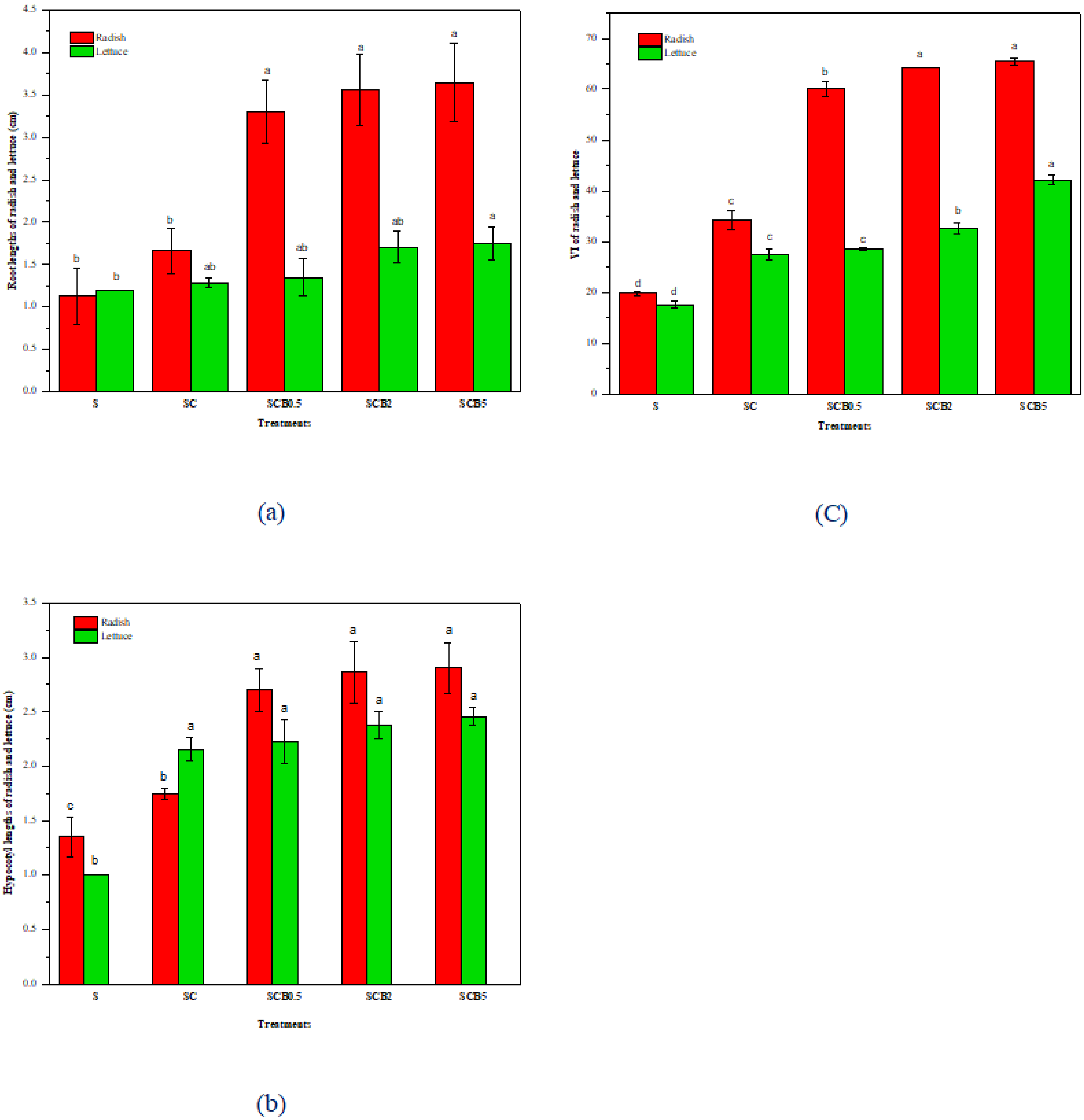
Images of control soil (a), Oscillatoria sp (b), inoculated soil (c), and surface soil after inoculation with Oscillatoria sp.




## Figure 3

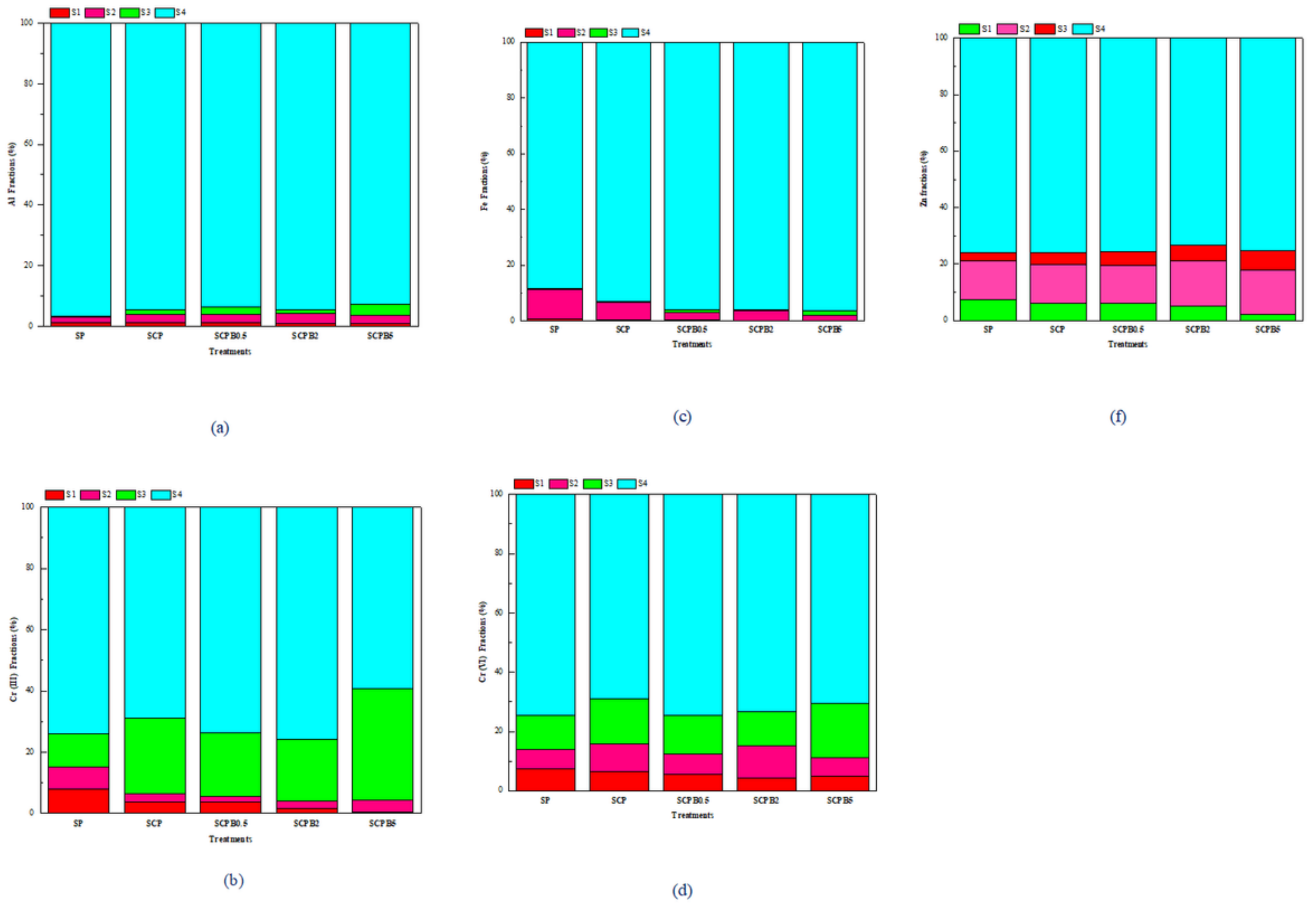
Heavy metals fractions changes in the soil bioaugmentation treatments: (S1, exchangeable; S2, Fe-Mn oxide bound; S3, organic matter bound; S4, residual).

Loading [MathJax]/jax/output/CommonHTML/fonts/TeX/fontdata.js



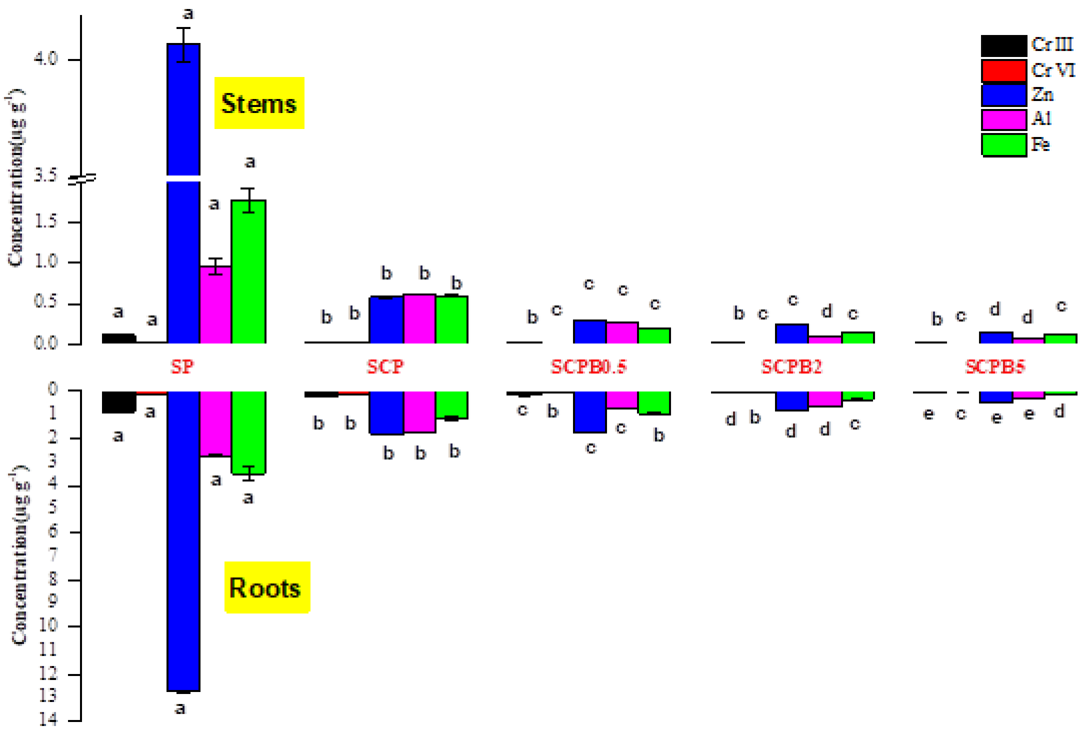

## Figure 4

Root and shoot lengths and VI of lettuces and radishes in the heavy metals contaminated soils under different treatments




## Figure 5

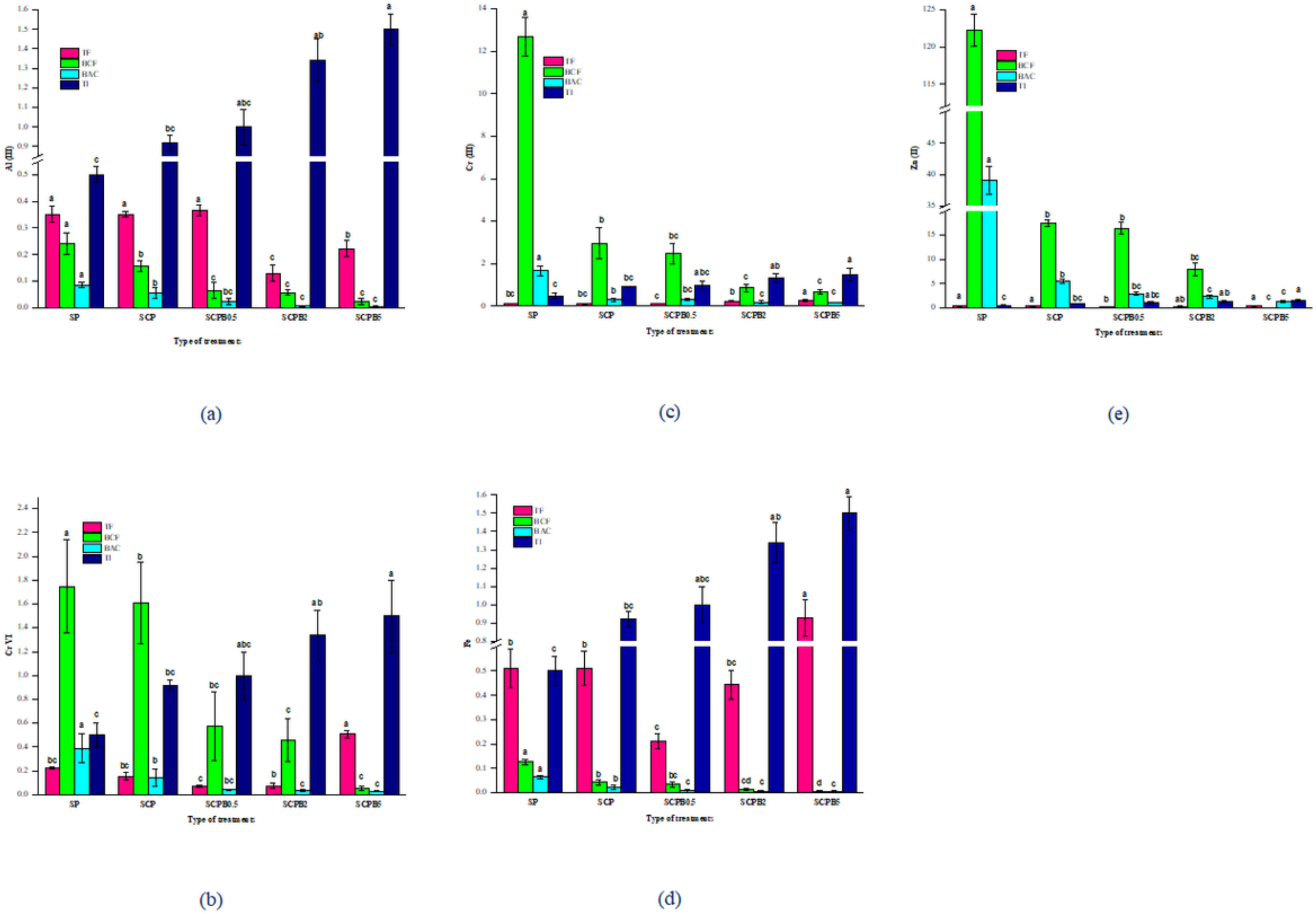
Heavy metals fractions changes in the soil bioaugmentation-assisted phytoremediation treatments: (S1, exchangeable; S2, Fe-Mn oxide bound; S3, organic matter bound; S4, residual).




## Figure 6

the concentrations of Cr (VI), Cr (III), Zn, Al, and Fe in shoots and roots of portulaca oleracea.

Loading [MathJax]/jax/output/CommonHTML/fonts/TeX/fontdata.js




### Figure 7

the estimated BCF, BAC, and TF of Portulaca oleracea grown on heavy metal contaminated soil under various treatments.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- GraphicalAbstract.pdf