STUDY ON EDTA-ASSISTED PHYTOREMEDIATION OF HEAVY METAL CONTAMINATED SOIL BY *PORTULACA OLERACEA*

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ABSTRACT

The removal of heavy metal from the soil is necessary in order to protect the environment. Phytoremediation is a process that uses plants to remove the pollutant and helps in the rehabilitation of damaged sites. In different circumstances, along with phytoremediation, chelating chemicals such as ethylenediaminetetraacetic acid (EDTA) are used to enhance the extraction of heavy metals from soil. Experiments were conducted to evaluate the potential role of *Portulaca oleracea* in the removal of heavy metals from contaminated soil, and to evaluate the effectiveness of EDTA on the removal of heavy metals by *Portulaca oleracea*. The results revealed that *Portulaca oleracea* accumulated 126.11 mg/kg of cadmium and 146.22 mg/kg of copper. Translocation factor (TF) for lead, cadmium, chromium, and copper was < 1, and bioconcentration factor (BCF) for cadmium, lead, chromium, and cooper was 4.254, 6.223, 4.385 and 10.97, respectively. After treatment with 0.1 mg/kg of EDTA, the accumulation of cadmium was 187.8 mg/kg, and the accumulation of copper was 196.2 mg/kg. TF for lead, cadmium, chromium, and cooper was > 1 and BCF for lead was 16.19, cadmium 10.75, chromium 8.141 and cooper 12.37. The accumulation of cadmium and lead after treatment with 0.3 mg/kg of EDTA was 222.49 mg/kg and 181.3 mg/kg, respectively. TF for lead, cadmium, chromium, and cooper was > 1 and BCF for lead was 16.34, cadmium 11.53, chromium 8.647 and cooper 7.005. Therefore, the results showed that 0.1 mg/kg of ETDA was the optimal dose for enhanced accumulation of heavy metals.

Keywords: *Portulaca oleracea, soil contamination, EDTA application, heavy metals, bioconcentration factor, translocation factor*

INTRODUCTION

The progressive increase in heavy metals (HMs) concentrations in soil ecosystems is an alarming global problem [1]. Due to the increasing growth of human activities, such as industrialisation, transportation, and urbanisation, heavy metal contamination is of vital importance for environmental management. Various activities, such as burning coal, fuel, mining, fertilisers and pesticides are just some of the sources of HMs that contribute to the environment [2, 3]. The following heavy metals are usually considered as hazardous: chromium (Cr), lead (Pb), aluminium (Al), zinc (Zn), cadmium (Cd), copper (Cu) and manganese (Mn) [4]. Heavy metals must be removed from the contaminated soil using the appropriate procedures due to the increasing contamination of the soil with harmful metal components; this requires an efficient and cost-effective solution. Biological, chemical, and physical methods have been applied in certain modern heavy metal cleaning strategies [5]. One biological technique used for soil remediation is phytoremediation. Since it is safer and cheaper than physical and chemical treatment, phytoremediation is a preferred option [5]. More than four hundred plants have been identified as hyperaccumulators of metals, i.e. plants with the ability to store large amounts of metal in the shoot. Plants include weeds, grasses, trees, and vegetable crops. According to [6] plants that are considered hyperaccumulators can accumulate more than 1000 mg/kg of Cu, Cr, Co, Ni, or Pb. Hyperaccumulators of Cu (24 species), Co (26), Pb (5), Mn (8), Ni (145), and Zn (4) have been identified [6]. Phytoremediation is a green technique that stabilises, transports, or degrades contaminants in water, soil, and the environment [7]. This technique is considered very effective, economically viable, and environmentally friendly [8, 9]. Five techniques for phytoremediation are used: phytodegradation, phytofiltration, phyto-extraction, phytostabilization, and phytovolatilization, depending on the soil characteristics, the contaminant, and the plant species used. Plant species that develop rapidly, produce a lot of biomass, can uptake and accumulate large amounts of heavy metals in their shoots, and do not show any toxic symptoms when growing in polluted soils, are categorised as HM-tolerant and/or hyperaccumulators [10]. Therefore, this environmentally friendly approach can be very useful for cleaning soils contaminated with heavy metals. Purslane, also known as *Portulaca oleracea* Linn., is a fleshy annual herb native to India and the Middle East and is established as an invasive plant in other countries [11]. *Portulaca oleracea L.*, one of these plant species, could grow rapidly and regenerate its stem and leaves, implying that it has the ability to clean soil and water [12]. Its capacity to regenerate and accumulate metals from soil and water habitats has been discussed in several studies [13, 14]. The most efficient chelating agent used for phytoremediation is ethylenediaminetetra-acetic acid (EDTA), which can chelate various metals, as well as increase the uptake and bioavailability of the metals from soil [15, 16]. According to [17], when EDTA is used, about 80 % of the total amount of metals in the soil is solubilized and available for phytoextraction. Despite not being considered hyperaccumulators, chelating chemicals are suitable for phytoremediation because they allow plants to absorb more heavy metals than usual [18]. Therefore, it is of crucial importance to improve the efficiency of phytoremediation of HMs using method of phytoextraction and phytostabilization. Hyperaccumulating plants, known as HM remedies, can store higher concentration of metals in their root and shoot tissues. Phytoextraction or phytoaccumulation is a process by which toxic heavy metals are absorbed in plant roots, transferred to aboveground biomass, and then deposited at cell walls, cell membranes, vacuoles, and other inactive parts in plant tissues [19]. Inactivation or immobilisation of toxicants or contaminants inside the roots or in the rhizosphere can be used to achieve remediation. The stabilising function of plant roots reduces the mobility and bioavailability of contaminants, reducing their harmful effects [20]. The goal of this research is to check the accumulation of the HMs in different parts of the plants, such as leaves, stem and roots of the *Portulaca oleracea*. In addition, the goal is to highlight the possible advantages of chemical additions in phytoremediation studies and to assess the potential use of *Portulaca oleracea* in EDTA-enhanced phytoremediation of metals (Pb, Cr, As, Cd and Cu) from the soil that was artificially contaminated. The bioconcentration factor and the translocation factor were both determined to evaluate the phytoremediation potential of the plant.

METHODS AND METHODOLOGY

In this experiment, soil from an agriculture field was used. The soil was air-dried for 6 days and sieved through a 6 mm mesh, then sealed in a polythene bag. To determine the metal concentration in the soil, 1 mg of soil was digested with 5 ml of HNO_3 and 3 ml of H_2O_2 and the solution was then diluted with mili-Q water to a final volume of 25 ml. Different heavy metals in the sample solutions were analysed on an inductively coupled plasma optical emission spectrometer (ICP–OES), Perkin Elmer Corporation, at SICART (Sophisticated Instrumentation Centre for Applied Research and Testing), Anand, Gujarat.

Experiments were conducted in a greenhouse at ambient temperature to analyse the accumulation of heavy metals by *Portulaca oleracea* with or without EDTA treatment. *Portulaca oleracea* was grown for 60 days in agricultural soil. The plants were treated with heavy metal solutions containing five heavy metals: As, Cr, Cd, Cu and Pb. To make the solutions, the following salts of Merck (AR grade) were used: pure sodium arsenate, potassium dichromate, cadmium sulphate, copper sulphate and lead sulphate. A mixture of heavy metal solutions was added to the soil in concentrations of 40, 80 and 100 mg/kg of Cr, As, Cd, Pb and Cu. The pots with the plants were divided into four groups. The control pots (only distilled water was added to the pot) were planted in garden soil, while a mixture of heavy metal solutions was added to the other three pots for 30 days except Sundays. Plants were periodically monitored for changes in growth parameters and toxic symptoms and were kept until they reached maturity. In another set of experiment, plants were treated with EDTA for 14 days along with the heavy metal treatment after maturity. After 90 days of growth, the plants were harvested (30 days after the addition of heavy metal solutions). After harvest, plants from each pot were excised, cleaned with 0.01 N HCL, and samples were then cleaned with deionized water after being rinsed with tap water. The separated parts of the plants (leaves, stem, and roots) were dried for 48 hours at 70° in an oven. Plants parts were digested with 5 ml of HNO_3 and 3 ml of H_2O_2 and then diluted to a volume of 25 ml using mili-Q water to analyse the content of metals (As, Cr, Cd, Cu, and Pb). Analysis was performed by inductively coupled plasma optical emission spectrometer (ICP–OES), Perkin Elmer Corporation, at Sophisticated Instrumentation Centre for Applied Research and Testing (SICART), Anand, Gujarat.

Data interpretation

In plants, metal concentrations vary depending on the plant species. Therefore, the efficiency of the species was evaluated using indices such as TF and BCF, which primarily classify plant species as extractors or excluders, and also indicate if the plant species is capable of phytoextraction or phytostabilization [21]. Plants showing high TF or BCF values > 1 are suitable for phytoextraction. If the plant shows a TF value < 1, then plant can be used for phytostabilization.

(i) Translocation factor (TF):
TF = concentration of heavy metal in the aboveground biomass / concentration of heavy metal in the root area

(ii) Bioconcentration factor (BCF):
BCF = concentration of heavy metal in the aboveground biomass / concentration of heavy metal in the soil

RESULTS AND DISCUSSION

Heavy metals accumulate in plant tissues and biomass in high concentration. Therefore, phytoremediation becomes interesting for remediation of polluted soil [22]. On agricultural soils contaminated with heavy metals due to industrial effluents, *Portulaca oleracea* can act as a phytoextracting and phytostabilizing species, especially when different related weed plants are also taken into account in the phytoremediation method [23].

In the leaves of *Portulaca oleracea*, the highest accumulation was observed for Cu (36.70 mg/kg in treatment with 80 mg/kg), followed by Cd (30.03 mg/kg in treatment with 100 mg/kg), Cr (24.93 mg/kg in treatment with 100 mg/kg), and Pb (24.70 mg/kg in treatment with 80 mg/kg) (Figure 1a). Compared to leaves and roots, the lowest accumulation was found in the stem (Figure 1b). Of all the analysed metals, Cu accumulated the most (22.00 mg/kg in treatment with 100 mg/kg). It was observed that the highest accumulation was in the roots (Cu = 91.35 mg/kg, Pb = 88.04 mg/kg, in treatment with 100 mg/kg) (Figure 1c). In reference [13], investigation of copper accumulation by plants showed that plants could regenerate up to 1,600 μg/g dw of copper in soil and accumulated more than 1,000 μg/g dw of Cu. Therefore, plants only accumulate high levels of Cu near Cd, even though Cu has a detrimental effect on the ability to regenerate.

As shown in Figure 2, TF of *Portulaca oleracea* for the 40 mg/kg treatment was less than 1 for all heavy metals (Cu, Pb, Cd and Cr). Low TF indicates that plants were unwilling to transfer the heavy metals. Wu et al., 2021 [24] also reported that the translocation factor allows determining the amount of heavy metal transport and redistribution between roots and shoots. Plants growing in soil with a high content of heavy metals, protecting the roots from toxicity, can transport heavy metals from the rhizosphere to the aerial parts of plants in significant quantities. According to Figure 2, BCF for the 40 mg/kg treatment was: Cu (10.97) > Cd (6.223) > Cr (4.385) > Pb (4.254). For the 80 mg/kg treatment, BCF was: Cd (4.901) > Cu (3.139) >Pb (3.002) > Cr (2.750). For the 100 mg/kg treatment, BCF had the following values: Cu (3.824) > Cd (3.721) > Pb (2.800) > Cr (2.024). According to [21], a plant is hyperaccumulator if its BCF value is greater than 1, while an excluder is indicated with a value below one.

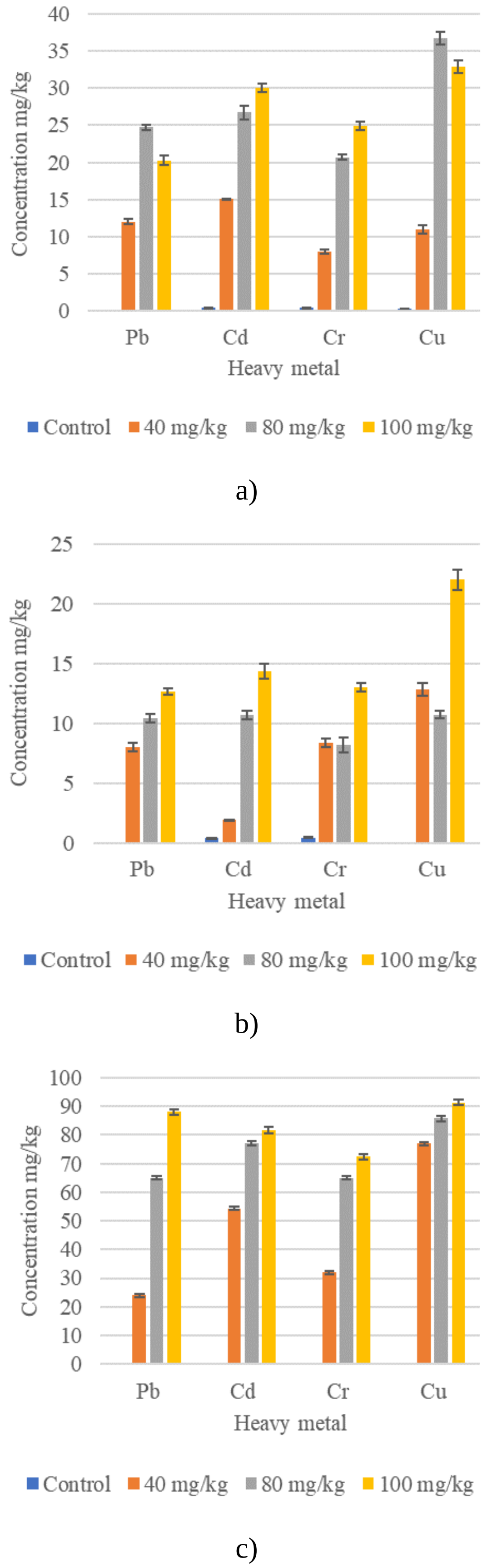


Figure 1. Content of heavy metals in leaves (a), stem (b) and roots (c) of *Portulaca oleracea* without EDTA treatment (n = 3)

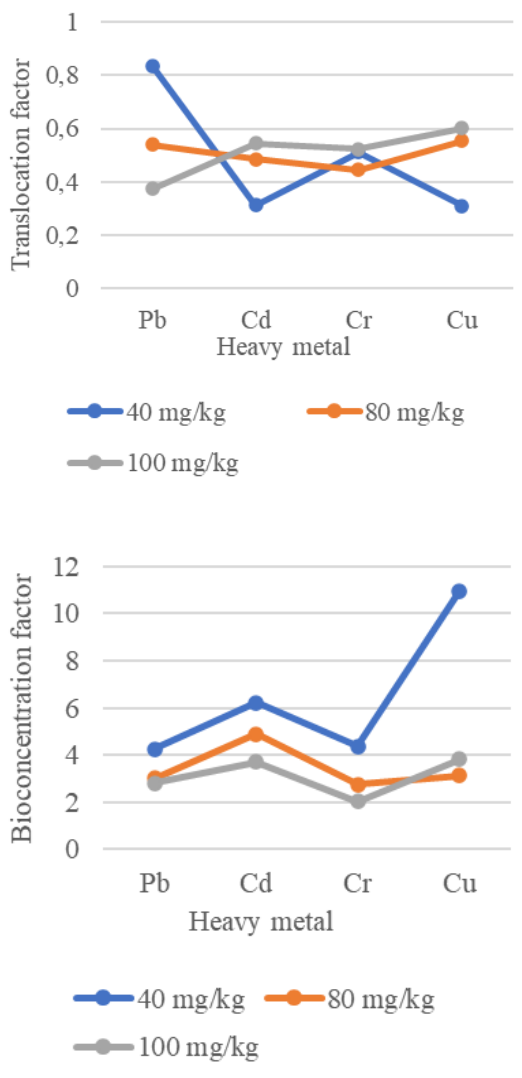


Figure 2. Translocation and bioconcentration factor without EDTA

As shown in Figures 3a, b and c, treatment with 0.1 mg/kg of EDTA increased the metal accumulation capacity of the *Portulaca oleracea*. Cu had the highest accumulation (113.8 mg/kg), then Cd (106.0 mg/kg), Cr (104.2 mg/kg), and Pb (59.37 mg/kg) in the treatment with 100 mg/kg. In the stem, the pattern of accumulation was: Cd > Pb > Cu > Cr. Pb showed the highest accumulation in the roots (90.36 mg/kg), followed by Cd > Cr > Cu. Application of two doses of 2.5 and 5 mM EDTA increased Cu concentration in the shoot of *T. angustifoli*. In 5 mM EDTA solution, the highest concentration of cooper was observed, which was 2.5 times higher than in the control sample, which is significant [25].

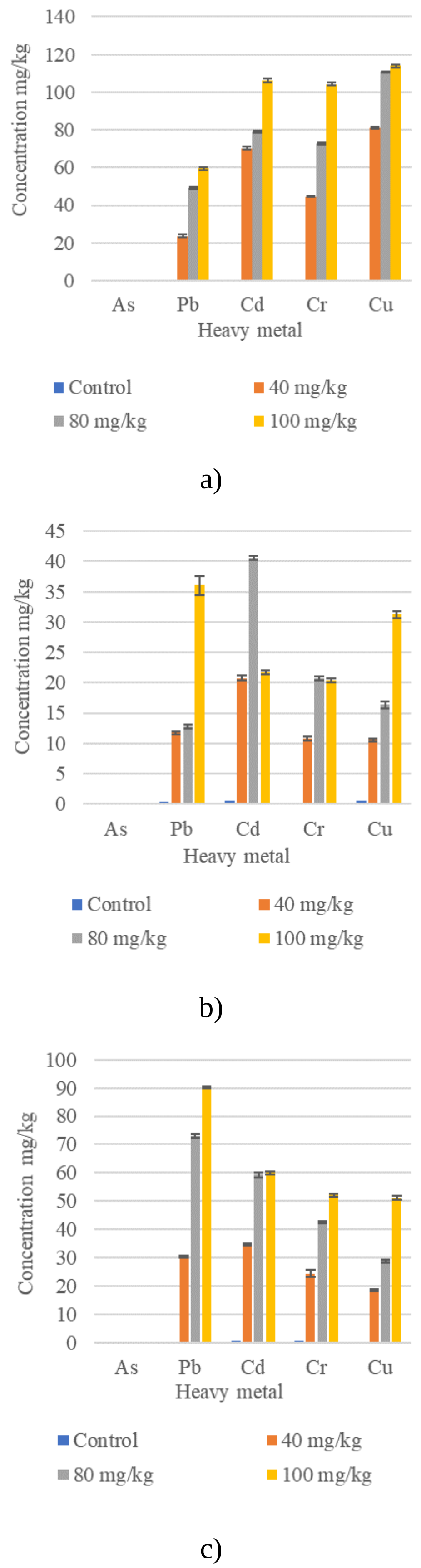


Figure 3. Content of heavy metals in leaves (a), stem (b) and roots (c) of *Portulaca oleracea* after treatment with 0.1 mg/kg of EDTA (n = 3)

The values of the translocation factors were as follows: for the 40 mg/kg treatment - Cu (4.923) > Cd (2.625) > Cr (2.275) > Pb (1.166), for the 80 mg/kg treatment - Cu (4.393) > Cr (2.189) > Cd (2.015) > Pb (0.846) and for the 100 mg/kg treatment - Cu (2.838) > Cr (2.393) > Cd (2.128) > Pb (1.056) (Figure 4).

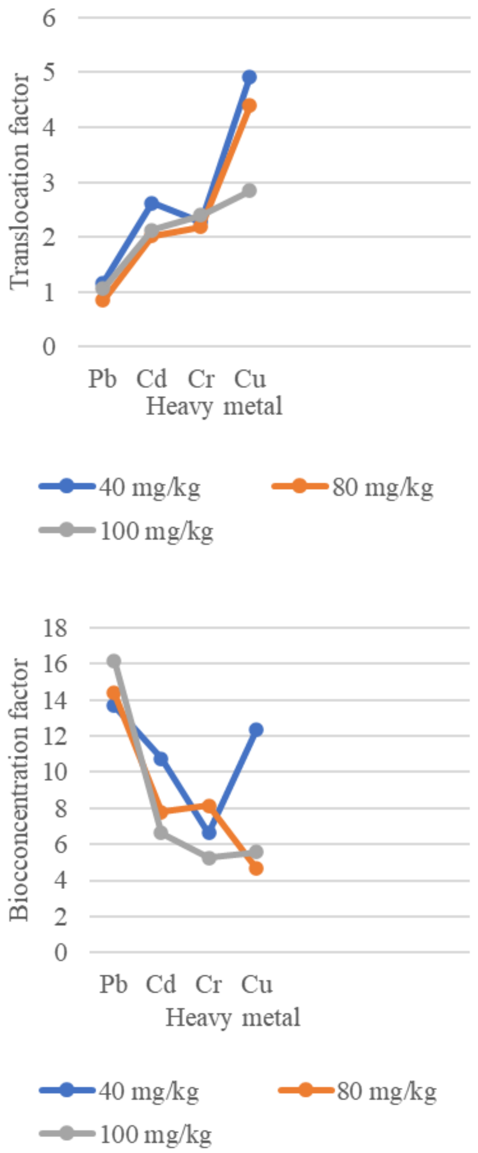
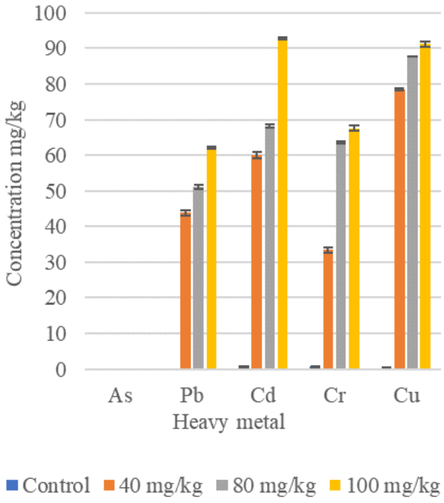


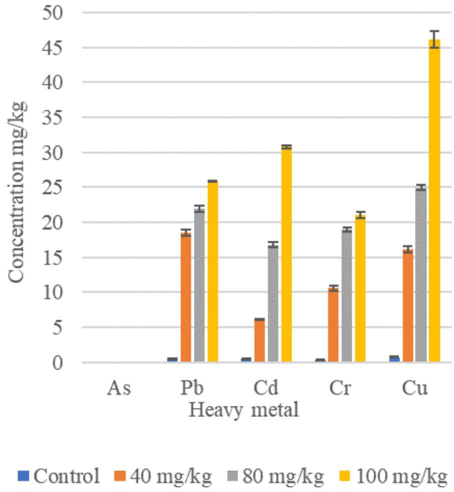
Figure 4. Translocation and bioconcentration factor after treatment with 0.1 mg/kg of EDTA

Phytoextraction is suitable for plant species with TF greater than 1. According to this index, the aerial parts of plants are the place where heavy metals are most efficiently translocated [26 - 31]. The values of the bioconcentration factor were as follows: for the 40 mg/kg treatment: Pb (13.69) > Cu (12.37) > Cd (10.75) > Cr (6.633), for the 80 mg/kg treatment: Pb (14.37) > Cr (8.141) > Cd (7.780) > Cu (4.645), and for the 100 mg/kg treatment: Pb (16.19) > Cd (6.614) > Cu (5.558) > Cr (5.248) (Figure 4). According to [21] *Plantago major* and *S. exaltata* had high BCF for Cu and Zn, respectively, and *G. pennelliana* for Zn, Cu and Pb.

The heavy metal accumulation profile of *Portulaca oleracea* after treatment with 0.3 mg/kg of EDTA is shown in Figures 5a, b and c. Leaves accumulated Cd (92.71 mg/kg), Cu (91.2 mg/kg), Cr (67.56 mg/kg), and Pb (62.04 mg/kg) in 100 mg/kg treatment. In the stem, Cu accumulation was 46.11 mg/kg. Pb (93.4 mg/kg) and Cd (98.70 mg/kg) accumulated mostly in the roots when treatment with 100 mg/kg was performed. The root of the purslane plant has accumulated more metals than the shoot. Therefore, it can be concluded that EDTA can enhance the transfer of heavy metals to the shoots [32]. In addition to their unwanted accumulation at higher soil levels, long-term persistence, and potential entry into the food chain, heavy metals are major environmental contaminants. Despite the strong affinity of EDTA for most of heavy metals, EDTA-enhanced remediation of heavy metals using high biomass species is a successful approach. Due to its ability to form highly soluble and stable metal-EDTA complexes, EDTA is the most effective organic ligand for enhancing metal solubilization, uptake, and translocation [33]. Therefore, in order to phytoextract heavy metals from contaminated soil, chemical amendments are helpful. However, the addition of EDTA or EGTA and SDS also has certain disadvantages [34], particularly harmful effects on soil microorganisms [35], soil-associated enzyme activities, and on grown plant species [36]. The potential of negative environmental consequences increases with chemically assisted phytoextraction because metals are mobilised over long periods of time. By dissolving minerals, chemical amendments can change the chemical composition of soil and its physical structures.



a)



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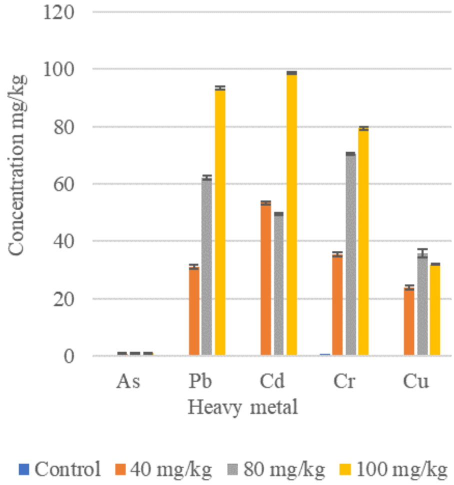

c)

Figure 5. Content of heavy metals in leaves (a), stem (b) and roots (c) of *Portulaca oleracea* after treatment with 0.3 mg/kg of EDTA (n = 3)

In order to check translocation of heavy metal in plant parts, the translocation factor was calculated. The values of the translocation factors were as follows: for the 40 mg/kg treatment - Cu (3.989) > Pb (2.010) > Cr (1.245) > Cd (1.241), for the 80 mg/kg treatment - Cu (3.131) > Cd (1.717) > Pb (1.175) > Cr (1.171) and for the 100 mg/kg treatment - Cu (4.296) > Cd (1.251) > Cr (1.117) > Pb (0.941) (Figure 6).

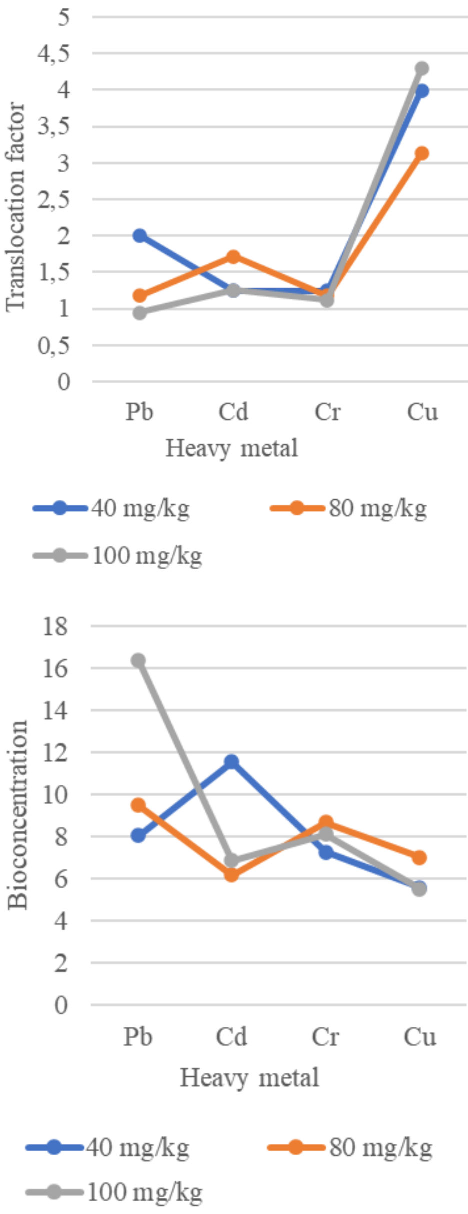

Figure 6. Translocation and bioconcentration factor after treatment with 0.3 mg/kg of EDTA

The values of the bioconcentration factors were as follows: for the 40 mg/kg treatment - Cd (11.53) > Pb (8.017) > Cr (7.264) > Cu

(5.576), for the 80 mg/kg treatment - Pb (9.484) > Cr (8.647) > Cu (7.005) > Cd (6.158) and for the 100 mg/kg treatment - Pb (16.34) > Cr (8.079) > Cd (6.837) > Cu (5.509) (Figure 6). Usman et al., 2019 [37] found that *T. qataranse* had BCF values greater than 1 for Cu, Cd, Cr and Ni, therefore BCF values of *T. qataranse* indicates phytostabilization of the Cu, Cd, Cr and Ni. Furthermore, Cd had a TF > 1 (1.6), while the TF of other metals such as Cu, Ba, Cr, Pb and Ni was < 1, suggesting that *T. qataranse* remediate Cd by phytoextraction.

CONCLUSION

Heavy metals can seriously affect the ecosystem because they can accumulate in the soil, survive for a long time, and potentially even enter the food chain. *Portulaca oleracea* showed a good ability to accumulate Cu, Cd, Cr and Pb when the soil was treated with 0.1 mg/kg of EDTA. The values of BCF and TF were >1 for all the heavy metals, which shows that the plant has a high bioaccumulation capacity and can transfer sequestered heavy metals from roots to aboveground parts. Based on the obtained results, it can be concluded that EDTA is effective for the removal of the heavy metals due to its strong ability to bind most heavy metals. Plant species that have been exposed to chemical amendments can effectively remove heavy metals from the environment. Therefore, it is concluded that *Portulaca oleracea* can be used to clean soil contaminated with heavy metals.

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