EDTA facilitated phytoextraction of Pb, Cd and Zn from a lead – zinc mine contaminated soil by three new accumulator plants (Marrubium cuneatum, Stipa arabica and Verbascum speciosum)

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Abstract

This study aims to evaluate the enhancement of phytoextraction of heavy metals Pb, Cd and Zn by species *M. cuneatum, S. arabica and V. speciosum*, through EDTA amendment. Assisted phytoextraction pot experiments were performed at different EDTA dosages (0, 1, 3 and 5 mmol kg^{-1} soil). Generally, applying EDTA decreased pH and increased EC, while no significant effect was observed on organic matter, nitrogen, and exchangeable K and P. Also the activity of soil dehydrogenase enzyme was inhibited at 5 mmol kg^{-1} EDTA. The phytoavailability of Cd, Pb and Zn increased significantly in the presence of EDTA, followed by the contents of them in the tissues of all three studied species amplified. Resulting from oxidative stress caused by the accumulation of metals, the activity of antioxidant enzymes such as, glutathione peroxidase (GPX), superoxide dismutase (SOD) and catalase (CAT) increased, when chelating agent was added. EDTA showed fluctuating effect on malondialdehyde (MDA) content and in higher doses it partially decreased chlorophyll concentration. EDTA at the level of 1 and 3 mmol kg^{-1} enhanced the plant growth, but 5 mmol kg^{-1} of that reduced the biomass of the studied species. The Bioconcentration factor (BCF) for Cd was notably high in all studied plants, and considerably elevated for Zn and Pb with the addition of EDTA in *M. cuneatum* and *S. arabica* (BCF>1), whilst accumulation factor greater one (AF>1) were found for Cd in all species and for Pb in the case of *S. arabica*. Predictive models for metal accumulation in plant tissues were developed using stepwise multiple linear regression, with pH and Cd showing the highest contribution to interpretation and variability of the dependent variables. In general, the results demonstrated that EDTA can be an effective amendment for phytoextraction of Cd, Zn and Pb by *M. cuneatum*, *V. speciosum* and *S. arabica* in contaminated soils.

1. Introduction

Recently, a rapid increase in soil metal-pollution has resulted from continuing urbanization and industrialization and as a serious global issue, the dispersion of potentially toxic metals throughout the environment poses a menace to humans and ecosystems (Attinti et al., 2017; Jiang et al., 2019; Vareda and Durães, 2019). There has been a growing concern regarding heavy metals pollution, which is primarily the result of anthropogenic activities such as smelting and mining, effluents discharging, solid waste disposal and industrial waste treatment (Ibrahim et al., 2017; Thuan et al., 2018; Guo et al., 2019). A negative effect of potentially toxic metals on soil microbial populations can result in a reduction of biological activity and it has been shown that a heavy metal's absorption and accumulation inhibit plant growth; these metals also interfere with root and leaf development (Tauqeer et al., 2019; Hosseinniaee et al., 2022). Heavy metal stress triggers a multitude of complex plant reactions; these mechanisms involve the concept of irritant as well as antioxidant defense mechanisms (Rehman et al., 2019; Saleem et al., 2020).

Metal-contaminated areas must be remediated due to the environmental and health implications resulting from heavy metal contamination to minimize their effects on the environment ecosystem. Research on various physicochemical and biological approaches has been conducted over the past few decades (Sarwar et al. 2017; Afonso et al., 2020). Heavy metal-contaminated soils are typically decontaminated using either encapsulation or dig-and-dump methods, but neither of these techniques address the issue of decontamination (Mkumbo et al., 2012). The traditional remediation technologies based on chemicals present disadvantages such as high costs, complex technical requirements, secondary pollution and in most cases, they are only suitable for small contaminated areas that need to be treated quickly and thoroughly (Xing et al., 2020; Wu et al., 2021). Moreover, traditional techniques degrade most of the inherent characteristics of the soil and killing its living organisms (Weber et al., 2011; Balazs et al., 2018). Currently, phytoremediation has been confirmed as the most economical and ecologically friendly option, which phytoextraction involves using accumulator species for the removal of pollutants from the soil via soil-root to shoot system and is applicable on large scales (Yang et al., 2018; Du et al., 2020). Additionally, plant species are an ideal candidate for remediation purposes are non-disrupting to ecosystems and have been proven to be useful for the in-situ phytoremediation process of a wide range of toxic metals (Aihemaiti et al., 2018).

As a phytoremediation process, phytoextraction is regarded as the most efficient method for removing soils contaminated with heavy metals and metalloids (Sarwar et al., 2017; Ghazaryan et al., 2019). It typically employs plants having a high growth rate, a developed root system, resistance to heavy metal toxicity and large biomass with high accumulation of metal contents in their aerial and harvestable parts (Al-Thani and Yasseen, 2020). Different plant species exhibit various phytoextraction potentials for heavy metals, depending on their species, metal availability, and growing environments (Anning and Akoto, 2018; Rehman et al., 2019). A plant's phytoextraction potential is mainly determined by its ability to absorb heavy metals into its harvestable parts and the bioavailability of metals in soil is a critical limitation of phytoextraction (Gul et al., 2019). Over the past decades, chelating agents have been extensively studied as a means of increasing the metals' solubility in soil and the absorption ability of plants. Common chelating agents for boosting Plants' uptake of heavy metals include nitrilotriacetic acid (NTA), ethylene diamine disuccinic acid (EDDS), Ethylenediaminetetraacetic acid (EDTA) and low-molecular-weight organic acids (LMWOAs) (Li et al., 2015; Guo et al., 2019; Tananonchai et al., 2019). In the meantime, EDTA is the most effective synthetic chelating agent for enhancing metal availability, sorption, and complexation (Nascimento et al., 2006; Najeeb et al., 2017; Kaurin et al., 2020). For the remediation of heavy metal-contaminated soils, EDTA has been widely used with plants such as *Bidens maximowicziana* (Wang et al., 2007), vetiver grass (Attinti et al., 2017) and *Brassica juncea, Coss* (Guo et al., 2019), indicating the its enhancement effect on the metal absorption.

Selecting the suitable plant species is the key factor to implementing phytoremediation successfully, as they must be tolerant of high metal concentrations and compatible with ecological conditions. Native species growing naturally in mining areas are usually metal-adapted, which makes them ideal candidates for phytoremediation. For this study, enhanced phytoextraction was performed on the following plant species: *Stipa arabica* (Poaceae), *Marrubium cuneatum* (Lamiaceae) and *Verbascum speciosum* (Scrophulariaceae) which are perennial with high biomass and metal-tolerant. In our previous study to identify native species with high phytoremediation potential, *M. cuneatum* serves as a stabilizer and accumulator for Cd and Zn, respectively, and also showed the highest accumulation of Pb in its aerial parts. *V. speciosum* and *S. arabica* with significant accumulation factor (AF) and translocation factor over one (TF > 1), are potentially appropriate plants for Pb and Zn removal through phytoextraction. In a comprehensive manner, Edta's effect on plants and soil has not been thoroughly investigated. Hence, the objective of this general research is to assess the effect of EDTA chelating agent on (1) chemical and biological properties of rhizosphere soil (2) Cd, Pb and Zn uptake by *M. cuneatum*, *S. arabica* and *V. speciosum* and (3) plant growth and physiological characteristics, (4) also establishment of prediction models for metal uptake by plants using stepwise multiple linear regression. To our knowledge, this is the first study that evaluates the metal uptake by the mentioned species in greenhouse conditions, as well as the effect of EDTA on the growth, physiological and phytoextraction capability of these plants.

2. Material And Methods

2.1. Growth conditions and treatments

The soil polluted with Zn, Pb and Cd was collected from Angouran Pb–Zn mine site, Iran (36 36'41" N and 47 23'32" E), air dried, sieved (≤ 2 mm) for removing gravel, stone and plant debris and homogenized. Physical-chemical characteristics of experimental soil including organic matter (OM), acidity (pH), total nitrogen (N%), electrical conductivity (EC), available potassium (K) and soluble phosphorus (P), texture and concentration of Zn, Pb and Cd were determined (Table 1). The seeds of *Stipa arabica*, *Marrubium cuneatum* and *Verbascum speciosum* species were purchased from the Research Institute of Forests and Rangelands, Tehran. After being surface sterilized with a 5% sodium hypochlorite for 10 minutes, the seeds were washed several times with distilled water. Due to the herbaceous life form of species *M. cuneatum* and *V. speciosum* one seedling per pot was sawn, but regarding the *S. arabica* because of the bunch growth system of this species to produce appropriate biomass, 0.1 g were planted. Each plastic pot was 18×17 cm, containing approximately 3000 g of contaminated soil. A Greenhouse experiment was carried out from 22 August to 22 February for six months on the agriculture and natural resources campus of Tehran University in a growth chamber with day (25 ± 5°C) and night (15 ± 5°C) temperatures and soil humidity of 60–80% of the field capacity. Three replications of each treatment were conducted in a completely randomized design and 36 pots were divided into 9 experimental groups randomly, and 50 days before harvesting, dosages of 0 (CK), 1 (E1), 3 (E2) and 5 (E3) mmol EDTA kg^{-1} soil were applied in all experiments according to previous studies (Li et al., 2020; Arshad et al., 2020).

Table 1
Physical-chemical properties of experimental soil.

Characteristic		Value
pH		7.12
EC (ds m^{-1})		0.31
Total N (%)		0.19
OM (%)		2.33
Texture		Loamy
Phosphorous (P)	Total (%)	0.06
	Soluble (mg/kg)	41.6
Potassium (K)	Total (%)	0.44
	Soluble (mg/kg)	494
Zn (mg/kg)		568.42
Pb (mg/kg)		472
Cd (mg/kg)		6.85

2.2. Plant harvesting and sampling

One day before harvest, the required amount of leaves of each plant were sampled in the pot and instantly transferred to liquid nitrogen, next kept at − 70 ∘C for physiological analysis such as antioxidant enzyme activities, chlorophyll and malondialdehyde. The plants were collected from the pots after 6 months of sowing and split into shoots and roots, then for removing the adhering soil particles, they were repeatedly washed with tap water and finally rinsed using de-ionized. The dry matter of the roots and shoots was recorded after drying at 70°C in the oven for 48 hours (to reach a constant weight). Soil samples were separated into two parts and some of those were stored in a refrigerator at 4°C for measuring dehydrogenase enzyme activity and another part were air-dried and sieved with 2 mm mesh for chemical analysis.

2.3. Measuring soil properties and determining the heavy metals concentration in soil and plant

Rhizosphere pH and EC were measured in a suspension of soil and water (1:2.5 ratio), available K and extractable P using a flame photometer and spectrophotometer (Shimadzu UV-160), respectively (Olsen and Somers, 1982), total nitrogen by the Kjeldahl method (Bremner., 1996) and a Walkley-Black titration was used to determine the OM (Nelson and Sommers, 1996). The activity of dehydrogenase enzyme by reducing triphenyltetrazolium chloride (TTC) to triphenylformazan (TPF) was measured with a spectrophotometer at 546 nm in µg TPF g^{-1} soil h^{-1} (Thalmann., 1968). Regrading the metal analysis in soil, the total concentration was extracted by digesting samples with a solution composed of HNO_3 and HCL (3:1 ratio) (USEPA, 2007) and bioavailable metals were determined using Diethylenetriamine penta-acetic acid (DTPA) 0.005 M (Lindsay and Norvell, 1978). In order to extract heavy metals from plants, 0.2 g of dried powder is heated in a furnace at 500 ∘C for 6 h, and then transferred into a beaker with 20 mL of 1 N HCl and kept at 150 ∘C for 20 minutes. A final filtration and dilution with distilled water to 100 mL is performed. Eventually the contents of heavy metals in soils and plants were measured using the ICP-OES (Spectro Arcos-Germany, 1999).

2.4. Translocation factor (TF), accumulation factor (AF), bioconcentration factor (BCF), and phytoremediation factor (PRF)

For investigating the accumulation and the translocation of every metal in plant, TF (the ration of metal content in aerial parts to concentration of that in the roots) (Eq. (1)) (Bonanno and Vymazal, 2017), BCF (root metal content ratio to that in rhizosphere soil) (Eq. (2)) (Marchiol et al., 2013), AF (An aerial part's metal concentration in relation to the soil's) (Eq. (3)) ((Mendez and Maier, 2008; Marrugo-Negrete et al., 2016) and PRF (The ratio of the total metals extracted by the aerial parts in the treated group to those extracted by the shoots in the control group) (Eq. (4)) (Li et al., 2020) were calculated. A phytoremediation factor greater than 1 (PRF > 1) indicates that the treatment has enhanced metal absorption by the plant, and the higher the value, the greater the effect. Also, higher values of BCF, AF and TF display a stronger ability of a certain metal to migrate from the soil to the plant's root and then to the aboveground parts.

(1) TF = $[C_{above}/C_{root}]$; (2) BCF = $[C_{root}/C_{soil}]$; (3) AF = $[C_{above}/C_{soil}]$

(4) $PRF = \frac{C_{above} \times M_{above}}{C'_{above} \times M'_{above}}$

Where, C_{above}, C_{root} and C_{soil} represent the metal contents in aerial part, root and soil, respectively; C'_{above}, M_{above} and M'_{above} are the metal concentration in the shoot of plant without treatment, the biomass of aerial parts with and without treatment, respectively.

2.5. Analyzing chlorophyll and oxidative stress (lipid peroxidation activity)

To determine chlorophyll content, 0.1 g of a fresh leaf sample was thoroughly ground in a mortar with 10 ml of 80% acetone and kept in the dark for 24 hours at a temperature of 4°C. Then, the extract was centrifuged for 10 minutes at a speed of 5000 × g and the absorbance was determined at 645, 480 and 663 nm using a spectrophotometer (Shimadzu UV-160) (Arnon, 1949). The concentration of membrane lipid peroxidation was measured based on malondialdehyde accumulation in leaves using Thiobarbituric acid. Optical absorption was determined at wavelengths of 450, 532 and 600 nm in a spectrophotometer (Shimadzu UV-160) and the content of MDA was obtained using its extinction coefficient (155 $mM^{-1}cm^{-1}$) in nmol g^{-1} fresh weight (FW) (Heath and Packer, 1968).

2.6. Influence of EDTA on antioxidant enzyme activities

The enzymatic extract was gathered by crushing 0.1 g of fresh tissue in liquid nitrogen and adding 1 mL of sodium phosphate buffer (50 mM, pH 7). After centrifuging the homogenate for 20 minutes at 10,000 rpm and 5°C, the activity of CAT, GPX (Maehly and Chance, 1954) and APX (Chen and Asada, 1989) was measured from the obtained supernatant; the activities were expressed in μmol min^{-1} mg protein. The superoxide dismutase (SOD) activity was measured based on (Dhindsa et al., 1981) at 560 nm. The main reaction buffer included 100 mM phosphate buffer (pH = 7.8), 26 mM methionine, 20 μM riboflavin, 0.1 μM EDTA, 750 μM nitroblue tetrazolium (NBT) and 20 μL of enzyme extract; expression of enzyme activity in U mg^{-1} protein.

2.7. Statistical analysis

In the present study, for the purpose of comparing soil and plant characteristics based on significance differences ($P < 0.05$), following the normality test (Kolmogorov–Smirnov), a one-way analysis of variance (one-way ANOVA) followed by a turkey test were performed. The correlation between soil and plant variables was investigated using Pearson's correlation coefficient and to predict the accumulation of heavy metals in the roots and shoots of the studied species, a stepwise linear multiple regression model was applied. SAS 9.4 and R 4.0.3 software were used to analyze all the statistics analysis

3. Results And Discussion

3.1. The influence of EDTA on soil characteristics

The soil characteristics cultivated by the studied species under EDTA amendment are shown in Fig. 1. Soil pH decreased smoothly under all investigated treatments except 1 and 3 mmol kg^{-1} EDTA in soil corresponding to *S. arabica*. When the EDTA concentration reached 5 mmol kg^{-1}, soil pH reduced in the control group from 7.09 to 7.02, 7.09 to 6.98 and 7.13 to 6.99 respectively for *S. arabica*, *V. speciosum* and *M. cuneatum*. By adding EDTA, the soil EC of *S. arabica*, *V. speciosum* and *M. cuneatum* by 10, 33 and 23%, respectively, compared to the control. As an acidic salt of hexacarboxylic acid, EDTA reduces pH because of its acidic nature (Li et al., 2020). Also, the reduction of soil acidity has been seen in the presence of EDTA (Arshad et al., 2020). The EDTA application showed a significant effect only on soil organic matter under *M. cuneatum* cultivation. Generally, this amendment increased the OM in habitats *M. cuneatum* and *V. speciosum* to some extent, while it was the opposite for the soil of *S. arabica*. The improvement of soil OM presumably is due to the effect of EDTA on increasing root exudates, that are preliminary sources of soil carbon (Ruf et al., 2006). The influence of EDTA on increasing soil nitrogen was negligible and at 1 and 5 mmol kg^{-1} EDTA, soil nitrogen cultivated with *S. arabica* strengthened slightly. The exchangeable K of the soil was not significantly affected by EDTA, but in general, the content of K decreased in high doses. However, the chelating agent did not show a significant effect on available P, but at low concentrations, it increased soil phosphorus content, particularly in *M. cuneatum* substrate. Kaurin et al. (2018), found that the nitrogen and carbon content of EDTA treated soil remained unchanged, while the amount of extractable phosphate (P2O5) rose, which is probably because strong chelating agents such as EDTA reduce phosphorus fixation in soil and boost its effectiveness by anion adsorption reactions (Ren et al., 2015). Dehydrogenase activity and microbial populations are highly correlated, and it is considered as a total microbial activity indicator (Ross, 1971; Alef and Nannipieri, 1995; Debnath et al., 2015). In this work, EDTA application initially enhanced the dehydrogenase enzyme activity, but by increasing the dose of this chelating agent, its activity decreased. The highest activity of this enzyme was seen in the soil of the habitat of species *S. arabica*, *V. speciosum* and *M. cuneatum* with 44, 72, and 24% increases compared to the untreated soil, respectively, in the concentrations of 1, 3, and 1 mmol kg^{-1} EDTA and the lowest for *M. cuneate* and *S. arabica* was related to 5 mmol kg^{-1}, whilst for *V. specious* in the control. 10 mmol EDTA kg^{-1} soil inhibited the development of arbuscular mycorrhiza (Grčman et al., 2001). Also, the addition of 1g (3.3 mmol) kg^{-1} soil of this amendment reduced the activity of the dehydrogenase, while it showed no significant effect on the b-glucosidase enzyme activity (Epelde et al., 2008). On the other hand, an improvement in catalase, urease, acid and alkaline phosphatase activity in the presence of EDTA has been reported (Rathika et al., 2021).

3.2. Effects of various EDTA doses on plant biomass

As shown in Table 2, EDTA affected the growth of the plants studied. The results demonstrated that EDTA treatment in low concentrations shows no significant effect on the root dry weight of the studied plants and even in *M. cuneatum*, *V. speciosum*, it slightly increased, while during the increase dose to 5 mmol kg^{-1}, the root biomass of *S. arabica, V. speciosum* and *M. cuneatum* decreased by 30, 20 and 22%, respectively, compared with those grow in untreated condition. The dry weight of the shoot was not significantly different between the control and doses of 1 and 3 mmol kg^{-1} EDTA for the studied species. Similar to the roots, at the level of 5 mmol, EDTA inhibited the growth of the aerial parts of all three species and decreased by 17, 6 and 11% respectively in *S. arabica*, *V. speciosum* and *M. cuneatum* compared to the control, however the negative effect of EDTA on root growth was more severe than that of the shoot. Considering that the roots are exposed to heavy metals first, they are more affected than the aerial parts (Liu et al., 2018). In this work, the decrease in plant growth due to the application of above doses of the chelating agent is the result of the high accumulation of potentially toxic metals, which is greater than the plant's capacity to activate the defense system (Li et al., 2015; Wei et al., 2018). In addition, EDTA could inhibit plant growth by reducing chlorophyll biosynthesis (Gu et al., 2019). The negative effects of this synthetic chelator because of its interference with soil fertility were observed by other researchers (Xiao et al., 2019; Li et al., 2020). In contrast, EDTA has been found to improve plant growth, according to authors (Weihong et al., 2009; Saifullah et al., 2009; Saleem et al., 2020; Rathika et al., 2021). Regarding this, Saleem et al. (2020) stated that EDTA can increase the chlorophyll concentration of the plant under the pollution stress and by increasing the photosynthetic pigments, it causes the effective conversion of light in the photochemical processes of photosynthesis and thus improves the growth and development of the plant. As a consequence, EDTA has variable effects on biomass depending on plant species, soil metal concentration, and dosage of chelating agents.

Table 2

Effects of the EDTA application on the dry mass of the studied species (Mean ± SD; n = 3).

Species	***M. cuneatum***		***V. speciosum***		***S. arabica***	
	Root	Shoot	Root	Shoot	Root	Shoot
Ck	$0.41^{a} \pm 0.02$	$2.56^{a} \pm 0.06$	$0.25^{b} \pm 0.002$	$1.77^{ab} \pm 0.06$	$1.30^{a} \pm 0.006$	$2.08^{a} \pm 0.01$
E1	$0.38^{a} \pm 0.003$	$2.62^{a} \pm 0.03$	$0.27^{a} \pm 0.009$	$1.90^{a} \pm 0.09$	$1.28^{a} \pm 0.04$	$2.13^{a} \pm 0.01$
E2	$0.42^{a} \pm 0.01$	$2.66^{a} \pm 0.06$	$0.24^{b} \pm 0.006$	$1.82^{ab} \pm 0.02$	$1.27^{a} \pm 0.02$	$2.07^{a} \pm 0.01$
E3	$0.32^{b} \pm 0.009$	$2.27^{b} \pm 0.07$	$0.20^{c} \pm 0.006$	$1.66^{b} \pm 0.05$	$0.90^{b} \pm 0.02$	$1.71^{b} \pm 0.05$

Different lowercase letters in a same column indicate significant differences existed among different plants or soils ($P < 0.05$, Tukey’s test).

3.3. Chelate-induced mobility of Cd, Zn and Pb

The high accumulation of heavy metals in plants and their solubility in the soil is a crucial factor in achieving phytoremediation goals (Kumar et al., 2017). Using EDTA, significantly increased soil Zn, Pb and Cd availability ($P < 0.05$) (Fig. 2). EDTA caused a promotion of 25, 19 and 34% of available Cd in the soil cultivated with *M. cuneatum*, *S. arabica* and *V. speciosum*, respectively, compared to the untreated group (Fig. 2). However, increasing the concentration of this amendment did not show a significant effect on exchangeable Cd content, which indicates that the applying of high EDTA doses in Cd phytoextraction studies does not seem reasonable; according to Liphadzi and Kirkham (2006), EDTA doses greater than 0.5 g kg^{-1} did not change the available Cd, probably due to the soil's weak absorption of this element (McLaughlin et al., 2000). The highest available Pb with an increase of 23, 13 and 13% compared to the untreated soil was obtained in the habitat of *M. cuneatum*, *S. arabica* and *V. speciosum*, respectively, at the concentration of 5 mmol kg^{-1} EDTA (Fig. 2). Regarding Zn, its solubility in soil was directly correlated with the dose of EDTA and the maximum value was obtained with chelating agents of 5 mmol kg^{-1} with an increase of 34, 43 and 35% compared to untreated soil for *M. cuneatum*, *S. arabica* and *V. speciosum* respectively (Fig. 2). Due to its acidic property, EDTA reduced the absorption of metals by soil minerals and organic clays by reducing pH of soil, and subsequently increasing the phytoavailability of metals (Wei et al., 2020). Arshad et al. (2020) and Shahid et al. (2014) reported that EDTA separated Pb from mineral and organic substances in soil, increasing its availability. Also, EDTA increases their solubility in soil by forming complexes with heavy metals (Jiang et al., 2019), thereby enhancing soil-to-plant biomass metal transfer efficiency (Li et al., 2015).

3.4. The uptake of Cd, Zn and Pb by the studied species

The concentration of Pb, Cd and Zn in the roots and aerial parts of the studied species upon the application of EDTA is shown in Fig. 3. The root Pb content was 68.77, 47.75, and 168.1 mg kg^{-1} in the untreated group, while the aerial Pb concentration was 46.06, 17.37, and 68.26 mg kg^{-1}, respectively for *M. cuneatum*, *S. arabica* and *V. speciosum.* Pb concentrations increased significantly in the roots of all three studied species when EDTA chelating agents were applied and was directly related to EDTA dose, so that at the level of 5 mmol kg^{-1}, the Pb content went up by 154, 536 and 525% in *S. arabica*, *V. speciosum* and *M. cuneatum*, respectively, compared to the control; this increase was 563, 376 and 211% in aerial parts. The increase in accumulation of Pb in plant tissues is due to the strengthening of phytoavailability of Pb because of the EDTA application (Arshad et al., 2020). Gul et al. (2019) claimed that the content of Pb in the roots of *Pelargonium hortorum* by 2.7 times increased in the presence of 5 mmol kg^{-1} EDTA, also in the study by Arshad et al. (2020) in 1000 mg kg^{-1} Pb contaminated soil, 10 mmol kg^{-1} EDTA increased root and shoot Pb up to 7.9 and 8.6 times respectively in *Pelargonium hortorum*. Without EDTA treatment, the Cd content in the roots of *S. arabica, V. speciosum and M. cuneatum* was 38.33, 45.35 and 35.66 mg Kg^{-1}, respectively. This concentration increased by 115, 60 and 23% with the addition of EDTA, however, the significant effect was not observed between different levels of EDTA and by increasing its dose, the absorption of Cd remained approximately unchanged. Regarding shoot Cd, EDTA enhanced its concentration from 6.65 to 10.75, 6.47 to 13.5, and 2.88 to 11.49 mg kg^{-1} with 61, 109 and 298% increase respectively for *S. arabica, V. speciosum and M. cuneatum.* EDTA increased the Zn content of roots from 423.01 to 648.27, 258.34 to 431.24 and 403.59 to 451.66 mg kg^{-1} by 53, 67 and 12% respectively for *S. arabica, V. speciosum and M. cuneatum* compared to the untreated group; this enhancement was 40, 67 and 99% in the studied plant's shoots respectively. Using the EDTA chelating agent and as a result of the formation of metal-EDTA complexes, all the routes of entry of metals into the root, such as mass flow, absorption on the root and binding to functional groups on the root surface, are affected (Shahid et al., 2014; García et al., 2017; Liu et al., 2018). As a result of the physical damage induced by the chelating agent and metal, the Pb-EDTA complex could penetrate the root endoderm as well as the Casparin strip (Bell et al., 2003), increasing metal absorption through the roots. Investigating the xylem sap of *Hordeum vulgare* cultivated in polluted soils treated with EDTA revealed metal-EDTA complexes but not individual EDTA (Collins et al., 2002).

3.5 Antioxidant enzyme system activity and contents of MDA and chlorophyll

As shown in Fig. 4, application of EDTA chelating agent remarkably influences the enzyme activity in the studied species. The linear increase in catalase activity was observed by adding EDTA, so that at 5 mmol kg^{-1}, its activity increased by 49, 23 and 40% in *S. arabica, V. speciosum and M. cuneatum*, respectively, compared with those grow in untreated group. Similar to catalase, the highest activity of GPX was achieved at 5 mmol kg^{-1} with 23, 7 and 26% stimulation for *S. arabica, V. speciosum and M. cuneatum*, respectively, compared to the untreated group. EDTA does not appear to have a significant effect on APX activity and its changes did not show a clear trend when different concentrations of this chelating agent were applied. SOD activity was not significantly different between EDTA doses in *M. cuneatum*, whilst, it showed a considerable increase up to 77 and 43% for *V. speciosum* and *S. arabica*, respectively, when chelated. Heavy metal concentrations increase in plants when EDTA is added, producing reactive oxygen species

(ROS) like H_2O_2 and OH^-. To combat ROS, SOD converts superoxide ($^{\bullet}O_2^-$) to H_2O_2 as a first line of defense and is decomposed by CAT into H_2O and O_2 to deal with oxidative stress (Gratão et al., 2005; Chakraborty and Pradhan, 2011). Peroxidase enzymes (APX and GPX) act as a defense molecule to eliminate H_2O_2 and prevent peroxidative damage by reducing and catalyzing ROS during severe stress (Cheng et al., 2016). Increasing antioxidant system activity is considered to be an adaptive response to environmental stress conditions, which these enzymes create synergistically to protect plants against oxidative stress (Weihong et al., 2009). There have been several studies reporting an increase in enzymatic activity under EDTA application (Khan et al., 2019; Saleem et al., 2020; Rathica et al., 2021). As a marker of lipid peroxidation, MDA is generally increased under metal stress due to increased ROS production (Chen et al., 2015; Kumar et al., 2017). In this research, EDTA demonstrated a negligible effect on the content of MDA, but in general, MDA decreased somewhat at lower concentrations (1 and 3 mmol kg^{-1}). Although EDTA increased the heavy metal stress in the studied species, the increase in antioxidant enzyme activity resulted in ROS being removed. Leaf chlorophyll concentration is usually considered an effective characteristic to quantify plant physiological reactions (Liu et al., 2016). The addition of EDTA did not show an obvious trend in the chlorophyll content of the studied species, however, its maximum amount was obtained in species *V. speciosum* and *S. arabica* at a level of 3 mmol kg^{-1}, and the lowest was related to the 5 mmol kg^{-1}. Saleem et al. (2020) found that applying 3 mM EDTA enhanced the chlorophyll content of *Corchorus capsularis L.* under pollution stress conditions. Also, Rathika et al. (2021) observed that EDTA significantly elevated the chlorophyll concentration in *Brassica juncea* planted in soil contaminated with Pb, while in a study, the application of 2.5 mM EDTA decreased the a, b and total chlorophyll concentration by increasing the content of heavy metals in the organs of the species *Petunia hybrida L* (Khan et al., 2019). EDTA may cause oxidative stress, leading to a decrement in photosynthetic pigments (Han et al., 2013).

3.6. Phytoremediation efficiency and PRF of Pb, Zn and Cd by studied plants

Bioconcentration, accumulation and translocation factors of studied species are listed in Table 3. Generally, EDTA enhanced the TF values of Pb, Zn, and Cd in all three species compared to the control, although the extent varied for the different metals and plants. The chelating treatment increased the TF of Cd, however, there was no significant difference between its concentrations, and the highest TF values for *S. arabica, V. speciosum and M. cuneatum* were 0.23, 0.18 and 0.15, respectively. Regarding Pb, at low EDTA dosage, the migration of metal to the aerial part of the studied species was strengthened, but with increasing its concentration, the value of TF decreased. The highest value of TF for all studied species was found at the 1 mmol kg^{-1} EDTA, so that the TF increased from 0.40 to 0.64, 0.36 to 0.48 and 0.68 to 0.95 respectively in *S. arabica, V. speciosum* and *M. cuneatum*. In comparison with the control, the chelating agent increased the TF of Zn in the studied species and induced the maximum value for *M. cuneatum* at the level of 5 mmol kg^{-1} with an increase in TF from 0.28 to 0.51, while the maximum TF between EDTA treatments for *V. speciosum* (0.51) and *S. arabica* (0.99) was observed at the concentration of 1 mmol kg^{-1}. *M. cuneaum* showed a higher TF for Pb than other species, while *S. arabica* was more effective in the transmission Cd and Zn. Several studies have found that metal translocation into the plant's aerial parts may be limited due to obstruction of casparian strip, accumulation in plasma membranes, deposition in intercellular spaces, deposition as insoluble salts, or fixation in root vacuoles and nodule cells (Shahid et al., 2014). In a research to investigate the ability of phytoremediation of Pb by five different bamboo species, Jiang et al. (2019) observed that *Arundinaria argenteostriata* and *Arundinaria fortunei* had a higher TF compared to other species. Zhivotovsky et al. (2011) stated that EDTA using decreases the metal accumulation in the root and transferred them more to the shoot. Also, the degradation of the root membrane by EDTA probably increases the mobility of metals into the plant's aerial parts (Bell et al., 2003). Moreover, EDTA increases the infusion of root metals into the vascular tissue via apoplast, thereby enhancing their uptake by shoot tissues (Hernández-Allica et al., 2007). However, the increase of TF by EDTA in the studied plant did not follow a clear trend, reflecting the complex nature of the metal transfer process in plants (Li et al., 2020), and it seems that at the treatment of 1 mmol kg^{-1} EDTA, the highest TF for metals is obtained. In fact, as the concentration of EDTA increases, the metal content in the roots increases, and most likely the plants roots prevent metals from emigrating into the aerial organs. With respect to this, Wei et al. (2008) pointed out that the roots of Italian ryegrass (*Lolium perenne L.*) reduced the transfer ability of some metals to the aerial parts by binding metals to the root walls.

EDTA's effect on BCF of Pb, Cd and Zn was incremental and there was a direct relationship with the dosage. The value of BCF for Cd was considerably high in all three studied species and reached its maximum value at 5 mmol kg^{-1} EDTA with 7.10, 12.26 and 14.77 for *S. arabica, V. speciosum* and *M. cuneatum*, respectively. This amendment increased the BCF of Pb in *M. cuneatum* and *S. arabica* to above 1 (BCF > 1), and with a tremendous increase from 0.11 to 0.76 it remained below one for *V. speciosum.* The BCF value of Zn by adding 5 mmol kg^{-1} EDTA for *M. cuneatum* and S. *arabica* reached 1.06 and 1.03 (BCF > 1), whilst the maximum value of that was 0.89 for *V. speciosum*. EDTA application enhanced the AF of studied species and except in a few cases, this increase was consistent with the dosage. The AF value of Cd in *M. cuneatum* at all levels of the chelating agent and even for *V. speciosum* in the control treatment was higher than one (AF > 1). This amendment increased the AF of Pb from 0.37 to 1.04, 0.04 to 0.24 and 0.11 to 0.81 for *S. arabica, V. speciosum* and *M. cuneatum*, respectively. EDTA also raised the AF value for the studied species, although the effect was not as strong as that of Pb and Cd. The maximum AF value of Zn with 0.84, 0.42 and 0.54 were found in *S. arabica, V. speciosum and M. cuneatum*, respectively, at 5 mmol kg^{-1} EDTA. As opposed to TF, the value of BCF as well as almost AF of the studied plants improved with increasing EDTA concentration; these results are compatible with the findings of Guo et al. (2019) and Li et al. (2020). Generally, according to TF, AF, and BCF factors, it can be concluded that *S. arabica* and *V. speciosum* species have the highest and lowest Zn accumulation and transmission capacity, respectively. Regarding Pb, the accumulation and translocation capacity of *M. cuneatum* for this element are higher than other studied species, and the lowest was related to *V. speciosum*. The species of *V. speciosum* was more effective in accumulating Cd in aerial organs than other species, followed by *M. cuneatum*.

Table 3
The effect of EDTA on the change of bioconcentration, translocation and accumulation factors (Mean; n = 3).

Species		TF				BCF				AF			
		CK	E1	E2	E3	CK	E1	E2	E3	CK	E1	E2	E3
M. cuneatum	Cd	0.08^{b}	0.12^{a}	0.12^{a}	0.15^{a}	5.94^{c}	11.19^{b}	13.17^{b}	14.77^{a}	0.48^{c}	1.43^{b}	1.66^{b}	2.32^{a}
	Pb	0.68^{b}	0.95^{a}	0.67^{b}	0.70^{b}	0.17^{d}	0.49^{c}	0.86^{b}	1.16^{a}	0.11^{c}	0.47^{b}	0.58^{b}	0.81^{a}
	Zn	0.28^{b}	0.49^{a}	0.44^{a}	0.51^{a}	0.73^{b}	0.75^{b}	0.85^{ab}	1.06^{a}	0.21^{c}	0.37^{b}	0.38^{b}	0.54^{a}
V. speciosum	Cd	0.14^{a}	0.17^{a}	0.18^{a}	0.16^{a}	7.17^{b}	11.55^{a}	12.80^{a}	12.26^{a}	1.02^{b}	1.97^{a}	2.40^{a}	2.04^{a}
	Pb	0.36^{b}	0.48^{a}	0.47^{a}	0.27^{c}	0.11^{d}	0.31^{c}	0.56^{b}	0.76^{a}	0.04^{c}	0.15^{b}	0.24^{b}	0.20^{ab}
	Zn	0.47^{a}	0.51^{a}	0.41^{a}	0.47^{a}	0.51^{b}	0.69^{b}	0.81^{a}	0.89^{a}	0.24^{b}	0.35^{a}	0.34^{ab}	0.42^{a}
S. arabica	Cd	0.17^{a}	0.23^{a}	0.21^{a}	0.23^{a}	5.94^{a}	6.90^{a}	6.66^{a}	7.10^{a}	0.15^{b}	0.46^{a}	0.45^{a}	0.51^{a}
	Pb	0.40^{c}	0.64^{a}	0.58^{ab}	0.50^{bc}	0.37^{c}	0.71^{b}	0.77^{b}	1.04^{a}	0.37^{c}	0.71^{b}	0.77^{b}	1.04^{a}
	Zn	0.73^{b}	0.99^{a}	0.75^{b}	0.66^{b}	0.81^{c}	0.75^{c}	1.03^{b}	1.26^{a}	0.59^{b}	0.75^{a}	0.77^{a}	0.84^{a}

Different lowercase letters in a same row indicate significant differences existed among different plants or soils ($P < 0.05$, Tukey's test).

Phytoremediation factor (PRF), which was considered as an index to determine the optimal concentration of EDTA utilization is shown in Table 4. The PRF value of metals is greater than one under all experimental treatments (PRF > 1) and is as Pb > Cd > Zn. EDTA also exhibited the highest promoting effect for *M. cuneatum* and the lowest for *S. arabica.* Based on the PRF, which considers the aboveground biomass, EDTA doses were not significantly different, however, but the highest value of that for Cd was found in 5, 3 and 1 mmol kg^{-1} EDTA for *M. cuneatum* (Cd = 3.62 and Pb = 5.93), *V. speciosum* (Cd = 2.15 and Pb = 5.39) and *S. arabica* (Cd = 1.65 and Pb = 3), respectively. Regarding Zn uptake, 1 mmol kg^{-1} EDTA with PRF of 1.75, 1.69 and 1.27 for *M. cuneatum*, *V. speciosum* and *S. arabica*, respectively, is suggested as the preferred level for phytoremediation through phytoextraction. Therefore, to determine the optimal concentration of EDTA for phytoextraction purposes, a balance must be maintained between plant growth and heavy metal accumulation (Li et al., 2020).

Table 4
The effect of different dosage of EDTA on the phytoremediation factor (Mean ± SD; n = 3).

		PRF			
		E1	E2	E3	Average
M. cuneatum	Cd	3.21^{a} ± 0.71	3.48^{a} ± 0.27	3.62^{a} ± 0.81	3.43
	Pb	4.33^{a} ± 0.50	5.50^{a} ± 0.91	5.93^{a} ± 0.70	5.25
	Zn	1.75^{a} ± 0.25	1.76^{a} ± 0.37	1.77^{a} ± 0.18	1.76
V. speciosum	Cd	2.03^{a} ± 0.32	2.15^{a} ± 0.12	1.69^{a} ± 0.38	1.95
	Pb	4.49^{a} ± 0.35	5.39^{a} ± 0.95	4.37^{a} ± 0.11	4.75
	Zn	1.69^{a} ± 0.48	1.46^{a} ± 0.30	1.58^{a} ± 0.22	1.57
S. arabica	Cd	1.65^{a} ± 0.41	1.42^{a} ± 0.37	1.36^{a} ± 0.46	1.47
	Pb	3.00^{a} ± 0.35	2.83^{a} ± 0.29	2.61^{a} ± 0.54	2.81
	Zn	1.27^{a} ± 0.07	1.27^{a} ± 0.11	1.15^{a} ± 0.02	1.23

3.7. Correlation analysis and modeling

Supplementary Figs. 1, 2 and 3 displays the results of correlation analysis between soil and plant variables. Soil available heavy metals were negatively correlated to pH and positively correlated to EC in soil of *V. speciosum* and *M. cuneatum*, and OM showed positive correlation with available Cd, Pb ($P < 0.05$ or 0.01), indicating the negative correlation between EDTA and pH and positive with OM, which is compatible with the Li et al. (2020). The results revealed a positive correlation between DTPA-extractable and the heavy metals concentration in plant tissues, while it demonstrated a negative correlation with dry biomass ($P < 0.05$ or 0.01), which is similar to the observations of Guo et al. (2019). BCF and AF were significantly correlated to DTPA-extractable and EC (Positive) and pH (negative) in soil of *M. cuneatum* and *V. speciosum*, while in *S. arabica* species they showed positive correlation with DTPA-extractable, P and N ($P < 0.05$ or 0.01). There was also a positive correlation between OM and BCF and AF of *M. cuneatum* ($P < 0.05$ or 0.01). According to previous studies, the BCF of Pb, Zn, and Cd was negatively correlated with pH and positively correlated with OM. (Dziubanek et al., 2017; Li et al., 2020). By forming organic complexes, organic materials increase the metal solubility in soil, thus increasing their absorption by plants (Kalbitz and Wennrich, 1998; Dube et al., 2001). No clear correlation was observed between the TF factor and other parameters, indicating the complex process of metal transport in plants.

To predict the accumulation of heavy metals in the roots and shoots of the studied species, a stepwise linear multiple regression model was applied. Soil characteristics and the available Pb, Zn, and Cd were considered independent variables. The reliable and significant regression formulas based on the input variables to the model are shown in Table 4 ($P < 0.05$ or 0.01). Most of the fitting formulas have a high explanatory coefficient (R^2), and Cd and pH showed the highest contribution to the interpretation and variability of dependent variables with 10 and 5 times, respectively. Based on these formulas, the regression relationship between the predicted and actual data was established (Supplementary Figs. 4, 5 and 6).

Table 5. Based on stepwise multiple linear regression of SPSS 26.0, prediction models for metal uptake were developed

Table 5
Based on stepwise multiple linear regression of SPSS 26.0, prediction models for metal uptake were developed

	Fitting Equation	P value	R^2
M. cuneatum	CR= -71.74 + 39.86 Cd	0.000	87
	PR = 3101.63 + 12.53 Pb – 586.29 pH	0.000	90
	ZR = 2851.04–343.36 pH	0.001	69
	CS= -17.31 + 7.52 Cd	0.000	78
	PS = 4008.78 + 160.77 Cd – 616 .49 pH	0.000	93
	ZS = − 323.81 + 79.27 Cd + 102.65 OM	0.000	89
V. speciosum	CR = 1178.34–158.72 pH	0.009	50
	PR = − 741.05 + 262.96 Cd	0.00	78
	ZR = 8.01 + 0.49 EC	0.007	53
	CS= -17.50 + 6.36Cd + 4.17DE	0.042	71
	PS= -266.68 + 76.93Cd + 43.00DE	0.009	87
	ZS = 26.71 + 0.2 EC	0.031	38
S. arabica	CR = -75.41 + 604.94 N	0.018	44
	PR= -436.46 + 210.28 Cd	0.00	81
	ZR = 5942.86 + 23.08 Zn – 840.92 pH	0.001	77
	CS= -12.62 + 2.53 Cd + 0.25 P	0.006	72
	PS= -283.77 + 127.52 Cd	0.000	75
	ZS = 61.45 + 14.24 Zn	0.000	73

Abbreviations: CR = Cd of root, PR = Pb of root, ZR = Zn of root, CS = Cd of shoot, PS = Pb of shoot, ZS = Zn of shoot

Conclusion

Plant properties and metal bioavailability in soil greatly influence phytoextraction. This study found that EDTA increased the availability of heavy metals in the soil, which subsequently enhanced their phytoextraction by the studied plants. Generally, the metals uptake by plants increased with the increase in the dose of EDTA, but at 5 mmol kg^{-1} EDTA phytotoxicity was evident due to the reduction in biomass production. To deal with oxidative stress, CAT and SOD increased significantly, GPX, although generally increased, but showed no particular change, especially in *M. cuneatum* and *V. speciosum*, while APX decreased in *S. arabica* and *M. cuneatum* and increased smoothly in *V. speciosum*. EDTA significantly increased BCF, AF and TF factors in the studied species, reflecting the high potential of this chelate for phytoextraction purposes. In this study, *S. arabica, M. cuneatum* and *V. speciosum* showed the highest capacity to accumulate and translocate Zn, Pb and Cd, respectively. EDTA concentration of 5, 3 and 1 mmol kg^{-1} are recommended for the extraction of Cd and Pb by *M. cuneatum, V. speciosum* and *S. arabica*, respectively. Also, 1 mmol kg^{-1} for all three studied species is suggested as the appropriate level for phytoremediation of Zn through phytoextraction. Therefore, due to its stable and highly soluble metal-EDTA complex, EDTA serves as an effective chelating agent to increase metal dissolution, absorption, and translocation.

Declarations

Author Contributions M. Jafari and S. Zare were the designer of the hypotheses; S. Hosseinniaee and A. Tavili performed greenhouse and laboratory works, also Sadegh Hosseinniaee wrote the original draft of the article; S. Hosseinniaee and G. Cappai did analysis and interpretation of data. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest

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

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Figures

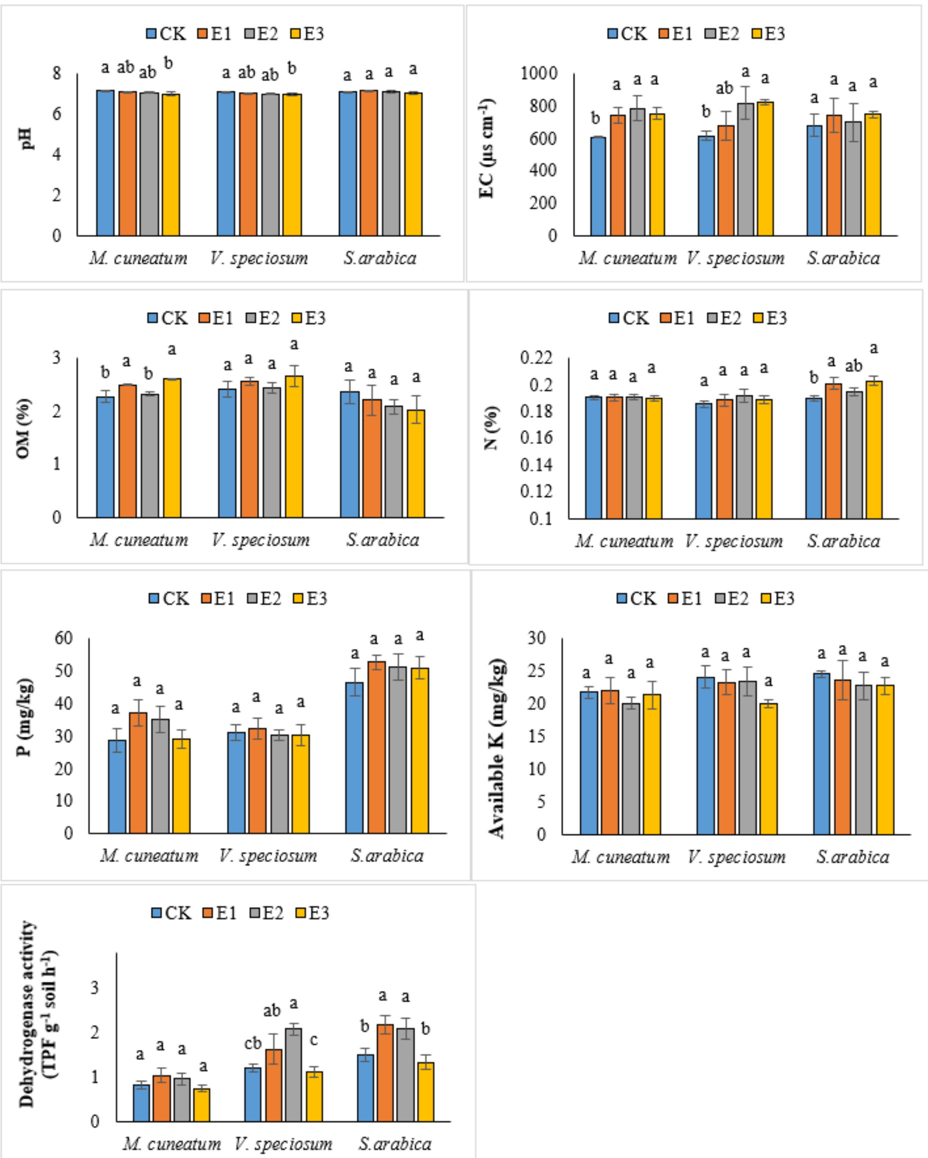

Figure 1

The effect of EDTA chelating agent on soil properties (Mean ± SD; n = 3). Different letters within a column denote significant difference from the corresponding control and other treatments (P < 0.05, Tukey's test). CK, E_1, E_2 and E_3 are represented the soil were treated with EDTA of 0, 1, 3, and 5 mmol kg^{-1}, respectively.

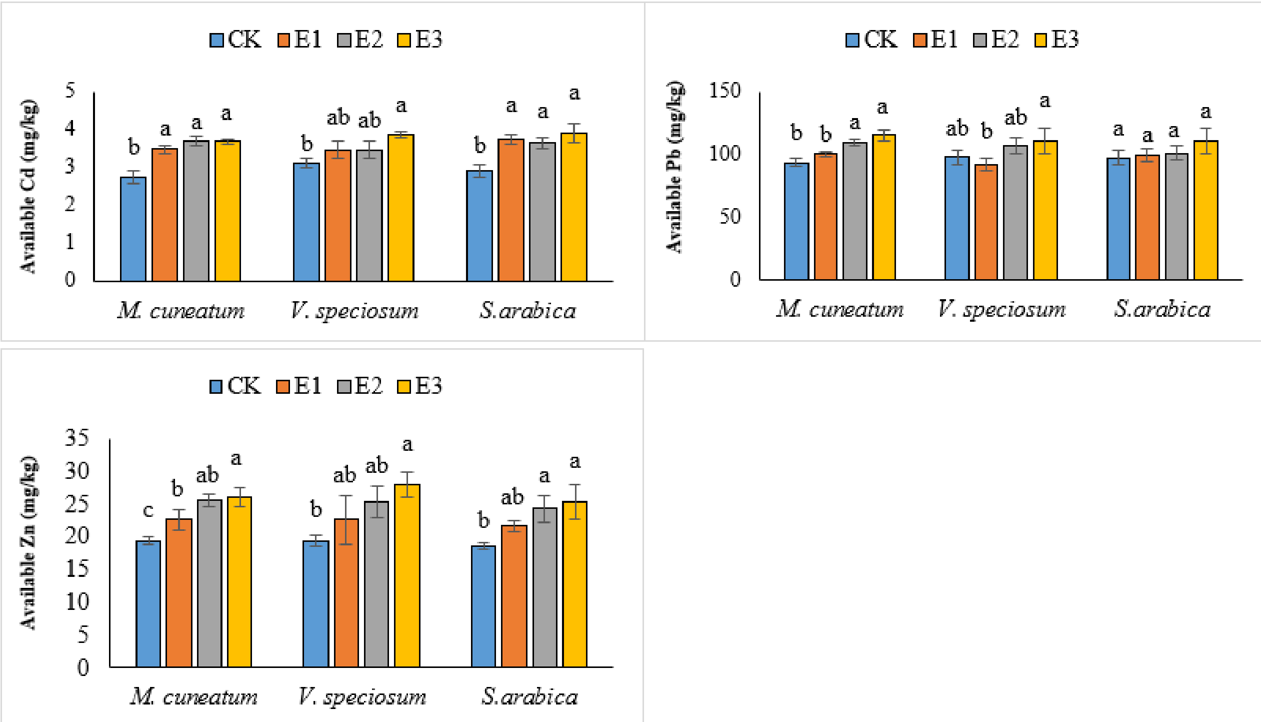

Figure 2

Phytoavailability of Pb, Zn and Cd metals under EDTA treatment (Mean ± SD; n = 3). Different letters within a column denote significant difference from the corresponding control and other treatments (P < 0.05, Tukey's test). CK, E_1, E_2 and E_3 are represented the soil were treated with EDTA of 0, 1, 3, and 5 mmol kg^{-1}, respectively.

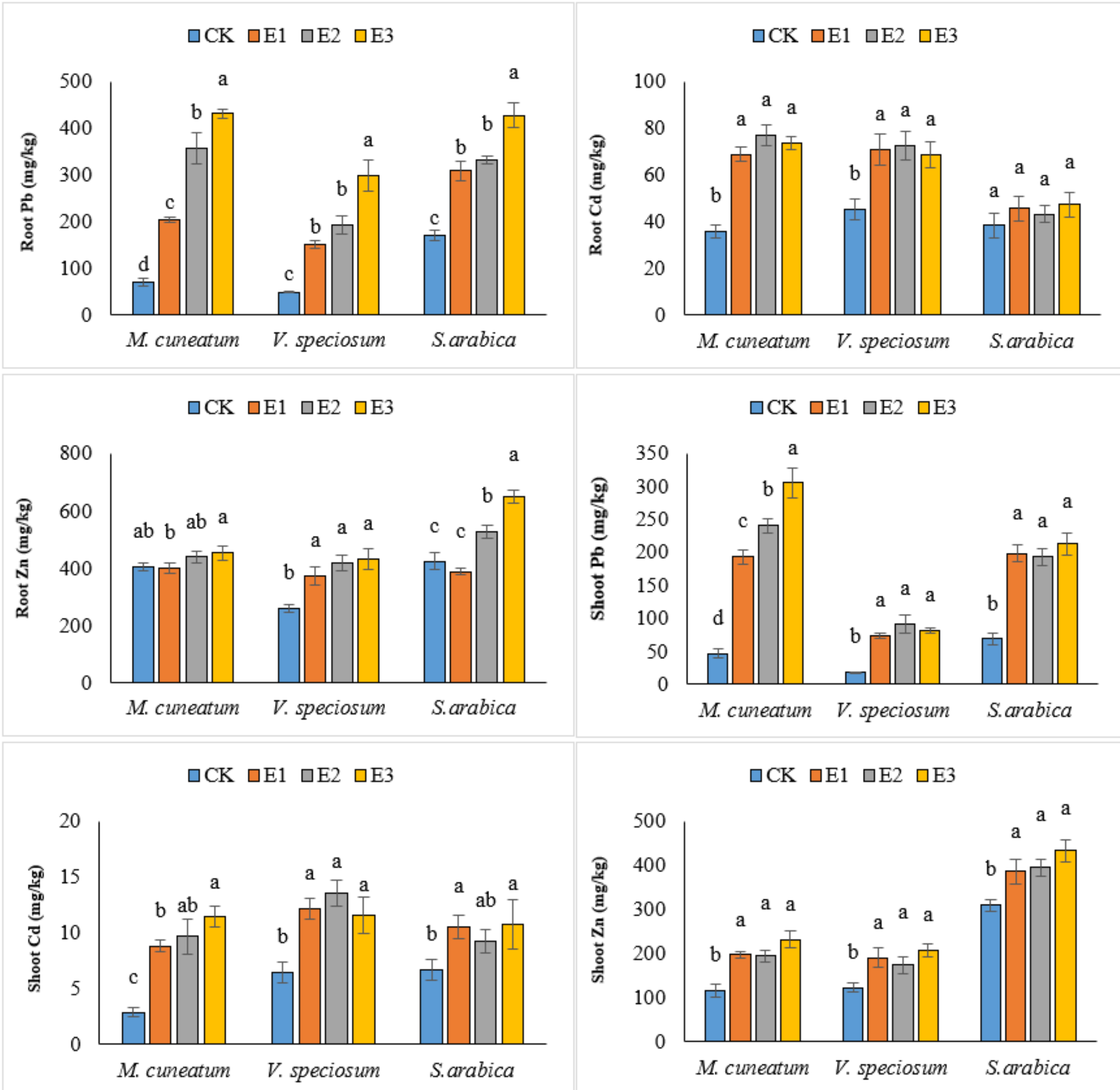

Figure 3

The effect of EDTA on the contents of Cd, Pb and Zn in the roots and shoots of the studied species (Mean ± SD; n = 3). Different letters within a column denote significant difference from the corresponding control and other treatments (P < 0.05, Tukey's test). CK, E_1, E_2 and E_3 are represented the soil were treated with EDTA of 0, 1, 3, and 5 mmol kg^{-1}, respectively.

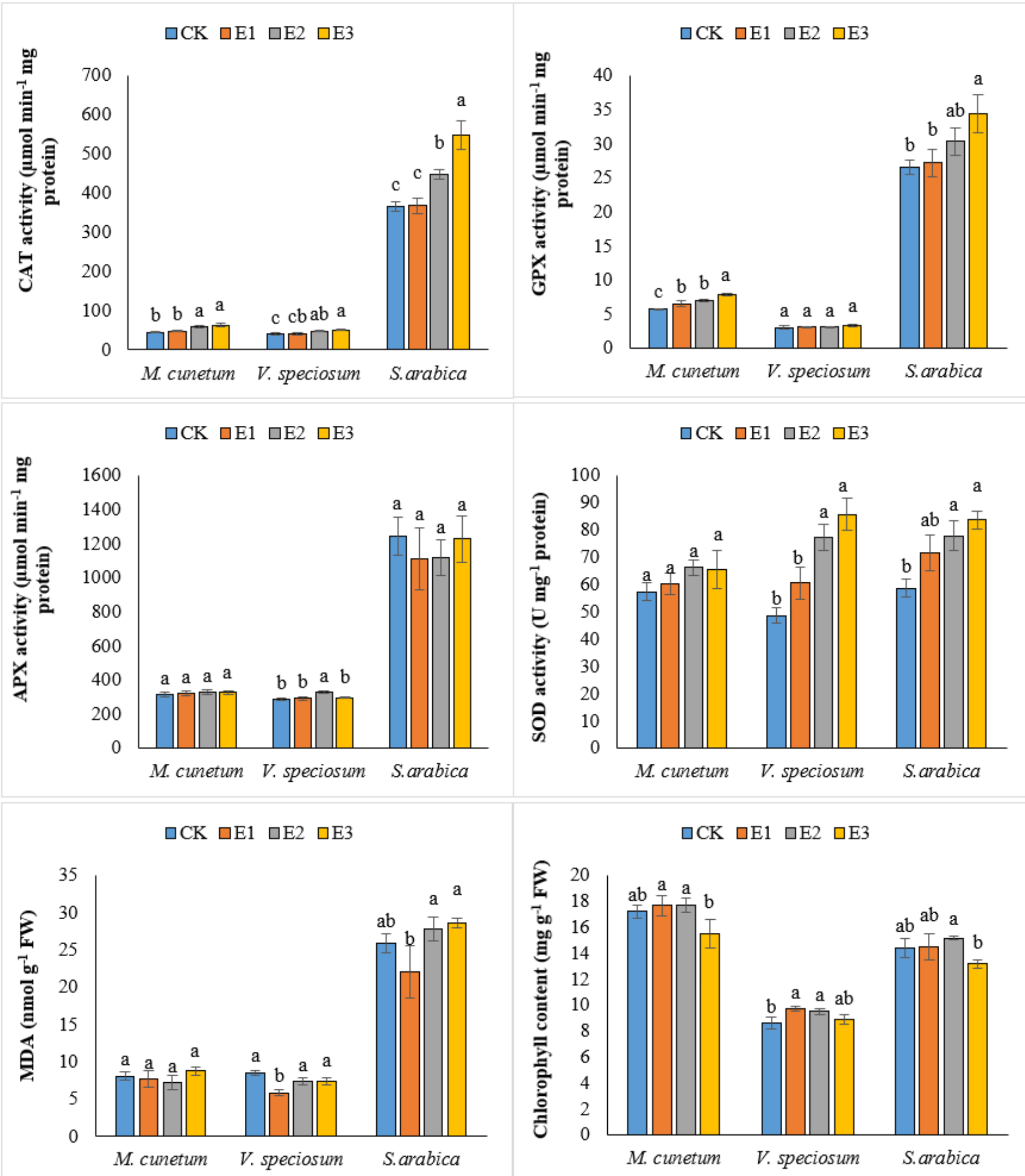

Figure 4

The effect of EDTA on antioxidant enzyme activities and the concentration of lipid peroxidation (MDA) and total chlorophyll (Mean ± SD; n = 3). Different letters within a column denote significant difference from the corresponding control and other treatments ($P < 0.05$, Tukey's test). CK, E_1, E_2 and E_3 are represented the soil were treated with EDTA of 0, 1, 3, and 5 mmol kg^{-1}, respectively.

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