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Assisted Phytoextraction of Arsenic and Cadmium by the Addition of Chemical Amendments and their Effect on Nutrient Ionome in *Sedum praealtum* Plants

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Abstract: Assisted phytoextraction involves the participation of plant species and supplementary agents (chemical or biological amendments) to increase the contaminant bioavailability and accumulation. The employment of many synthetic chelators, increase the mobility and bioavility of the heavy metal uptake by plants and favoring their accumulation in aerial parts of phytoextracting plants. The present work evaluates the effect of two chemical amendments: ethylenediamine tetraacetic acid (EDTA) and oxalic acid, as combine assisted phytoextraction of arsenic and cadmium and their effect on ionome of some nutrients of *Sedum prealtum* plants. At the concentrations tested for As and Cd; the nutrient elements: Ca and Mn accumulated in leaves, Fe, Cu and Mo accumulated in roots and for Mg and Zn concentrations these were almost equally distributed in leaves, stems and roots of this plant species. Even there was a diminished growth of them in presence of both heavy metals; ionomic profiles obtained as response of exogenous addition of As and Cd and both chelating agents were efficiently, increasing the bioavailability of some elements, showing a synergistic effect.

Keywords: Assisted Phytoextraction, *Sedum praealtum*, Arsenic, Cadmium

INTRODUCTION

According to Marschner (2011), Pii et al. (2015a) reported that inorganic elements for plants are classified as macronutrients (nitrogen (N), sulphur (S), phosphorous (P), calcium (Ca), magnesium (Mg), potassium (K)), micronutrients (nickel (Ni), molybdenum (Mo), cupper (Cu), zinc (Zn), manganese (Mn), boron (B), iron (Fe), chloride (Cl)) and beneficial elements (sodium (Na), cobalt (Co), aluminum (Al), selenium (Se), silicon (Si)). Park et al. (2011) defined another term 'heavy metal(loid)s' that in general includes elements (both metals and metalloids) with an atomic density greater tan $6 g/cm^3$, with the exception of arsenic (As), B and Se. This group includes both biologically essential like Co, Cu, chromium (Cr), Mn and Zn and the non-essential elements cadmium (Cd), lead (Pb) and mercury (Hg). Williams and Salt (2009) noted that a balanced supply of these elements ensures an optimal plant growth and development, regulating their homeostasis. It is know that mineral elements interact with one another, according to their similarities in chemical properties and interrelated metabolic pathways (Patterson 1971; Feng, et al. 2017). The development of ionomics contributes to explore interactions between them (Salt 2004; Lyubenova, et al. 2013; Chu, et al. 2015) and this strategy evaluates their adsorption and accumulation in plants by an elemental analysis (Salt 2004). Baxter, et al. (2012) and Chen, et al. (2014) proposed that studying these interactions via ionomics; it allows the knowledge of migration rules for heavy metals and regulating their accumulation by the correlation of elements in plants, giving significant differences in the obtained ionomes from different parts of the plant. Salt, et al. (2008) defined the ionome as: "the mineral nutrient and trace element composition of an organism, representing the inorganic component of cellular and organismal

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systems; giving a dynamic network that complete the physiology and biochemistry of plants". According to Mimmo, et al. (2014) and Pii, et al. (2015b), for this strategy, the availability and consequently plant uptake, translocation and allocation of any element are affected by the type of nutrient source and also the physical, chemical and biological soil characteristics. Guerrero and Rodríguez (2013) mention that the bulk of soil heavy metal concentrations present in contaminated soils are commonly found as insoluble compounds and are unavailable for been absorbed and transported into roots, but the employment of many synthetic chelators like ethylenediamine tetraacetic acid (EDTA), diethylene triamine penta-acetic acid (DTPA), ethylenediamine disuccinic acid (EDDS) and nitrilotriacetic acid (NTA), increase the mobility and bioavailability of the heavy metal uptake by plants, favoring their translocation from roots to shoots (Blaylock, et al. 1997; Huang, et al. 1997; Cooper, et al. 1999; Wu, et al. 1999; Shen, et al. 2002). EDTA has been the most widely used chelating agent in studies of phytoremediation because of its high efficiency in extracting many metals and also organic acids play an important physiological role in conferring metal tolerance, involved in a number of mechanisms that implies metal translocation or accumulation (Wang, et al. 2007). The aim of this study was to evaluate the effect of two chemical amendments: ethylenediamine tetraacetic acid (EDTA) and oxalic acid, as combine assisted phytoextraction of arsenic and cadmium and their effect on ionome of some nutrients of *Sedum prealtum* plants.

MATERIALS AND METHODS

Hydroponic culture of *Sedum praealtum* plants

Branches from a shrub of *Sedum prealtum* A. DC. were collected from the gardens of the Escuela Nacional de Ciencias Biológicas and cutting buds of 4.5cm were obtained, washed with tap water and surface sterilized with 10% sodium hypochlorite for 3minutes, rinsed with sterile distilled water and placed separately in sterile baby food flasks with Magenta SIGMA caps with 100mL of diluted (1/4) of concentrate mineral medium (0.20 M $NH_4H_2PO_4$, 1.15 M $Ca(NO_3)_2$, $MgSO_4{\cdot}7H_2O$, 1.2 M KNO_3, 1.2 × 10^{-2} M H_3BO_3, 1.2 × 10^{-4} M $CuCl_2{\cdot}H_2O$, 2.3 × 10^{-3} M $ZnCl_2$, 4.4 × 10^{-4} M $MnCl_2{\cdot}4H_2O$, 6 × 10^{-6} M $Na_2MoO_4{\cdot}H_2O$, Fe-EDTA (7.1 X 10^{-3}M $FeSO_4{\cdot}7H_2O$ + 7.2 X 10^{-3}M EDTA-Na_2), pH = $\pm$ 6.0). Indole butyric acid (IBA) was added to this mineral medium at 0.1mg/L to induce roots formation in each cutting buds and maintained under greenhouse conditions for 35 days. After this time, rooted plantlets were deposited in concentrate mineral medium and maintained again under greenhouse conditions for another 25 days, for their final exposition to As and Cd supplemented with the chemical amendments.

Sedum praealtum plants exposed to arsenic and cadmium supplemented with chemical amendments

The rooted plantlets were root surface sterilized with 96% ethanol and 10% sodium hypochlorite for one minute, respectively, rinsed with sterile distilled water and transfer again to baby flasks according to the following experimental conditions: control (C) with plants grown only in mineral medium, plants grown in mineral medium with arsenic (As) (1mM $Na_2HAsO_4{\cdot}7{\cdot}H_2O$), plants grown in mineral medium with cadmium (Cd) ($3CdSO_4{\cdot}8{\cdot}H_2O$), plants grown with mineral medium supplemented with oxalic acid (5mM OX) and experimental conditions with the corresponding amendment and heavy metal: As+OX, Cd+OX, As+EDTA and Cd+EDTA (5mM EDTA-Na_2). All the experiments were performed with six replicates and maintained under greenhouse conditions for 10 days. At the end of this time, plants were harvest; leaves, stems and roots were cut and keep separately obtaining their fresh weight and after dried at 70°C for 24 hours to obtain their dry biomass.

Ionic profiles determination in *Sedum praealtum* plants

Ionic profiles of *S. paraealtum* plants were obtained by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) technique. 1g of dry biomass from leaves, stems and roots were finely ground and digested with and acid mixture (2mL of H_2SO_4 + 1mL of H_2O_2 + 3mL of HNO_3 + 2mL of HCl), in a microwave (CEM, MARSX press). The contents of As, Ca, Cd, Cu, Fe, Mg, Mn, Mo and Zn, were determined by employing a ICP-OES spectrometer, 4600DV-Perkin Elmer, USA, taking three lectures of each sample (nine total samples), expressing their final concentration as mg /Kg dry weight (DW). All the plastic containers employed for the analysis were 24 hours previously treated with 2% HNO_3.

The translocation factor (TF) proposed by Cabello-Conejo et al. (2014) was calculated to analyze the translocation of each element tested, considering the total quantity of them in leaves and roots, for each experimental condition expressed as leaves/roots index.

Satistical Analysis

All data obtained were analyzed by one-way analysis of variance and the mean differences were compared applying a Tukey-Kramer Method using the statistics program Graph Pad Instat Ver. 2.03.

RESULTS AND DISCUSSION

Content and behavior of As and Cd in *Sedum praealtum* plants

Figure 1a shows that the addition of EDTA do not favored the absorption of As and finally diminished its concentration. Plants grown only with arsenic, accumulated the highest concentration of it (778mg/Kg DW) and plants grown in experimental condition of As+OX, diminished the As concentration accumulated in plants (289 mg/Kg DW). Arsenic was not mobilized to the leaves of *S. praealtum* plants; it concentrated in roots, particularly only in the presence of this element and supplemented with EDTA as a chemical amendment. In Figure 1b, contrary to the behavior in *S. praealtum* plants, Cd increased its concentration in roots and stems of this plant species and the supplement of both chemical amendments increase it compared with the experimental conditions where only Cd was present.

Distribution in *Sedum praealtum* plants of analyzed elements with or without the chemical amendments

Calcium bioavailability and mobility were present and the total concentration of this element was obtained in plants tested with As+EDTA (26,456mg /Kg DW), followed by the experimental conditions of OX > As > C > Cd+EDTA > As+OX > Cd+OX > Cd. It was evident that the mobility of this macroelement was favored and its quantity in leaves, following the next order according to its accumulation for both heavy metals and chemical amendments tested: As+EDTA > C > OX > Cd+EDTA > Cd+OX > As+OX > As > Cd, showed in Figures 2a and 2b.

The same behavior according to calcium mobility's of an element inside *S. praealtum* plants, was for Mn, (Figures 3a and 3b), where not only the presence of As and Cd favored its mobility from roots to leaves; the chemical amendments supplied also increased it, following the next order: C > As+EDTA > As+OX > Cd > Cd+EDTA > OX > As > Cd+OX.

Fe, Mo and Cu (Figures 4, 5 and 6) showed the same behavior according to their distribution inside *S. praealtum* plants; where in all the experimental conditions, these elements were accumulated and immobilized in roots of this plant species. Total Fe content, showed in Figures 4a and 4b that the highest concentration accumulated in plants was in presence of As in the experimental conditions with the following order: As > OX > Cd > C > As+OX > Cd+OX > As+EDTA > Cd+EDTA. In Figures 5a and 5b, the accumulation of Mo inside roots of *S. praealtum* plants showed again that the presence only of As and Cd plus the supply of both chemical amendments increase it according to the next order: As > As+ OX > OX > C > Cd > Cd+ OX > Cd+ EDTA > As + EDTA.

Even Cu mobility was less but according to the pattern showed by Fe and Mo; this element increased its accumulation in leaves and stems, compared to those elements and the total Cu quantified in this plant species followed the next experimental order: OX > As > C > Cd+OX > Cd > As+OX > Cd+EDTA > As+EDTA (Figures 6a and 6b).

Particular response was obtained for Mg and Zn elements; where the concentration quantified in each part of *S. praealtum* plants, was distributed almost equal in leaves, stems and roots. For Mg element, according to Figures 7a and 7b, the highest mobility to leaves was present in OX and Cd+OX experimental conditions, but the highest concentration of Mg quantified in plants followed the next order: C > Cd+EDTA > Cd > As > As+OX > OX > Cd+OX.

For Zn element, according to the highest total concentration quantified in plants, the order of it was as follows: C > As > Cd > As+OX > As+EDTA > OX > Cd+EDTA > Cd+OX (Figures 8a and 8b).
For Mg and Zn elements, almost the highest quantity of them was determined in stems; particularly for Mg in presence of As and Cd and As+EDTA and Cd+EDTA; EDTA was the convenient chemical amendment for it. Regarding to Zn, the experimental conditions: As, Cd, As+OX and Cd+OX , showed that the oxalic acid favored its accumulation.

DISCUSSION

Eide, et al. (2005) mention that it is well known that the mechanisms underlying the homeostatic control of ions in an organism are strongly interrelated; Baxter (2015) and Baxter, et al. (2012) resume the ionome is not a simply collection of elements, because it allows a clear relationship between elements tested. Regarding to the interspecific relationships between elements in this work As and Cd were closely related to Cu, Fe and Mo. Regarding to their mobility, it was agree with the results reported by Feng, et al. (2017), where Cd transport mechanisms also favored Cu, Zn and Mg translocations. According to Turgut, et al. (2005), the FT is employed to evaluate the capacity of heavy-metal transport from roots to shoots; in this study there was a particular association between elements with FT > 1, for Mn, Mg, Ca and Zn. And a FT < 1, between the elements: Cu, Fe and Mo, with the inclusion of As and Cd in this group, according to Table 1. It is important to note that the presence of As and Cd favored the translocation of Cu Fe and Mo, with the less values obtained; also for the immobilized elements Mn, Ca, Mg and Zn, accumulated in the roots of *S. prealtum* plants.

Berndnowack and Robinson (2006) showed in their results a simplified schematic accumulation of Cu and Zn in shoots or translocation from roots to shoots in the absence and presence of chelating agents based on pot and hydroponic experiments (Tandy, et al.; 2006a, 2006b) based on hydroponic data on Cu and Zn uptake, it mostly shows a decrease in metal uptake in the presence of chelants (Tandy, et al. 2006b; Wenger, et al. 2003). In the present study for Zn, the accumulation of this metal was equally distributed, thus the variability in uptake of chelants by different plant species needs to be considered.

Lou, et al. (2004) mention that metals are accumulated at first in roots of plants and after transferred to the rest of the parts by a disruption of the metabolism, which regulates the transport of metal to shoots; these authors founded large amounts of Cu absorbed and accumulated in roots of plants. In this study, it occurs in *S. praealtum* plants.
Verbruggen, et al. (2009) noted that the metalloid arsenic and the heavy metal cadmium are often considered to be biologically nonessential. Essential or beneficial functions for Cd or As have not been reported in higher plants. However, the possibility cannot be excluded, give a growth promoting effects of Cd as an indirect effect of interference with the plant-internal availability of nutritional elements. These authors remark the fact that Cd and As are potentially toxic for the plant cells because they tend to substitute for Zn and P, respectively. Particularly, it is known that P is present as phosphate in cells and cellular As can be present as arsenate, As (V), probably as a phosphate chemical analog, but also this could be found as arsenite, As (III), which behaves as a sulphur-seeking heavy metal ion, rather like Cd^{2+}. As(V) is easily incorporated into plant cells through the high-affinity Pi transport system and the uptake of Cd from the soil seems to occur mainly via Ca^{2+}, Fe^{2+}, Mn^{2+} and Zn^{2+} transporters (Clemens 2006).

Schaider, et al. (2006) resume that a number of hydroponic and soil experiments have demonstrated a strong correlation between free-ion activity and plant uptake of metals (Pavan and Bingham 1982; Bingham, et al. 1983; Checkai, et al.1987; Cabrera, et al. 1988; Sauvé, et al. 1996); particularly in hydroponic experiments, metal uptake has been found to increase with increasing total metal concentrations even when constant free-ion activities were maintained (Bell, et al. 1991; McLaughlin, et al. 1997). Uptake of metal-EDTA complexes has been observed in a variety of plant species including *Brassica juncea* (Vassil, et al. 1998), *Phaseolus vulgaris* (Sarret, et al. 2001), *Hordeum vulgare* (Collins, et al. 2001) and *Solanum tuberosum* (Collins, et al. 2002), where these studies demonstrate that EDTA-bound metals can be transported to the shoots of plants. Liu, et al. (2008) mention that synthetic chelators and low molecular weight organic acids (LMWOA) are the most common chemical amendments used in chemical assisted phytoextraction of heavy metals; because such substances are capable of forming chemical complexes with metal ions modifying the bioavailability of them (Wu, et al. 2004; Quartacci, et al. 2006).

Two particularly works done by Liu, et al. (2008) and Yang, et al. (2004) with *Sedum alfredii* Hance plants, reported interesting results. Liu, et al. (2008) compared the performance of the synthetic chelators: EDTA, DTPA and EDDS with citric, oxalic and tartaric acid, in the metal solubility and enhancing phytoextraction of Zn, Cu and Cd; showing that synthetic chelators were more effective than LMWOA on increasing the solubility of Zn, Cu and Cd, which were consistent with earlier studies (Chaney 1988; Evangelou, et al. 2006; do Nascimento, et al. 2006). The results of this study final showed that although LMWOA had mild effects on the heavy metals removal when compared with synthetic chelators, their advantages of being cheaper and safe in phytoremediation practices, could be tried in further studies.

Yang, et al. (2004) analyze the growth response and Cd uptake, distribution, and accumulation at varied Cd supply levels in *S. alfredii* in nutrient solution; these authors found that concentrations of other essential mineral nutrients in the leaves, stems and roots of this plant species significantly influenced by Cd treatments. Particularly, Mn concentration decrease when plants grown at Cd concentrations $\leq$ 0.4mM/L. for Ca, its concentration in leaves increased as Cd supply was up to 0.4mM/L. Concentrations of Mg decreased in the roots, but was not affected in leaves and stems of *S. alfredii*; with increasing concentration of Cd up to 0.4 mM/L. Concentration of Cu dramatically raised in the roots while decreased in the leaves at the external Cd higher than 0.5 mM/L. In this plant species, the root Fe concentration increased over 2-fold with increasing Cd levels. Zinc concentrations increased in the leaves but decreased in the roots by more than two times with increasing Cd supply levels up to 0.4 mM/L; whereas stem Zn concentrations remained unchanged for different Cd levels. The authors also report a remarkable decrease for Ca in the leaves and those of Mg, Cu, and Mn in the roots only at high Cd concentrations as 0.8 mM/L and conclude that tolerance of *S. alfredii* is closely associated with its exceptional ability of maintaining balanced nutrition of essential elements in the plant. It is known by experimental reports that Cd is highly toxic to plants, where the critical concentration of Cd in nutrient solution for conventional crop plants are reported to be 0.8 mM/L for white clover and maize, 0.014 mM/L for cabbage and 0.28 mM/L for ryegrass (Yang, et al. 1995). For *S. alfredii*, there were no visible symptoms in plants regarding to some kind of metal-induced toxicity, because the authors founded no reduced shoot and root dry matter yields in plants grown at concentration of Cd up to 0.2 mM/L. Figure 9 shows that the growth of *S. praealtum* plants in this study presented a particularly response regarding to the promotion of development in plants, according to each experimental conditions the effect of Cd and the amendments significantly reduce the growth of leaves in 6% for Cd, 8% and 14% for Cd+OX and Cd+EDTA, respectively and OX condition with 136%, compared to control plants. For stems of this plant species their growth diminished in experimental conditions of As, plus amendments employed with 56% for this metal, 54% for As+OX and 40% for As+EDTA. There was a promotion of root growth of this plant species with an increase from 3 to 9-fold in experiments with the presence of Cd as follows: Cd+EDTA (882%) > Cd+OX (587%) > Cd (343%), all the experimental conditions compared to the control plants.

Thus, in this plant species compared to *S. alfredii* reports, the effect of both metals showed an increase in plants growth, particularly in the stems and roots development.

Welch and Norvell (1999) mention that it is generally knowing that Cd uptake by non-accumulator plants may through the same carrier as for other divalent cations such as Zn^{2+}, Cu^{2+} or Fe^{2+}, or via cation channels of Ca^{2+} and Mg^{2+} and inhibitory effects of Cd on the uptake and accumulation of Zn, Cu, Mn, and Ca were noted in conventional crop plants as Wong, et al. (1984) and Yang, et al. (1996) noted. Finally, Yang, et al. (2004) reported as a conclusion, that according to the Cd concentration tested; at ≤ 0.4 mM/L of Cd concentration in mineral medium, the concentrations of Mn and Cu in leaves and Mg and Zn in roots considerably decreased; while concentrations of Ca and Zn in leaves and Fe and Cu in roots significantly increased as Cd concentration increase.

In this study, *S. praealtum* plants at the concentrations tested for As and Cd; the nutrient elements: Ca and Mn accumulated in leaves, Fe, Cu and Mo accumulated in roots and for Mg and Zn concentrations these were almost equally distributed in leaves, stems and roots of this plant species. Some of these results were similar to those obtained by Liu, et al. (2008) and Yang, et al. (2004) for this species of the *Sedum* genera tested, showing that this plant species at these concentrations of the metals tested demonstrate a particularly response regarding to the ionomic interactions between nutrient elements.

CONCLUSIONS

Finally, in this study, even *S. praealtum* plants diminished their growth in presence of both heavy metals; ionomic profiles obtained as response of exogenous addition of As and Cd and the addition of both chelating agents was efficiently, increasing the bioavailability of some elements, showing a synergistic activity with Mo, Cu, Fe and Zn.

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REFERENCES

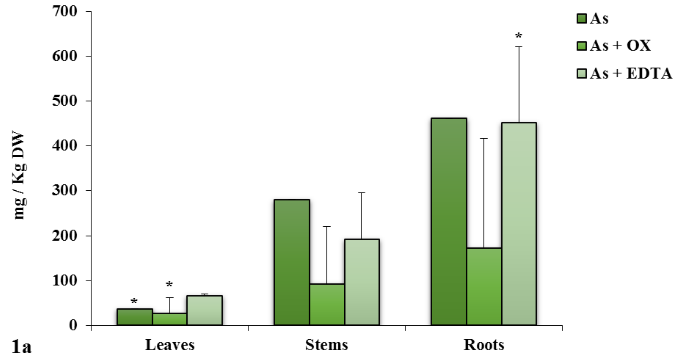
1. Baxter, I. (2015). “Should we treat the ionome as a combination of individual elements: or should we be deriving novel combined traits?”. Journal of Experimental Botany. **66:** 2127-2131. https://doi.org/10.1093/jxb/erv040
2. Baxter, I., C. Hermans, B. Lahner, E. Yakubova, M. Tikhonova, N. Verbruggen, D.Y. Chao, D.E. Salt. (2012). “Biodiversity of mineral nutrient and trace element accumulation in *Arabidopsis thaliana*”. PLoS One **7:** 35121. https://doi.org/10.1371/journal.pone.0035121
3. Bell, P.F., R.L. Chaney, J.S. Angle. (1991). “Free metal activity and total metal concentrations as indices of micronutrient availability to barley (*Hordeum vulgare* (L.) cv. ‘Klages’)”. Plant and Soil. **130:** 51-62. https://doi.org/10.1007/BF00011855
4. Berdnowack, R. and B. Robinson. (2006). “Critical assessment of chelant-enhanced metal phytoextraction”. Environmental Science & Technology.**40:** 5225-5232. https://doi.org/10.1021/es0604919
5. Bingham, F.T., J.E. Strong, G. Sposito. (1983). “Influence of chloride salinity on cadmium uptake by Swiss chard”. Soil Science. **135:**160-165. https://doi.org/10.1097/00010694-198303000-00005
6. Blaylock, M.J., D.E. Salt, S. Dushenkov, O. Zakharova, C. Gussman, Y. Kapulnik, B.D. Ensley, I. Raskin. (1997). “Enhanced accumulation of Pb in Indian mustard by soil applied chelating agents”. Environmental Science and Technology. **31:** 860-865. https://doi.org/10.1021/es960552a
7. Cabello-Conejo, M.I., Á. Prieto-Fernández, P.S. Kidd. (2014). “Exogenous treatments with phytohormones can improve growth and nickel yield of hyperaccumulating plants”. Science of the Total Environment.**1-8:** 494-495.
8. Cabrera, D., S.D. Young, D.L. Rowell. (1988). “The toxicity of cadmium to barley plants as affected by complex formation with humic acid”. Plant and Soil. **105:** 195-204. https://doi.org/10.1007/BF02376783
9. Cooper, E.M., J.T. Sims, S.D. Cunningham, J.W. Huang, W.R. Berti. (1999). “Chelate-assisted phytoextraction of lead from contaminated soils”. Journal of Environmental Quality. **28:** 1709-1719. https://doi.org/10.2134/jeq1999.00472425002800060004x
10. Chaney, R.L. 1988. Metal speciation and interactions among elements affect trace element transfer in agricultural and environmental food-chains, in: Kramer, J.R, H.E. Allen (Eds.), Metal Speciation: Theory, Analysis and Applications, Lewis Publishers, Chelsea, MI. pp. 218-260.

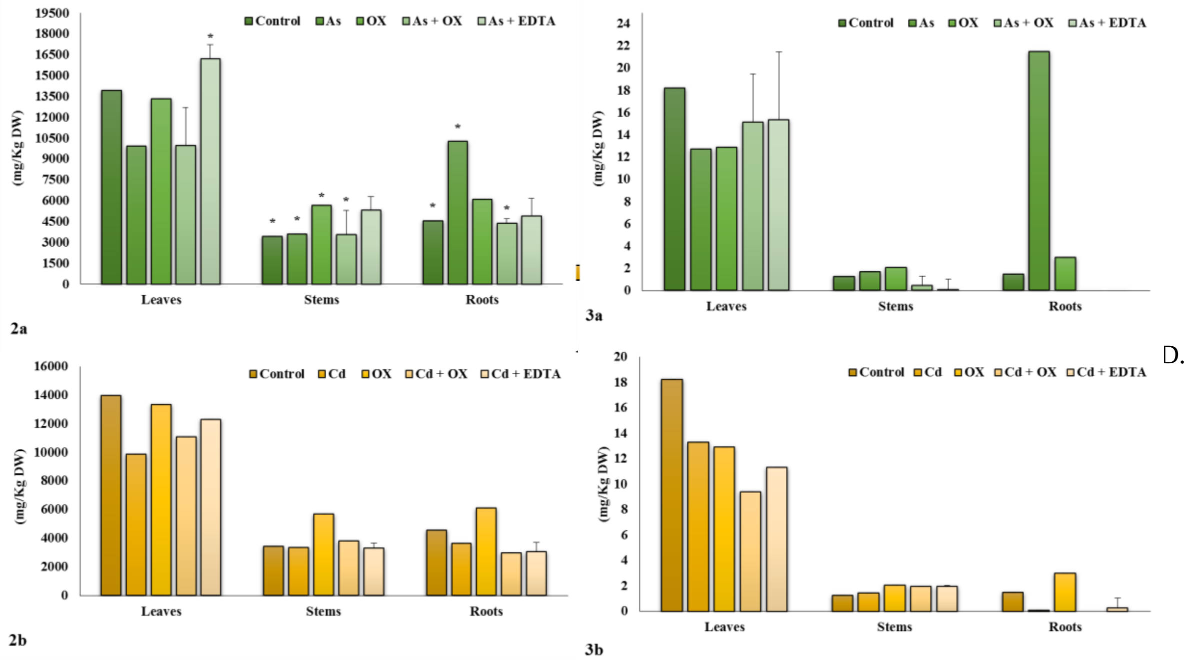
11. Checkai, R.T., R.B. Corey, P.A. Helmke. (1987). "Effects of ionic and complexed metal concentrations on plant uptake of cadmium and micronutrient metals from solution". Plant and Soil. **99:** 335-345. https://doi.org/10.1007/BF02370879
12. Chen, J.G., Chao, D.Y. 2014. Advances in mineral element interactions and heavy metal pollution. Plant Physiol. J. 50: 585-590.
13. Chu, Q.N., T. Watanabe, Z.M. Sha, M. Osaki, T. Shinano. (2015). "Interactions between Cs Sr, and other nutrients and trace element accumulation in amaranthus shoot in response to variety effect". Journal of Agricultural and Food Chemistry. **63:** 2355-2363. https://doi.org/10.1021/jf5058777
14. Clemens, S. 2006. "Toxic metal accumulation, responses to exposure and mechanisms of tolerance in plants". Biochimie. **88:**1707-1719. https://doi.org/10.1016/j.biochi.2006.07.003
15. Collins, R.N., B.C. Onisko, M.L. McLaughlin, G. Merrington. (2001). "Determination of metal–EDTA complexes in soil solution and plant xylem by ion chromatography electrospray mass spectrometry". Environmental Science and Technology. **35:** 2589-2593. https://doi.org/10.1021/es001893y
16. Collins, R.N., G. Merrington, M.J. McLaughlin, C. Knudsen. (2002). "Uptake of intact zinc-ethylenediamine tetraacetic acid from soil is dependent on plant species and complex concentration". Environmental Toxicology and Chemistry. **21:**1940-1945.
17. do Nascimento, C.W.A., D. Amarasiriwardena, B.S. Xing. (2006). "Comparison of natural organic acids and synthetic chelates at enhancing phytoextraction of metals from a multi-metal contaminated soil". Environmental Pollution. **140:** 114-123. https://doi.org/10.1016/j.envpol.2005.06.017
18. Eide, D.J., S. Clark, T.M. Nair, M. Gehl, M. Gribskov, M. Guerinot, M. Lou, J.F. Harper. (2005). "Characterization of the yeast ionome: a genome-wide analysis of nutrient mineral and trace element homeostasis in *Saccharomyces cerevisiae*". Genome Biology. **6:** R77. https://doi.org/10.1186/gb-2005-6-9-r77
19. Evangelou, M.W.H., M. Ebel, A. Schaeffer. (2006). "Evaluation of the effect of small organic acids on phytoextraction of Cu and Pb from soil with tobacco *Nicotiana tabacum*". Chemosphere. **63:** 996-1004. https://doi.org/10.1016/j.chemosphere.2005.08.042
20. Feng, X., L. Hana, D. Chao, Y. Liu, Y. Zhang, R. Wang, J. Guo, R. Feng, Y. Xua, Y. Ding, B. Huang, G. Zhang. (2017). "Ionomic and transcriptomic analysis provides new insight into the distribution and transport of cadmium and arsenic in rice". Journal of Hazardous Materials. **331:** 246-256. https://doi.org/10.1016/j.jhazmat.2017.02.041
21. Guerrero-Zúñiga, L.A., A. Rodríguez-Dorantes. (2013). "Release of metabolites and enzymatic activity as physiological response to cadmium by *Sedum praealtum* roots inoculated with a siderophore producing bacteria". Annals of Biological Research. **4:** 39-47.
22. Huang, J.W., J.J. Chen, W.R. Berti, S.D. Cunningham. (1997). "Phytoremediation of lead-contaminated soils: role of synthetic chelates in lead phytoextraction". Environmental Science and Technology. **31:** 800-805. https://doi.org/10.1021/es9604828
23. Liu, D., E. Islam, T. Li, X. Yang, X. Jin, Q. Mahmooda. (2008). "Comparison of synthetic chelators and low molecular weight organic acids in enhancing phytoextraction of heavy metals by two ecotypes of *Sedum alfredii* Hance". Journal of Hazardous Materials. **153:** 114-122. https://doi.org/10.1016/j.jhazmat.2007.08.026
24. Lou, L.Q., Z.G. Shen, X.D. Li. (2004). "The copper tolerance mechanisms of *Elsholtzia haichowensis*, a plant from copper enriched soils". Environmental and Experimental Botany. **51:** 111-120. https://doi.org/10.1016/j.envexpbot.2003.08.002
25. Lyubenova, L., P. Pongrac, K. Vogel-Mikůs, G.K. Mezek, P. Vavpetič, N. Grlj, M. Regvar, P. Pelicon, P. Schroder. (2013). "The fate of arsenic cadmium and lead in *Typha latifolia*: a case study on the applicability of micro-PIXE in plant ionomics". Journal of Hazardous Materials. **248-249:** 371-378. https://doi.org/10.1016/j.jhazmat.2013.01.023
26. Marschner, P. (2011). Marschner's Mineral Nutrition of Higher Plants, third ed.London
27. McLaughlin, M.J., E. Smolders, R. Merckx, A. Maes. (1997). Plant uptake of Cd and Zn in chelator-bufered nutrient solution depends on ligand type, in: Ando, T. (Ed.) Plant Nutrition-for Sustainable Food Production and Environment. Kluwer Academic Publishers, Japan. pp. 113-118. https://doi.org/10.1007/978-94-009-0047-9 20
28. Mimmo, T., D. Del Buono, R. Terzano, N. Tomasi, G. Vigani, C. Crecchio, R. Pinton, G. Zocchi, S. Cesco. (2014). "Rhizospheric organic compounds in the soil microorganism-plant system: their role in iron availability". European Journal of Soil Science. **65:** 629-642. https://doi.org/10.1111/ejss.12158
29. Park, J.H., D. Lamb, Y. Paneerselvam, G. Choppala, N. Bolan, J.W. Chung. (2011). "Role of organic amendments on enhanced bioremediation of heavy metal(loid) contaminated soils". Journal of Hazardous Materials. **185:** 549-574. https://doi.org/10.1016/j.jhazmat.2010.09.082
30. Patterson, C.C. 1971. "Native copper, silver and gold accessible to early metallurgists". American Antiquities. **36:** 283-321. https://doi.org/10.2307/277716
31. Pavan, M.A., F.T. Bingham. (1982). "Toxicity of aluminum to coffee seedlings grown in nutrient solution". Soil Science Society of America Journal. **46:** 993-997. https://doi.org/10.2136/sssaj1982.03615995004600050021x
32. Pii, Y., S. Cesco, T. Mimmo. (2015a). "Shoot ionome to predict the synergism and antagonism between nutrients as affected by substrate and physiological status". Plant Physiology and Biochemistry. **94:** 48-56. https://doi.org/10.1016/j.plaphy.2015.05.002
33. Pii, Y., T. Mimmo, N. Tomasi, R. Terzano, S. Cesco, C. Crecchio. (2015b). "Microbial interactions in the rhizosphere: beneficial influences of plant growthpromoting rhizobacteria on nutrient acquisition process. A review". Biology and Fertility of Soils. **51:**403-415. https://doi.org/10.1007/s00374-015-0996-1
34. Quartacci, M.F., A. Argilla, A.J.M. Baker, F. Navari-Izzo. (2006). "Phytoextraction of metals from a multiply contaminated soil by *Indian mustard*". Chemosphere. **63:** 918-925. https://doi.org/10.1016/j.chemosphere.2005.09.051
35. Salt, D.E. (2004). "Update on plant ionomics". Plant Physiology. **136:** 2451-2456. https://doi.org/10.1104/pp.104.047753
36. Salt, D.E., I. Baxter, B. Lahner. (2008). "Ionomics and the study of the plant ionome". Annual Review of Plant Biology. **59:** 709-733. https://doi.org/10.1146/annurev.arplant.59.032607.092942
37. Sarret, G., J. Vangronsveld, A. Manceau, M. Musso, J. D'Haen, J.J. Menthonnex, J.L. Hazemann. (2001). "Accumulation forms of Zn and Pb in *Phaseolus vulgaris* in the presence and absence of EDTA". Environmental Science and Technology. **35:** 2854-2859. https://doi.org/10.1021/es000219d
38. Sauvé, S., N. Cook, W.H. Hendershot, M.B. McBride. (1996). "Linking plant tissue concentrations and soil copper pools in urban contaminated soils". Environmental Pollution. **94:**153-157. https://doi.org/10.1016/S0269-7491(96)00081-4
39. Schaider, L.A., D.R. Parker, D.L. Sedlak. (2006). "Uptake of EDTA-complexed Pb, Cd and Fe by solution and sand-cultured *Brassica juncea*". Plant and Soil. **286:** 377-391. https://doi.org/10.1007/s11104-006-9049-8
40. Shen, Z.G., X.D. Li, C.C. Wang, H.M. Chen, H. Chua. (2002). "Lead phytoextraction from contaminated soil with highbiomass plant species". Journal of Environmental Quality. **31:** 1893-1900. https://doi.org/10.2134/jeq2002.1893
41. Tandy, S., R. Schulin, B. Nowack. (2006a). "Uptake of metals during chelant-assisted phytoextraction with EDDS related to the solubilized metal concentration".

Environmental Science and Technology. **40:** 2753-2758. https://doi.org/10.1021/es052141c

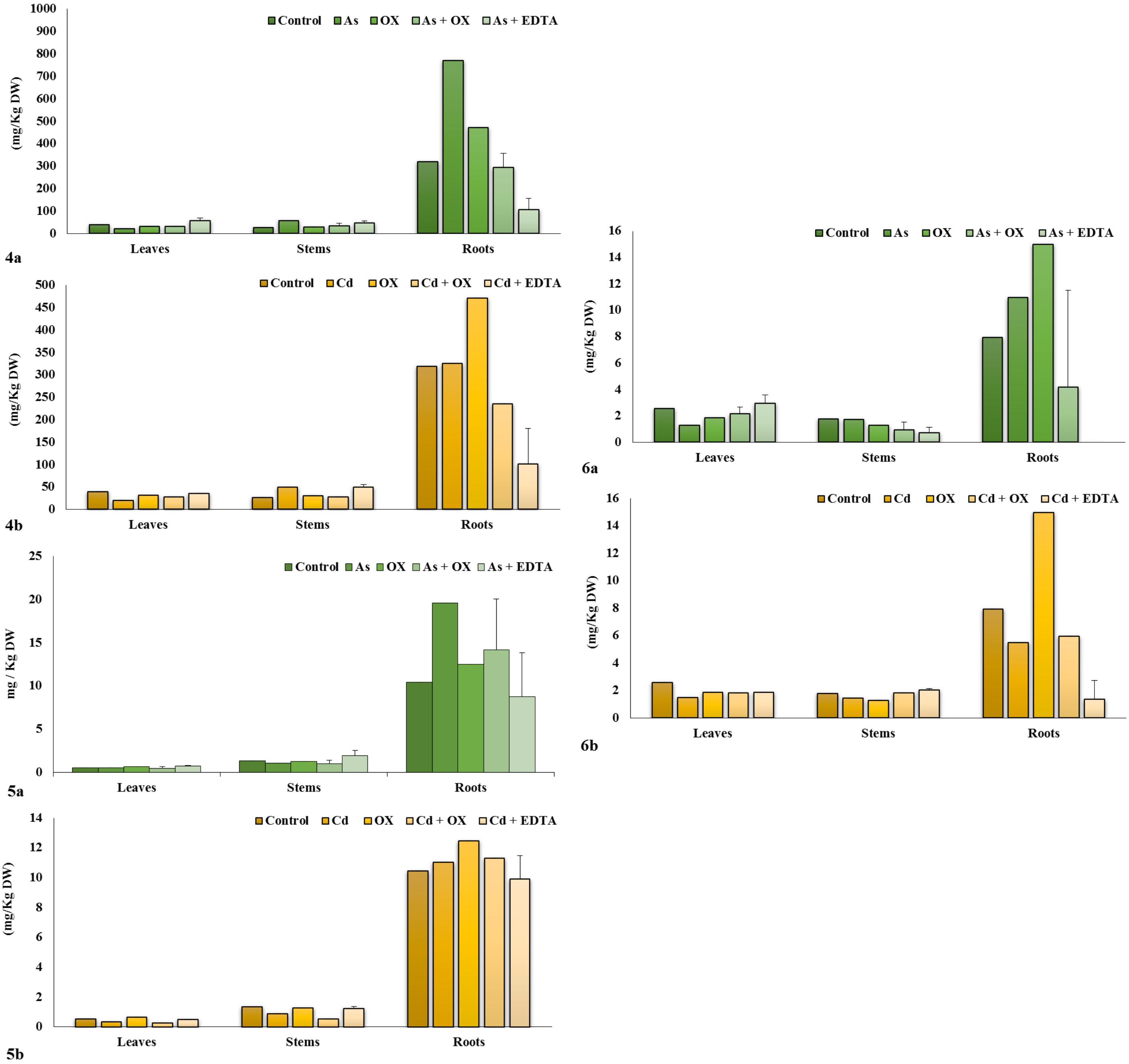
42. Tandy, S., R. Schulin, B. Nowack. (2006b). "The influence of EDDS on the uptake of heavy metals in hydroponically grown sunflowers". Chemosphere. **62:**1454-1463. https://doi.org/10.1016/j.chemosphere.2005.06.005
43. Turgut, C., M.K. Pepe, T.J. Cutright. (2005). "The effect of EDTA on *Helianthus annuus* uptake, selectivity, and translocation of heavy metals when grown in Ohio, New Mexico and Colombia soil". Chemosphere **58:** 1087-1095. https://doi.org/10.1016/j.chemosphere.2004.09.073
44. Vassil, A.D., Y. Kapulnik, I. Raskin, D.E. Salt. (1998). "The role of EDTA in lead transport and accumulation by Indian mustard". Plant Physiology. **117:** 447-453. https://doi.org/10.1104/pp.117.2.447
45. Verbruggen, N., C. Hermans, H. Schat. (2009). "Mechanisms to cope with arsenic or cadmium excess in plants". Current Opinion in Plant Biology. **12:** 364-372. https://doi.org/10.1016/j.pbi.2009.05.001
46. Wang, H., X. Shan, T. Liu, Y. Xie, B. Wen, Sh. Zhang, F. Han, M.Th. van Genuchten. (2007). "Organic acids enhance the uptake of lead by wheat roots". Planta. **225:**1483-1494. https://doi.org/10.1007/s00425-006-0433-7
47. Welch, R.M., W.A. Norvell. (1999). Mechanisms of cadmium uptake, translocation and deposition in plants, in: Cadmium in Soils and Plants. McLaughlin, M.J., B.R. Singh (Eds.) Kluwer Academic Publishers, Dordrecht. pp. 125-150. https://doi.org/10.1007/978-94-011-4473-5 6
48. Wenger, K., S.K. Gupta, G. Furrer, R. Schulin. (2003). "The role of nitrilotriacetate in copper uptake by tobacco". Journal of Environmental Quality. **32:** 1669-1676. https://doi.org/10.2134/jeq2003.1669
49. Williams, L., D.E. Salt. (2009). "The plant ionome coming into focus". Current Opinion in Plant Biology. **12:** 247-249. https://doi.org/10.1016/j.pbi.2009.05.009
50. Wong, M.K., G.K. Chuah, L.L. Koh, K.P. Ang, C.S. Hew. (1984). "The uptake of cadmium by *Brassica chinensis* and its effect on plant zinc and iron distribution". Environmental and Experimental Botany. **24:** 189-195. https://doi.org/10.1016/0098-8472(84)90021-2
51. Wu, J., F.C. Hsu, S.D. Cunningham. (1999). "Chelate-Assisted Pb phytoextraction: Pb availability, uptake and translocation constraints". Environmental Science and Technology. **33:** 1898-1904. https://doi.org/10.1021/es9809253
52. Wu, L.H., Y.M. Luo, X.R. Xing, P. Christie. (2004). "EDTA-enhanced phytoremediation of heavy metal contaminated soil with *Indian mustard* and associated potential leaching risk". Agriculture, Ecosystems & Environment. **102:** 307-318. https://doi.org/10.1016/j.agee.2003.09.002
53. Yang, X.E., V.C. Baligar, D.C. Martens, R.B. Clark. (1995). "Infux, transport, and accumulation of cadmium in plant species grown at different Cd^{2+} activities". Journal of Environmental Science and Health. B. **30:** 569-583. https://doi.org/10.1080/03601239509372954
54. Yang, X.E., V.C. Baligar, D.C. Martens, R.B. Clark. (1996). "Cadmium effects on influx and transport of mineral nutrients in plant species". Journal of Plant Nutrition. **19:** 643-656. https://doi.org/10.1080/01904169609365148
55. Yang, X.E., X.X. Long, H.B. Ye, Z.L. He, D.V. Calvert, P.J. Stoffella. (2004). "Cadmium tolerance and hyperaccumulation in a new Zn-hyperaccumulating plant species (*Sedum alfredii* Hance)". Plant and Soil. **259:** 181-189. https://doi.org/10.1023/B:PLSO.0000020956.24027.f2

FIGURES

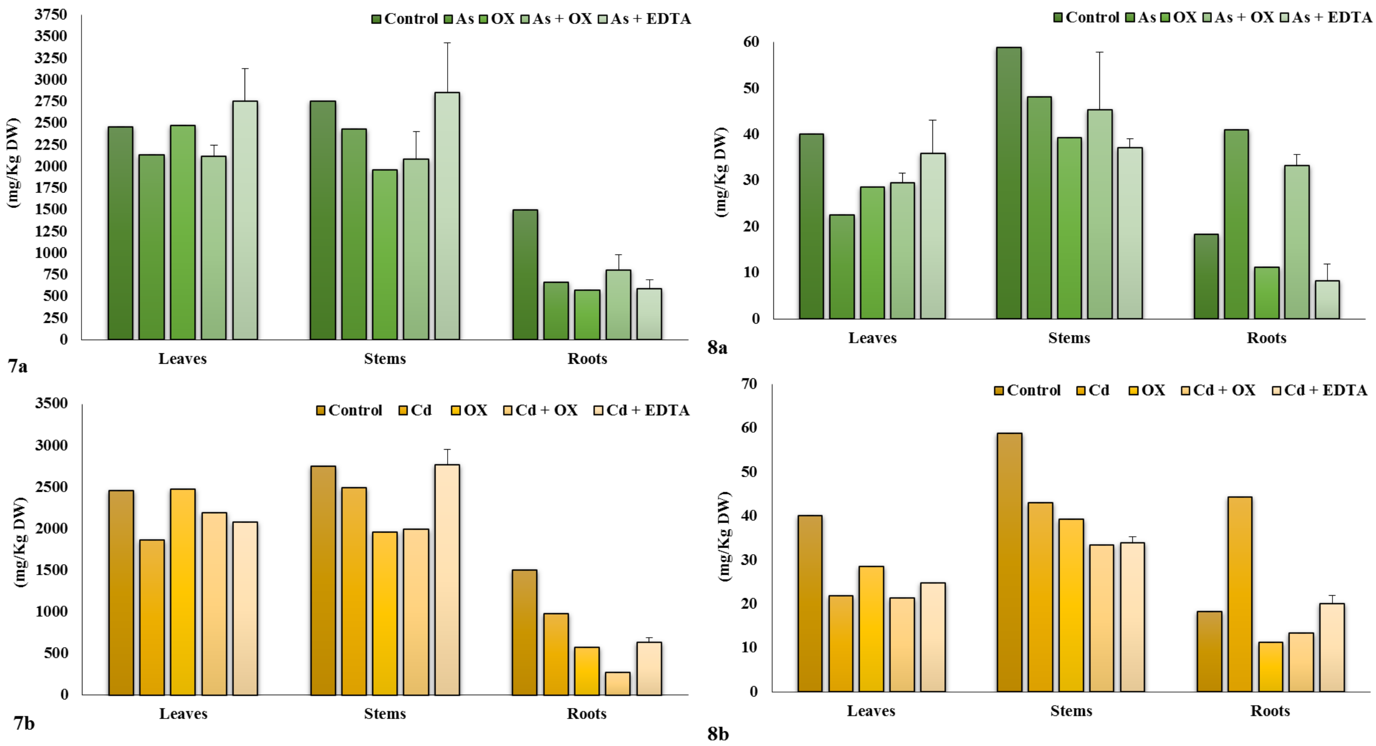




Figures 2 and 3: 2a and 2b) Calcium and 3a and 3b) Manganese concentrations in *Sedum praealtum* plants. Mean values + S.D. from six replicates. Asterisks showed the significant differences between treatments ($p < 0.05$).



Figures 4, 5 and 6: 4a and 4b) Iron, 5a and 5b) Molibdenum and 6a and 6b) Cupper concentrations in *Sedum praealtum* plants. Mean values + S.D. from six replicates. No significant differences were found between plant species treatments ($p > 0.05$).



Figures 7 and 8: 7a and 7b) Magnesium and 8a and 8b) Zinc concentrations in *Sedum praealtum* plants. Mean values + S.D. from six replicates. No significant differences were found between plant species treatments ($p > 0.05$).

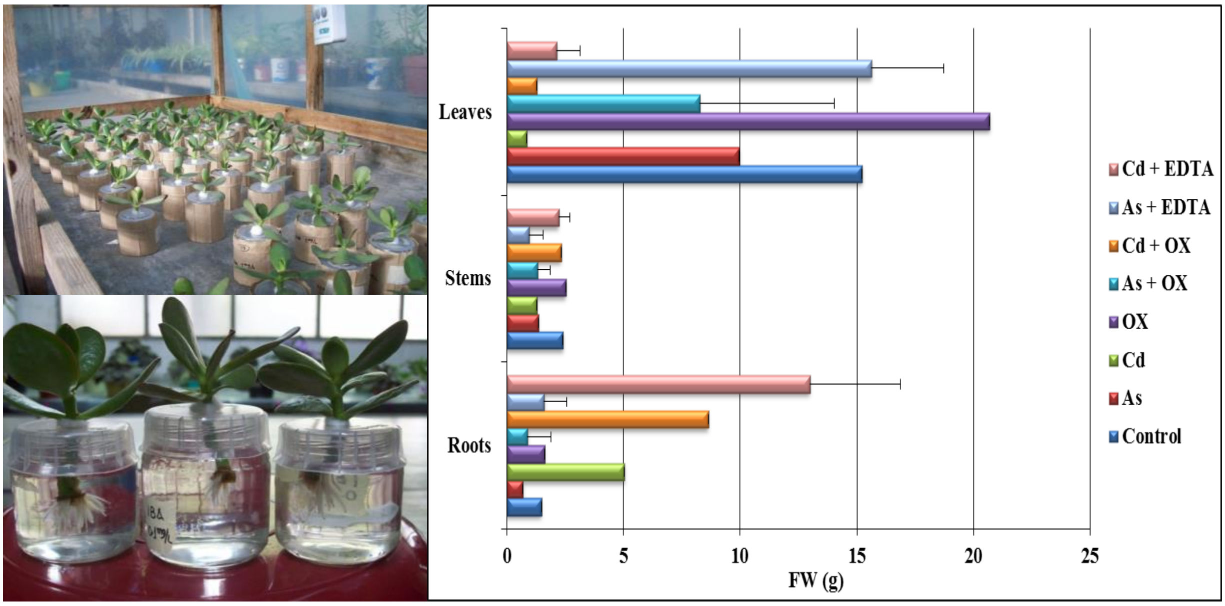


Figure 9: Growth of *Sedum praealtum* plants regarding to gain of fresh biomass in leaves, stems and roots. Mean values + S.D. from six replicates. No significant differences were found between plant species treatments ($p > 0.05$).

Table 1. Factor of Translocation (FT) of tested elements in *Sedum praealtum* plants in all the experimental conditions

	Element	C	OX	As	As + EDTA	As + OX	Cd	Cd + EDTA	Cd + OX
Immobilized (>1)	**Mn**	12.41	4.34	0.59			195.86	43.08	
	Ca	3.05	2.18	0.96	3.29	2.28	2.7	4	3.69
	Mg	1.63	4.31	3.23	4.7	2.62	1.91	3.3	8.08
	Zn	2.19	2.54	0.55	4.31	0.88	0.49	1.24	1.59
Mobilized (<1)	**Cu**	0.32	0.12	0.11		0.51	0.26	1.36	0.3
	Fe	0.12	0.067	0.027	0.52	0.1	0.062	0.35	0.11
	Mo	0.049	0.051	0.026	0.082	0.032	0.031	0.05	0.024
	As			0.079	0.14	0.14			
	Cd						0.013	0.17	0.05

TABLES

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