

Succulent species differ substantially in their tolerance and phytoextraction potential when grown in the presence of Cd, Cr, Cu, Mn, Ni, Pb and Zn

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**Abstract** Plants for the phytoextraction of heavy metals should have the ability to accumulate high concentrations of such metals, and exhibit multiple tolerance traits to cope with adverse conditions such as co-existence of multiple heavy metals, high salinity and drought which are the characteristics of many contaminated soils. This study compared 14 succulent species for their phytoextraction potential of Cd, Cr, Cu, Mn, Ni, Pb, and Zn. There were species variations in metal tolerance and accumulation. Among the 14 succulent species, an Australian native halophyte *Carpobrotus rossii* exhibited the highest relative growth rate (20.6–26.6 mg plant<sup>-1</sup> day<sup>-1</sup>) and highest tolerance index (78–93 %), whilst *Sedum* “Autumn Joy” had the lowest relative growth rate (8.3–13.6 mg plant<sup>-1</sup> day<sup>-1</sup>), and *Crassula multicava* showed the lowest tolerance indices (<50%). *Carpobrotus rossii* and *Crassula helmsii* showed higher potential for phytoextraction of these heavy metals than other species. These findings suggest that *C. rossii* is a promising candidate for phytoextraction of multiple heavy metals and the aquatic or semiterrestrial *C. helmsii* is suitable for phytoextraction of Cd and Zn from polluted waters or wetlands.

**Keywords:** Halophyte, Multiple heavy metals, Phytoremediation, Sedum, species variation, Tolerance

## Introduction

Some plant species (defined as hyperaccumulators) can take up and accumulate extraordinarily higher (10 - 100 times) concentrations of heavy metals in shoots than do most plants (Brooks et al. 1977), and this has led to the development of a cleanup technology termed phytoextraction (Chaney 1983). As an environment-friendly and cost-effective approach compared to chemical and physical techniques (Moffat 1995, Salt et al. 1995a), this phytoextraction approach has attracted much

attention in remediation research of soils contaminated with heavy metals. Selection of suitable plant species is the most important step in the development of a phytoextraction approach.

Most heavy-metal-contaminated sites are characterized by high concentrations of multiple heavy metals (Baun & Christensen 2004, Moffat 1995), especially landfills typically polluted with common heavy metals like Cd, Cr, Cu, Mn, Ni, Pb and Zn. Although hyperaccumulators can accumulate high amounts of heavy metals in shoots, most hyperaccumulators are not effective for practical phytoextraction applications due to their specificity to a particular metal, low biomass production and their requirement for specific management under field conditions (Gleba et al. 1999, Hassan & Aarts 2011). Recently, much interest has been focused on the utilization of crop plants capable of producing high biomass with high concentrations of heavy metals in shoots (Doty 2008) to improve phytoextraction efficiency. Indian mustard (*Brassica juncea*) has been considered as one such candidate species for phytoextraction (Belimov et al. 2005, Ebbs et al. 1997, Kochian & Ebbs 1998) because it is reasonably tolerant to and accumulate multiple heavy metals (Blaylock et al. 1997, Quartacci et al. 2006, Salt et al. 1995a) including Pb in shoots (Kumar et al. 1995) and produces high shoot biomass (Salt et al. 1995b, Singh et al. 2010). It is also tolerant to drought and salinity (Baudh & Singh 2012, Moffat 1995, Novo et al. 2014).

More recently, two succulent species of Crasulaceae have been identified as potential candidates for the phytoextraction of heavy metals. These are *Sedum alfredii* which is a Cd/Zn co-hyperaccumulator (Yang et al. 2004) and Pb accumulator, and *Sedum plumbizincicola* as a Zn hyperaccumulator (Wu et al. 2012). As many succulent plant species are tolerant to drought, they are likely to be promising candidates for phytoextraction of metals in dry polluted sites like rural landfills in Australia, which have regular dry periods. This present study selected seven *Sedum* species and investigated their tolerance and shoot uptake of heavy metals. In addition, another seven succulent species were selected and investigated on the basis of their apparent metal tolerance (Baker 1984). Thus 14 succulent species were exposed to multiple heavy metals (Cd, Cr, Cu, Mn, Ni, Pb, and Zn) at various concentrations. The objective was to assess their tolerance to these metals and to determine their ability to extract these metals from soil in their shoot.

## Materials and methods

### Soil

A silt loam soil was collected from the 0-25 cm surface layer at the agricultural reserve of La Trobe University. It contained 21.3% clay, 54.5% silt and 24.1% sand. The soil had 2.4% organic C, 2.75 g kg<sup>-1</sup> total N, 44 mg kg<sup>-1</sup> Colwell P, 126 mg kg<sup>-1</sup> Colwell K, 0.076 dS m<sup>-1</sup> electrical conductivity (1:5 water) and pH 5.41 (1:5 soil: 0.01 M CaCl<sub>2</sub>). The concentrations of total Cd, Cr, Cu, Mn, Ni, Pb, and Zn in the unamended soil were 0.55, 4.2, 18, 303, 5.0, 28, and 119 mg kg<sup>-1</sup>, respectively.

### Experiment design and treatments

The experiment was conducted using a completely randomized block design with four replicates. It consisted of 15 plant species and three levels of heavy metals. The three levels of heavy metals (Cd, Cr, Cu, Mn, Ni, Pb and Zn in a mixture) were selected based on the results of a preliminary experiment which grew geranium (*Pelargonium zonal*, tolerant) (Dan et al. 2002), radish (*Raphanus sativus*, sensitive) (Chaney 1983) and Indian mustard (*Brassica juncea*). These were T0 (without

addition of heavy metals, as control), T1 and T2 (addition of designed concentrations of heavy metals as shown in Table 1).

The 15 species include 14 succulent plant species, and geranium as a reference plant. Geranium showed some tolerance to the mixture of heavy metals in the preliminary experiment. The 14 succulent plant species belong to four families and six genera. The seven *Sedum* (Crasulaceae) species were collected from Melbourne Royal Botanic Garden, and these included *S. rubrotinctum*, *S. X Graptosedum* “Bert Swanwick”, *S. stahlii*, *S. mexicanum*, *S. sediforme*, *S. spectabile*, and *S. “Autumn Joy”*. Three *Crassula* species (Crasulaceae) (*C. ovate*, *C. helmsii* and *C. multicava*), one *Senecia* [*S. serpens* (Asteraceae)] and a *Portulacaria* [*P. afra* (Portulacaceae)] species were collected from residential gardens. Two species of Aizoaceae were *Carpobrotus rossii* collected from a rural landfill site (37°36'S, 143°35'E) and *Disphyma crassifolium* collected from a Melbourne beach.

### *Plant growth*

Uniform cuttings of each species were prepared for propagation in plastic nursery cells (5×5×8 cm) filled with the soil. Osmocote fertilizer (nutrient composition as N 15.3%, P 3.56%, K 12.6%, Scotts Australia Pty Ltd) was mixed with the soil at 10 g kg<sup>-1</sup> and an automatic water spray was used for irrigation. After one month, root systems of the cuttings were well developed and then transplanted into the experiment pots.

Each of experiment pots was prepared by mixing 1.5 kg soil in a plastic bag. Nutrients were added together with heavy metals at the desired concentrations. Copper and Mn were added as different metallic compounds (Table 1) to avoid excess of companion elements (Cl and S). All the chemicals were of analytical grade. Various amounts of KNO<sub>3</sub>, KCl, NH<sub>4</sub>Cl, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, K<sub>2</sub>SO<sub>4</sub>, and NH<sub>4</sub>NO<sub>3</sub> were added to balance the amounts of Cl, S, N, and K between the treatments with final additions of 160, 234, 144, and 253 mg kg<sup>-1</sup>, respectively. This was followed by incubation at 80% of field capacity in a constant-temperature room (25±0.2 °C) for two weeks, and during this period the soil was mixed daily.

Rooted cuttings of uniform size for each species were planted into the pots and the number of plant cuttings in each pot varied from 2 to 6 depending on plant size of the individual species. Plants were grown in a glasshouse with minimum and maximum temperatures of 19 and 33 °C, respectively. The pots were irrigated with deionized water to 80% of field capacity for 84 days.

### *Plant harvest*

After 84 days of growth, the plants were harvested. Shoots and roots were separated except for *C. helmsii* and *S. stahlii* which produced very fine roots. The shoots were first rinsed with running tap water, then immersed in 0.01 M HCl for approximately 5 s in order to remove dust from the shoot surface (Papazoglou 2011) and then washed with deionized water twice. After removing the soil particles, the roots were given the same washing procedure as the shoots.

### *Measurements*

Total root length was determined using a root scanner at 400 dpi (Epson Perfection 4990 Scanner, model J131B, Epson Inc.) with WinRhizo software (Regent Instruments Inc.). Shoots and roots were placed in paper bags and oven-dried at 80°C for 72 h. Dry weight was recorded. The dried plant samples were then ground to pass a 0.25-mm sieve.

Ground plant samples were digested in a mixture of concentrated HNO<sub>3</sub> and HClO<sub>4</sub> (4:1 by volume) (Monsant et al. 2008). The samples were then diluted to 25 mL using 5% HCl for further analysis. Metal concentrations in digests were determined using inductively coupled plasma–optical emission spectrometry (ICP-OES) analysis (Varian Vista AX CCD, Australia Pty Ltd.). For quality control, three reference samples and three blanks were used for every batch.

#### *Assessment of tolerance and accumulation and statistical analysis*

The relative growth rate (RGR) (mg plant<sup>-1</sup> day<sup>-1</sup>) was calculated as

$$RGR = 1000 \times \frac{\ln W_2 - \ln W_1}{t_2 - t_1}$$

where W<sub>1</sub> and W<sub>2</sub> are the estimated initial (t<sub>1</sub>) (an average biomass of 5-10 cuttings per species at the beginning of the experiment) and final (t<sub>2</sub>) shoot biomass (g) per plant, respectively.

The concentrations of individual heavy metals were compared between the species using one-way analysis of variance (ANOVA) using SPSS (version 18.0, Chicago, Illinois, USA). To assess the phytoextraction potential of these plant species, two indexes were calculated (Fitz & Wenzel 2002, Yoon et al. 2006).

$$\text{Bioaccumulation factor (BF)} = \frac{\text{Metal concentration in shoots}}{\text{Metal concentration in soil}}$$

$$\text{Translocation factor (TF)} = \frac{\text{Metal concentration in shoots}}{\text{Metal concentration in roots}}$$

The total phytoextraction potential of the chosen seven heavy metals was characterized by the following equation:

$$\text{Phytoextraction potential} = \sum_{i=1}^7 R_i$$

where i=1, 2, ..., 7, represent Cd, Cr, Cu, Mn, Ni, Pb and Zn, respectively; R<sub>i</sub> represents the ranking value of one species for each heavy metal.

## **Results**

### *Biomass production*

There was a large variation in growth parameters between the species grown in the control soil. Dry shoot weights ranged from 0.04 to 1.05 g plant<sup>-1</sup> at the commencement of treatment, and from 0.6 to 6.4 g plant<sup>-1</sup> at harvest. The relative growth rate (RGR) of shoots ranged from 13.6 to 37.7 mg plant<sup>-1</sup> day<sup>-1</sup> during the experimental period (Table 2). Exposure to multiple heavy metals decreased shoot biomass and RGR, depending on species and the level of treatment (Table 2). At the highest level of treatment (T2), *C. helmsii*, *D. crassifolium*, *C. rossii* and *S. mexicanum* had higher RGR than other species, within a range of 20.6-24.6 mg plant<sup>-1</sup> day<sup>-1</sup>, while *Sedum* “Autumn Joy” had the lowest RGR.

Exposure to multiple heavy metals also decreased root biomass production of all species and root length of selected species, except for *C. rossii* at the low level of heavy metals (T1). Compared to the control (T0), root biomass and length of *C. rossii* grown in T1 treatment increased by 31% and 17%, respectively (Table 3), but in T2 treatment, decreased by 15% and 18 %, respectively.

Among the 14 succulent species grown in T2 treatment, *C. rossii* and *D. crassifolium* (24%) exhibited the lowest decrease in root biomass and length whilst *C. multicava* and *C. ovate* showed the great decrease in root biomass, i.e. 81% and 76%, respectively.

#### *Tolerance to multiple heavy metals*

Overall, *C. helmsii*, *C. rossii*, *S. mexicanum*, *S. Graptosedum*, and *D. crassifolium* showed a higher tolerance to Cd, Cr, Cu, Mn, Ni, Pb and Zn than the other species studied, with tolerance indices ranging from 88 to 93% in T1 treatment and from 64 to 78% in T2 treatment (Fig. 1). *Carpobrotus rossii* had the highest tolerance indices, i.e. 93% and 78% in T1 and T2 treatments, respectively, whilst *C. multicava* showed the lowest tolerance indices, less than 50%. However, the tolerance rankings of some species were inconsistent between T1 and T2 treatments.

In the T1 treatment, there were seven species with significantly less tolerance than the reference species, and others had a similar tolerance to the reference species (Fig. 1). In the T2 treatment, only four species, namely *S. sediforme*, *S. stahluii*, *S. spectabile*, and *C. multicava* showed significantly less tolerance than the reference species (Fig. 1).

#### *Concentrations of heavy metals in shoots and roots*

The concentrations of heavy metals in shoots (Fig. 2) and roots (Fig. 3) showed a substantial species variation in the T1 and T2 treatments. The highest concentration of each metal varied with species, i.e. Cd: 143  $\mu\text{g g}^{-1}$  in shoots of *C. helmsii* and 571  $\mu\text{g g}^{-1}$  in roots of *S. rubroinctum*; Cr: 3.0  $\mu\text{g g}^{-1}$  in shoots of *P. zonal* and 561  $\mu\text{g g}^{-1}$  in roots of *S. rubroinctum*; Cu: 30  $\mu\text{g g}^{-1}$  in shoots of *C. rossii* and 601  $\mu\text{g g}^{-1}$  in roots of *D. crassifolium*; Mn: 597  $\mu\text{g g}^{-1}$  in shoots of *D. crassifolium* and 1087  $\mu\text{g g}^{-1}$  in roots of *D. crassifolium*; Ni: 55  $\mu\text{g g}^{-1}$  in shoots of *C. rossii* and 213  $\mu\text{g g}^{-1}$  in roots of *C. multicava*; Pb: 35  $\mu\text{g g}^{-1}$  in shoots of *C. helmsii* and 714  $\mu\text{g g}^{-1}$  in roots of *C. multicava*; and Zn: 976  $\mu\text{g g}^{-1}$  in shoots of *C. helmsii* and 1544  $\mu\text{g g}^{-1}$  in roots of *S. rubroinctum*.

Concentrations of heavy metals in plants increased as their concentrations increased in the soil. An exception was that the Cu concentrations in the shoots of most species decreased with increasing the soil concentration of this metal (Fig. 2C) although the opposite was generally observed with the Cu concentrations in roots (Fig. 3C).

#### *Bioaccumulation factor*

The values for bioaccumulation factor (BF) showed a great variation between the heavy metals and the 14 succulent species grown in the two treatments (Fig. 4). Among the seven heavy metals, Cd and Zn had BF greater than 1.0 for most plant species, followed by Ni and Mn, while Cr, Cu and Pb exhibited the lowest BF's of less than 1.0 for all selected species in the two treatments. Irrespective of plant species, BF values generally decreased with increasing the concentration of heavy metals in soil.

In particular, *C. rossii*, which was identified as tolerant to heavy metals, had BF of 2.3-2.7 for Cd, 2.3-2.8 for Zn, 1.2-1.6 for Ni, 0.8-1.1 for Mn, 0.2-0.7 for Cu, 0.06-0.11 for Cr, and 0.02-0.03 for Pb. Similarly, *C. helmsii* had BF of 6.9-7.4 for Cd, 2.0-2.3 for Zn, 0.5-1.1 for Ni, 0.3-1.0 for Mn, 0.3-0.4 for Cu, 0.05-0.07 for Cr, and 0.04-0.11 for Pb (Fig. 4).

#### *Translocation factor*

The translocation factor (TF) values of seven heavy metals varied among the 14 succulent species (Figure 5). Similar to BF, the TF values of Cd, Mn and Zn were greater than 1.0 in more than half of the species grown in the T1 treatment, while TF values of the other four heavy metals were less than 1.0 in almost all of the species grown in the T1 and T2 treatments.

*Carpobrotus rossii* had TF of 0.23-0.29 for Cd, 1.57-1.66 for Zn, 1.79-2.56 for Mn, 0.35-0.53 for Ni, 0.12-0.35 for Cu, 0.06-0.07 for Cr, and 0.03-0.05 for Pb. Due to insufficient root samples, TF was not determined for *C. helmsii*.

#### *Shoot uptake of heavy metals and the phytoextraction potential*

Shoot uptake of heavy metals was a function of shoot biomass and concentration of heavy metals in shoots, and is related to phytoextraction efficiency. *Carpobrotus rossii* and *C. helmsii* had greater uptake of Cu, Mn and Zn than the other species (Fig. 6-C, D and G). *Carpobrotus rossii* showed the greatest uptake of Cr and Cu in the T1 treatment (Fig. 6-C and D) while *C. helmsii* had the highest uptake of Cd, Pb and Zn (Fig. 6-A, F and G).

The phytoextraction potential of the plants grown in the T1 and T2 treatments varied widely between the 14 succulent species (Fig. 7). The ranking pattern was similar between the two treatments. Obviously, *C. rossii* and *C. helmsii* had the highest phytoextraction potential whereas *C. multicava* had the lowest. *Carpobrotus rossii* exhibited the potential for phytoextraction as a result of its higher tolerance and biomass production as well as its higher concentrations of heavy metals than other species.

## **Discussion**

#### *Species variation in metal tolerance*

The 14 succulent species showed significant differences in shoot (RGR) and root growth in response to the two treatments (Tables 2-3). Their tolerance to the combination of seven heavy metals, as indicated by the corresponding tolerance indices also varied (Fig. 1). Moreover, the metal tolerance reflected co-tolerance to multiple heavy metals rather than single-metal tolerance. It should be noted that the metal concentrations in shoots (Fig. 2) exceeded the critical toxic levels of Cd ( $5-10 \mu\text{g g}^{-1}$ ) (White & Brown 2010) in all species, of Zn ( $300-600 \mu\text{g g}^{-1}$ ) (Long et al. 2003) in some species, and of Ni ( $25 \mu\text{g g}^{-1}$ ) (Uren 1992) in two species. The co-tolerance to multiple heavy metals has been shown in species (Baker et al. 1999) such as *Deschampsia cespitos* which is co-tolerant to Zn, Pb, Ni and Cu (Cox & Hutchinson 1979), *Silene vulgaris* - co-tolerant to Cu, Zn, Cd, Ni and Co (Schat & Vooijs 1997), and *Chloris barbata* - co-tolerant to Hg, Cd and Zn (Patra et al. 1994). The co-tolerance in plants may result from the sharing of transporters among these metals and their sharing of a similar fate in the plants (e.g. binding to cell walls) (Fernando et al. 2013, Guerinot 2000, Oomen et al. 2009, Thomine et al. 2000). But it is still unclear why these co-tolerant species still exhibit element-specific tolerances and differences in accumulation patterns. Further work is needed to elucidate whether the element-specific tolerance is related to some secondary metabolites in plants.

The four species with the highest shoot RGR, when grown in the two multi-metal treatments, were *C. helmsii*, *D. crassifolium*, *C. rossii* and *S. mexicanum* (Table 2). They also had higher tolerance indices than most of the other species studied. Additionally, it is interesting to note that

among these four species, only *C. rossii* showed significant increases in both root biomass and length in the T1 treatment (Table 3). The growth stimulus by heavy metals (Cox & Hutchinson 1980) has also been documented in many metal-tolerant species like Indian mustard, rapeseed, barley, and tumbleweed (Shi & Cai 2009 and literatures therein), and especially in hyperaccumulators like *Arabidopsis halleri* and *S. alfredii* (Hu et al. 2009, Tang et al. 2009, Yang et al. 2004, Zhao et al. 2000). In contrast, the root growth of other species generally decreased in the presence of heavy metals (Table 3), which was consistent with their shoot growth.

The reference plant *Pelagonium zonal* was a good indicator plant for distinguishing the metal tolerances among the 14 succulent species studied because it showed a moderate metal tolerance (Fig. 1). In previous studies, *P. zonal* showed high tolerance to Cd, Pb and Ni (Arshad et al. 2008, Dan 2001, Dan et al. 2002). For example, when grown in a solution culture, 8.9 mM Cd did not decrease the photosynthesis in *P. zonal* (Dan et al. 2000). The high tolerance of *P. zonal* has been attributed mainly to a low TF and to increased lignification in its tissues leading to an increase in the formation of metal-lignin complexes on cell walls (Dan et al. 2002).

Although the amount of Cd added to the soil was moderate, and the addition of other heavy metals exacerbated the metal toxicity effects, *P. zonal* still exhibited relatively higher metal tolerance (Fig. 1), which might be attributed to low translocation ability of these metals from roots to shoots ( $TF < 1$ ) (Fig. 5). The low translocation ability of *P. zonal* was also observed in other studies (Dan 2001, Dan et al. 2000, Dan et al. 2002, KrishnaRaj et al. 2000). By comparison with this reference plant grown in the T2 treatment, the 14 succulent species could be classified into three groups: 1) highly tolerant group with  $>78\%$  tolerance indices, *C. rossii* and *S. X Graptosedum*; 2) sensitive group with 39-45% tolerance indices, *S. stahlii*, *S. spectabile* and *C. multicava*; and 3) moderately tolerant group with 53-65% tolerance indices, the remaining species (Fig. 1B).

The species variation in metal tolerance could result from a number of different mechanisms. These include the metal detoxification by complexation of heavy metals with metabolites acting as high-affinity ligands. Other mechanisms involve compartmentalization in vacuoles or cell walls, their distribution to insensitive tissues (e.g. leaf epidermis) (Baker et al. 1999, Callahan et al. 2006), their exclusion from the roots, or their translocation to shoots (Baker 1981). In the present study, root and/or shoot growth of *C. helmsii*, *D. crassifolium*, *C. rossii*, *S. mexicanum*, *S. X Graptosedum*, *S. rubrotinctum*, and *S. stahlii* were not affected by the T1 treatment. Among these seven species, the tolerance of *S. X Graptosedum*, *C. rossii* and *D. crassifolium* was associated with both high translocation ability of metals (e.g. Cd, Mn and Zn) from roots to shoots, which was evidenced by TF values of  $>1$ , and high storage ability of metals (Cd, Cr, Cu, Ni and Pb) (Fig. 5). *Disphyma crassifolium* and *C. rossii* are from the same family Aizoaceae, and may share the same mechanisms of metal tolerance. It is interesting to note that *Sedum* species had higher TF values than most other species (Fig. 5), indicating that high translocation ability of heavy metals from roots to shoots plays an important role in their metal tolerance. However, the information on mechanisms of metal tolerance of individual species is limited.

#### *Species variation in metal extraction*

The present study showed that the 14 succulent species differed greatly in their ability to accumulate heavy metals. Interestingly, *C. rossii* exhibited not only high metal-tolerance (Fig. 1), but also had high concentrations and bioaccumulation factors for Cr, Cu, Mn, Ni, Pb and Zn in its

tissues (Figs. 2 and 3), indicative of the phytoextraction potential in soils contaminated with multiple heavy metals. However, due to competition of multiple heavy metals, the metal concentrations recorded in the present study are lower than those in plants with high accumulation capacity. Therefore, further experiments are needed to investigate tolerance and accumulation of the respective heavy metals in some promising species, especially *C. rossii*, and to elucidate the associated mechanisms.

In comparison, most *Sedum* species in the T1 treatment had higher concentrations of metals in shoots (Fig. 2), and higher bioaccumulation factors for Cd, Cu, Ni, Pb, and Zn (Fig. 4). However, these *Sedum* species are not suitable for phytoextraction of Cu and Pb or Cr and Mn due to bioaccumulation factors of less than 1 (Fig. 4). Plants with lower metal concentrations in shoots than that in soils are not useful for phytoextraction (Lottermoser et al. 2008). Additionally, compared to two identified *Sedum* Cd hyperaccumulators *S. alfredii* (Yang et al. 2004) and *S. plumbizincicola* (Wu et al. 2012), the *Sedum* species in the present study had much lower concentrations of Cd, Pb and Zn, which might partly result from the metal competition in the multiple heavy metal experiments. Similarly, *C. helmsii* has been reported as a Cu hyperaccumulator with Cu concentration of up to 9000  $\mu\text{g g}^{-1}$  in shoots at 10  $\mu\text{M Cu}^{2+}$  in a nutrient solution (Küpper et al. 2009) but the concentration of Cu in shoots ranged only from 15 to 30  $\mu\text{g g}^{-1}$  in the present study (Fig. 2), indicating that Cu uptake and accumulation may have been inhibited by the other heavy metals present. The reduced accumulation due to competition of multiple heavy metals was also reported with a Cu accumulator (up to 355  $\mu\text{g g}^{-1}$  in shoots) *Hirschfeldia incana* (L.) that did not have high Cu concentrations (7.5-12.2  $\mu\text{g g}^{-1}$ ) in contaminated soils containing 35-72, 190-2345, and 68-2602  $\text{mg kg}^{-1}$  of Cu, Pb and Zn, respectively (Gisbert et al. 2006). The competition of heavy metals has been attributed to their sharing metal transporters in plants (Guerinot 2000, Rivetta et al. 1997, Sasaki et al. 2012, Zornoza et al. 2010) or to non-specific metal uptake (Baker et al. 1999).

The accumulation of heavy metals in shoots was proportional to the species translocation factor (TF). In the present study, most *Sedum* species had higher translocation ability than the other species studied, with *Sedum* “Autumn Joy” having the highest TF values for all heavy metals (Fig. 5). The high translocation ability of heavy metals in *Sedum* species is consistent with other studies involving *S. alfredii* (Yang et al. 2004) and *S. plumbizincicola* (Wu et al. 2012). Thus, the high TF in *Sedum* species was responsible for the high concentrations of Cd, Cu, Ni, Pb and Zn in shoots relative to roots but these species had a low uptake and hence low phytoextraction potential due to their shoot biomass (Table 3) being lower than that of the remaining succulent species. In comparison, although the higher metal tolerant *C. rossii* had lower TF for Cd and Ni, intermediate TF for Cr and Zn, and higher TF only for Cu, Pb and Mn, the combination of higher shoot biomass and higher concentrations of heavy metals (except for Cd) made it the species with the highest shoot uptake of heavy metals (Fig. 6) and therefore the species with the highest phytoextraction potential which was similar to that of the *C. helmsii* (Fig. 7).

#### *Candidates for phytoextraction of multiple heavy metals*

Most contaminated soils and sites are characterized by contamination with multiple heavy metals, high salinity and deficiency of nutrients (Prasad et al. 2006). Therefore, potential plants used for phytoextraction should have at least five traits including (i) tolerance to multiple heavy



metals; (ii) fast growth and high biomass production; (iii) great accumulation of metals in harvestable parts; (iv) deep rooting, and (v) tolerance to soil constraints (Garbisu & Alkorta 2001, Lu et al. 2014). A high bioaccumulation factor is also important for plants used for phytoextraction (1998). Based on these requirements and realistic performance in the field, we consider that *C. rossii* and *C. helmsii* have phytoextraction potential for soils contaminated with multiple metals. Due to the presence of multiple heavy metals in the soil in the present study, further work is needed to investigate tolerance and accumulation of specific heavy metals by *C. rossii* and to study the associated mechanisms. Additionally, in consideration of both high salinity in most polluted sites and the halophytic nature of *C. rossii* (Pirie et al. 2013), experiments should be carried out to examine phytoextraction behaviour of this species at sites with high salinity. It is worth noting that *C. helmsii* is aquatic or has semiterrestrial traits, and thus may be suitable for remediation of polluted waters or wetlands.

## Conclusions

This study showed that *C. rossii* is a promising candidate for phytoextraction of multiple heavy metals in contaminated soils for a number of reasons. First, among the 14 succulent species, *C. rossii* had the highest relative growth rate and the highest tolerance index when grown in soil containing a mixture of multiple heavy metals. Second, *C. rossii* showed the second highest phytoextraction potential ranking just behind aquatic or semiterrestrial *C. helmsii* at the two levels of the mixture of seven heavy metals. The latter may be suitable for phytoextraction of Cd and Zn in polluted waters or wetlands.

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**Table 1** Added concentrations of heavy metals in three treatments (mg kg<sup>-1</sup> soil)

Element	Compound	T0 (control)	T1	T2
Cd	CdCl <sub>2</sub> •2.5H <sub>2</sub> O	0	10	20
Cr	K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub>	0	10	20
Cu	CuCl <sub>2</sub> •2H <sub>2</sub> O/ CuSO <sub>4</sub> •5H <sub>2</sub> O	0	37	74
Pb	Pb(NO <sub>3</sub> ) <sub>2</sub>	0	150	300
Mn	MnSO <sub>4</sub> •H <sub>2</sub> O/ MnCl <sub>2</sub> •4H <sub>2</sub> O	0	100	200
Ni	NiCl <sub>2</sub> •6H <sub>2</sub> O	0	15	30
Zn	ZnSO <sub>4</sub> •7H <sub>2</sub> O	0	150	300

**Table 2** Dry weights of initial cuttings and shoots at harvest, and shoot relative growth rate (RGR) of 14 succulent plant species and the reference plant *Pelargonium zonal* exposed for 84 days to multiple heavy metals (T0-control, T1-low, T2-high)

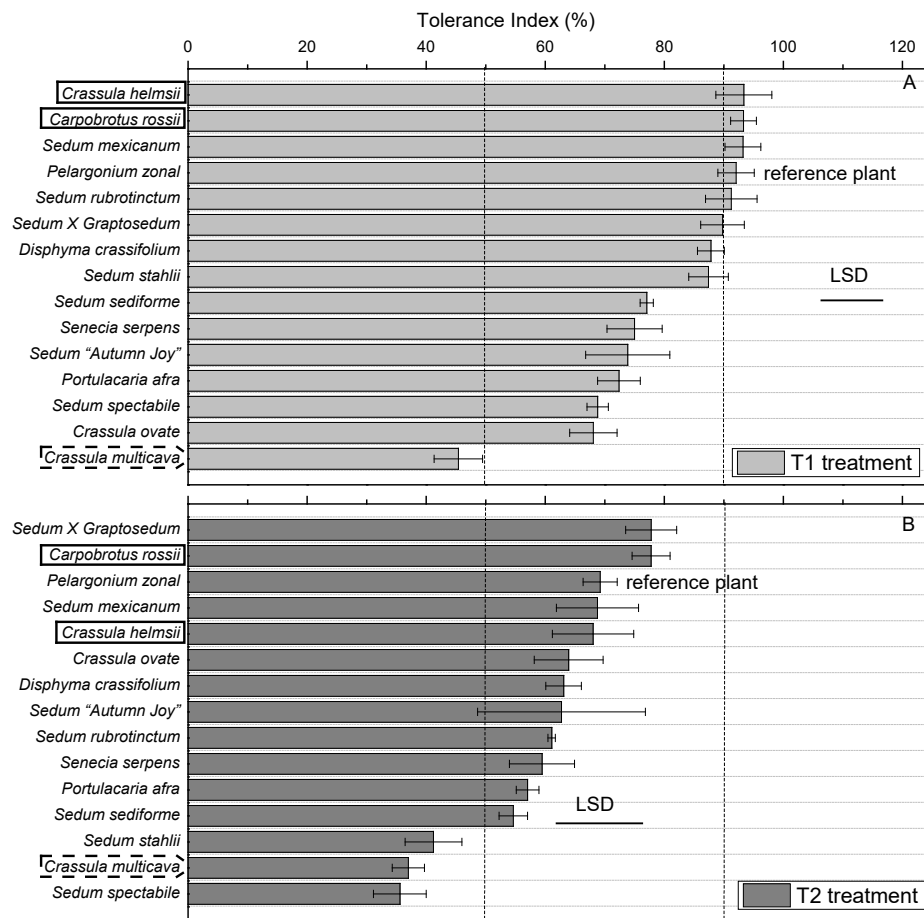
Species	Initial biomass (g plant <sup>-1</sup> )	Shoot biomass (g plant <sup>-1</sup> )				RGR (mg plant <sup>-1</sup> day <sup>-1</sup> )			
		T0	T1	T2	LSD	T0	T1	T2	LSD
<i>Crassula helmsii</i>	0.22	0.8	0.6	0.3	0.3	36.6	34.0	24.6	4.6
<i>Disphyma crassifolium</i>	0.04	5.3	3.6	1.7	0.5	37.7	33.1	23.7	2.6
<i>Carpobrotus rossii</i>	0.65	6.4	5.8	3.9	0.7	26.6	24.8	20.6	1.9
<i>Sedum mexicanum</i>	0.14	1.3	1.1	0.7	0.3	27.7	25.9	19.3	6.4
<i>Sedum X Graptosedum</i>	0.38	2.2	1.8	1.5	0.3	20.5	18.3	15.9	2.3
<i>Portulacaria afra</i>	0.18	1.7	0.9	0.6	0.1	26.4	19.0	15.0	1.6
<i>Pelargonium zonal</i>	1.05	5.1	4.5	3.0	0.6	20.7	19.1	14.4	3.0
<i>Sedum rubrotinctum</i>	0.28	2.1	1.8	1.0	0.3	23.4	21.4	14.3	2.3
<i>Crassula ovate</i>	0.45	2.9	1.6	1.5	0.5	21.3	14.5	13.6	2.3
<i>Sedum spectabile</i>	0.28	4.9	2.3	1.0	0.9	30.0	20.7	10.7	3.0
<i>Senecia serpens</i>	0.89	4.1	2.8	2.3	0.8	18.0	13.5	10.6	3.8
<i>Sedum sediforme</i>	0.82	3.6	2.5	1.8	0.4	17.7	13.6	9.7	1.2
<i>Crassula multicava</i>	0.34	3.0	0.9	0.8	0.4	25.9	11.9	9.6	3.6
<i>Sedum stahlii</i>	0.10	0.6	0.5	0.2	0.1	21.9	19.2	9.0	3.0
<i>Sedum "Autumn Joy"</i>	0.20	0.6	0.5	0.4	0.1	13.6	9.9	8.3	3.5
LSD (P=0.05)	0.06	0.5	0.4	0.3		2.5	2.7	3.4	

Note: Initial cutting biomass was calculated as the mean of 5 - 10 cuttings depending on species at the commencement of transplanting into treatments. The species highlighted in solid-line rectangles had the lowest reduction in RGR at T1 compared to at T0 and the species highlighted in a dash-line rectangle had the highest reduction in RGR at T1.

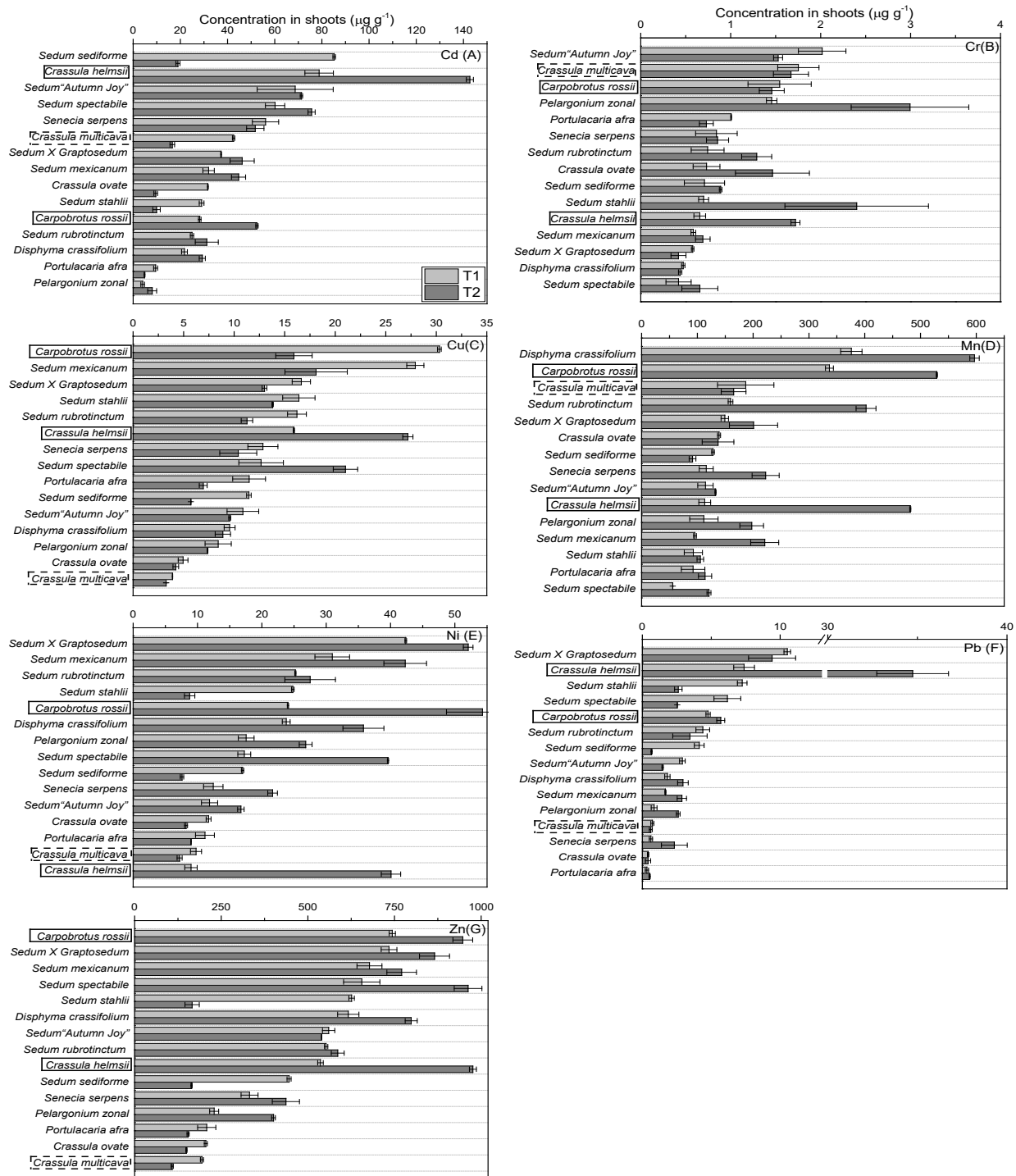
**Table 3** Root dry weights and length of succulent plant species and *Pelargonium zonal* exposed for 84 days to multiple heavy metals (T0-control, T1-low, T2-high). The values in parentheses are the percentage of root biomass or length in treatment relative to the control.

Species	Root biomass (g DW plant <sup>-1</sup> )				Root length (m plant <sup>-1</sup> )			
	T0	T1 (%)	T2 (%)	LSD	T0	T1 (%)	T2 (%)	LSD
<i>Carpobrotus rossii</i>	0.41	0.54(131)	0.34(82)	0.10	3.7	4.2(117)	3.1(85)	0.6
<i>Disphyma crassifolium</i>	0.38	0.36(96)	0.29(76)	0.07	3.3	2.2(67)	1.7(53)	0.6
<i>Sedum X Graptosedum</i>	0.32	0.30(94)	0.22(69)	0.27	0.7	0.6(94)	0.3(52)	0.4
<i>Sedum sediforme</i>	0.12	0.09(79)	0.06(54)	0.05				
<i>Portulacaria afra</i>	0.13	0.08(64)	0.07(50)	0.00				
<i>Senecia serpens</i>	0.31	0.21(69)	0.14(47)	0.13				
<i>Sedum rubrotinctum</i>	0.06	0.04(70)	0.03(41)	0.09	0.6	0.3(59)	0.2(41)	0.1
<i>Pelargonium zonal</i>	0.65	0.36(55)	0.26(40)	0.11	1.4	0.5(37)	0.4(26)	0.2
<i>Sedum "Autumn Joy"</i>	0.53	0.28(52)	0.15(28)	0.09				
<i>Crassula ovate</i>	0.22	0.12(52)	0.05(24)	0.05				
<i>Crassula multicava</i>	0.37	0.10(27)	0.07(19)	0.05				
<i>Sedum mexicanum</i>	0.14	0.08(60)	0.03(18)	0.00	1.2	1.0(85)	0.4(35)	0.2
<i>Sedum spectabile</i>	0.33	0.18(56)	0.03(10)	0.14				
<i>Sedum stahlii</i>	0.03	0.02(78)						
LSD (P=0.05)	0.13	0.07(43)	0.08(23)		0.5	0.3(21)	0.1(25)	

Note: The roots of *C. helmsii* were too fine to be collected and the roots of *S. stahlii* in T2 treatment were too small to be collected. The species highlighted in a solid-line rectangle had the lowest reduction in RGR at T1 compared to at T0, and the species highlighted in a dash-line rectangle had the highest reduction in RGR at T1.

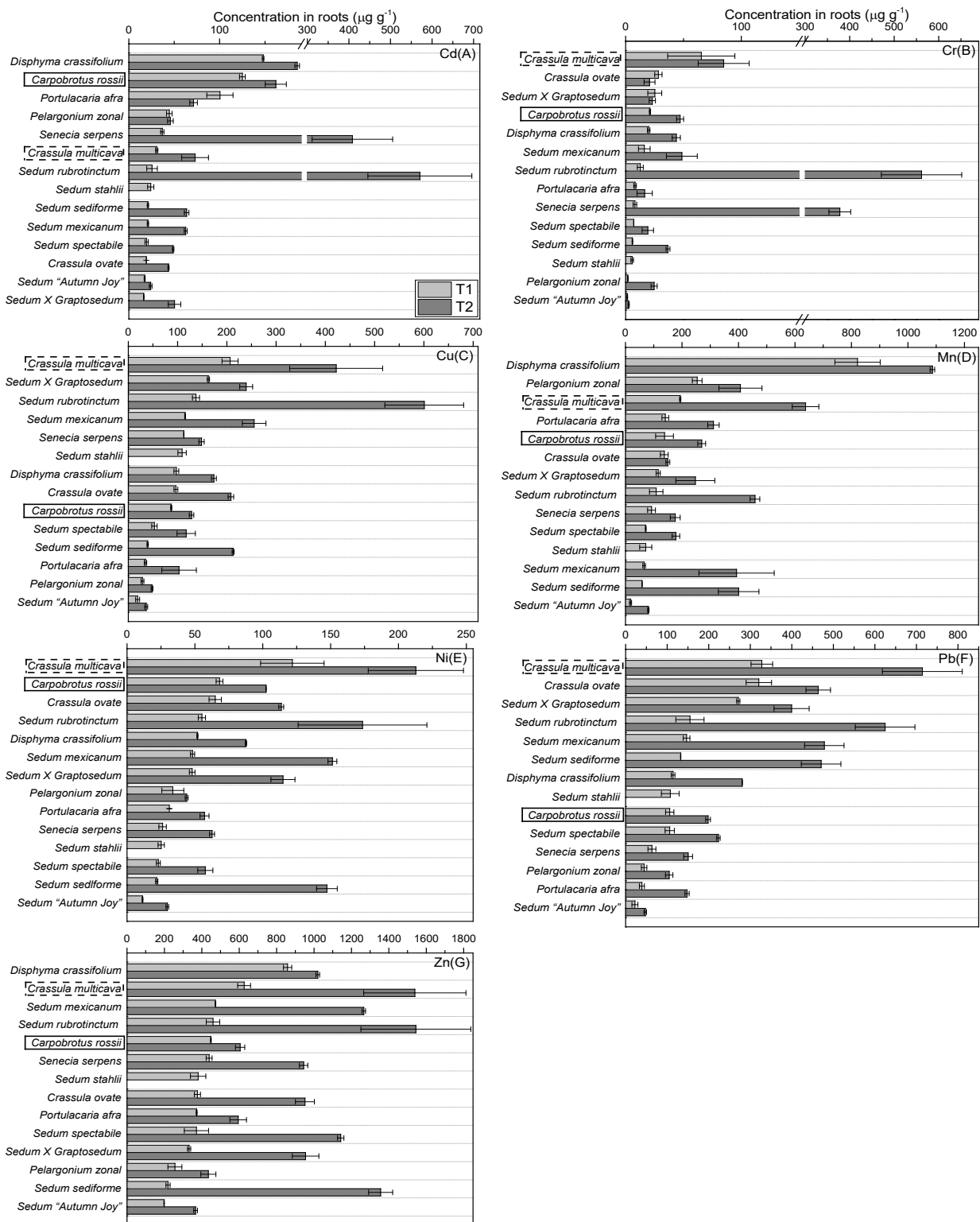


**Fig. 1** Tolerance indices of 14 succulent species and *Pelargonium zonal* exposed for 84 days to low (T1) (A) and high levels (T2) (B) of multiple heavy metals. Tolerance index = (shoot RGR in treatment/shoot RGR in control)  $\times$  100. Error bars represent  $\pm$  SE (n = 4). The LSD bars are also shown. The species highlighted in a solid-line rectangle had the lowest reduction in RGR at T1 compared to at T0, and the species highlighted in a dash-line rectangle had the highest reduction in RGR at T1. The dotted lines are based on 50% and 90% of the control (100%), respectively.

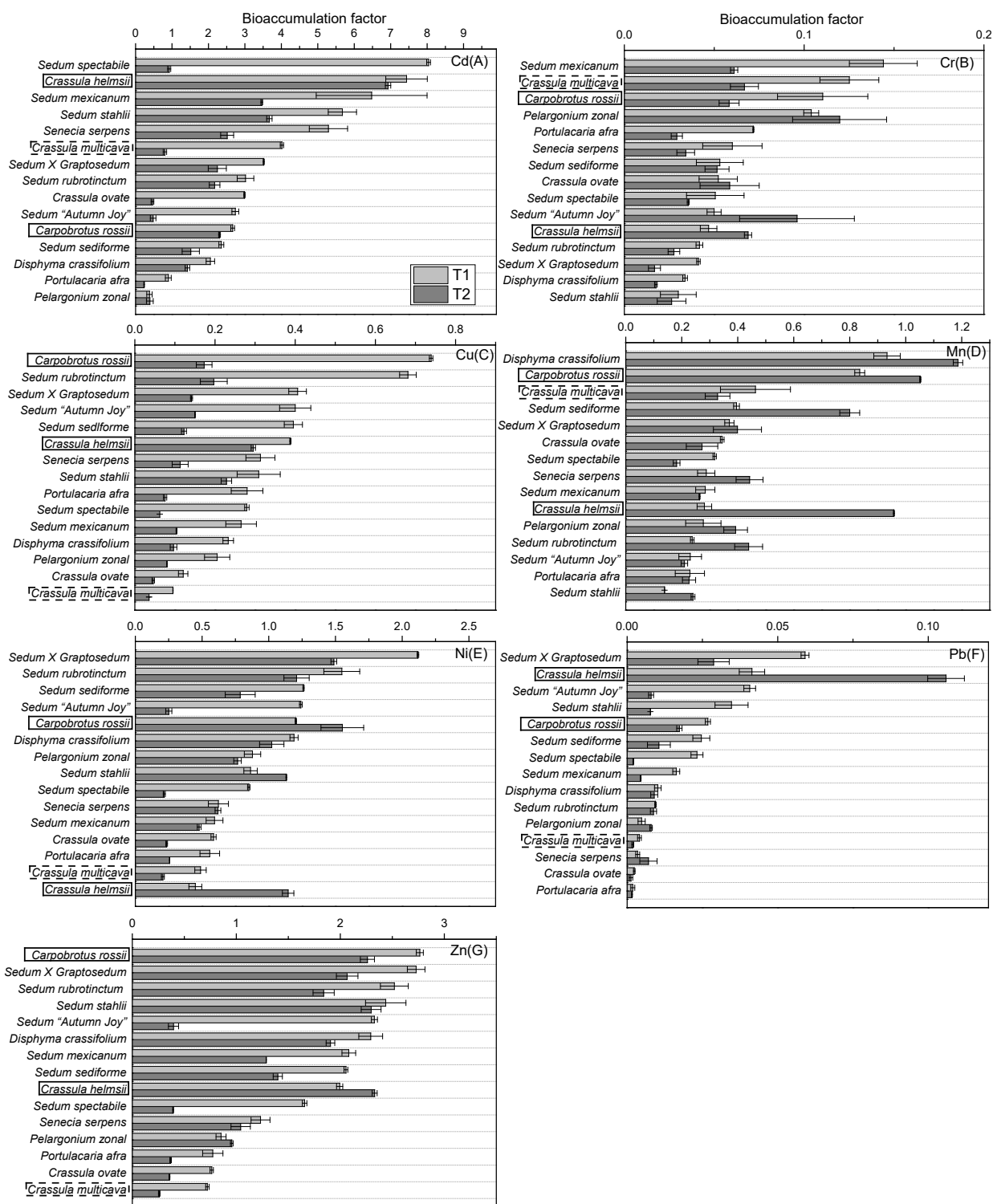


**Fig. 2** Concentrations of Cd (A), Cr (B), Cu (C), Mn (D), Ni (E), Pb (F) and Zn (G) in the shoots of succulent species and *Pelargonium zonal* exposed for 84 days to low (T1) and high (T2) concentrations of multiple heavy metals. Error bars represent  $\pm$  SE (n = 4). The species were ordered based on their concentrations at low level (T1). The species highlighted in a solid-line rectangle had the lowest reduction in RGR at T1 compared to at T0, and the species highlighted in a dash-line rectangle had the highest reduction in RGR at T1.

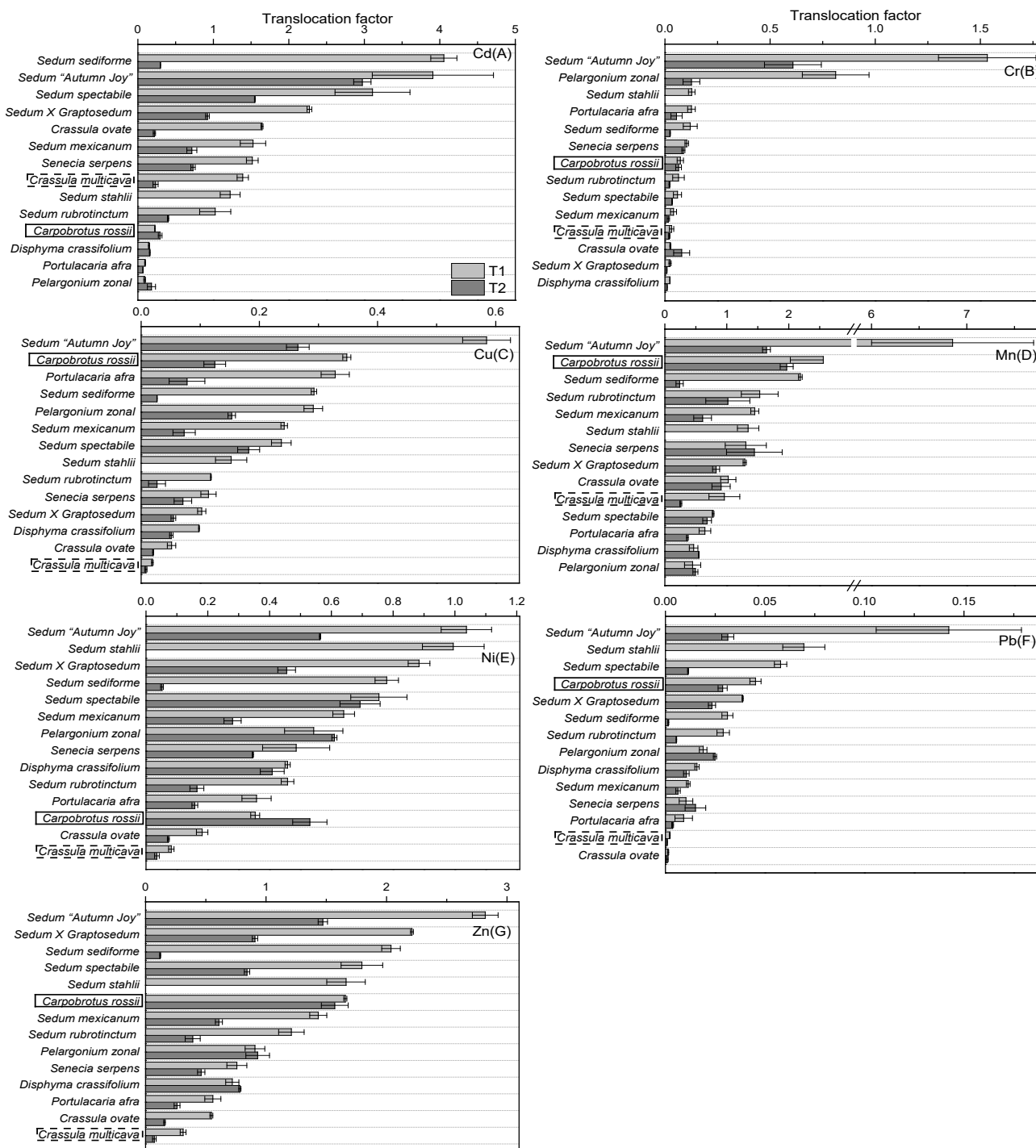




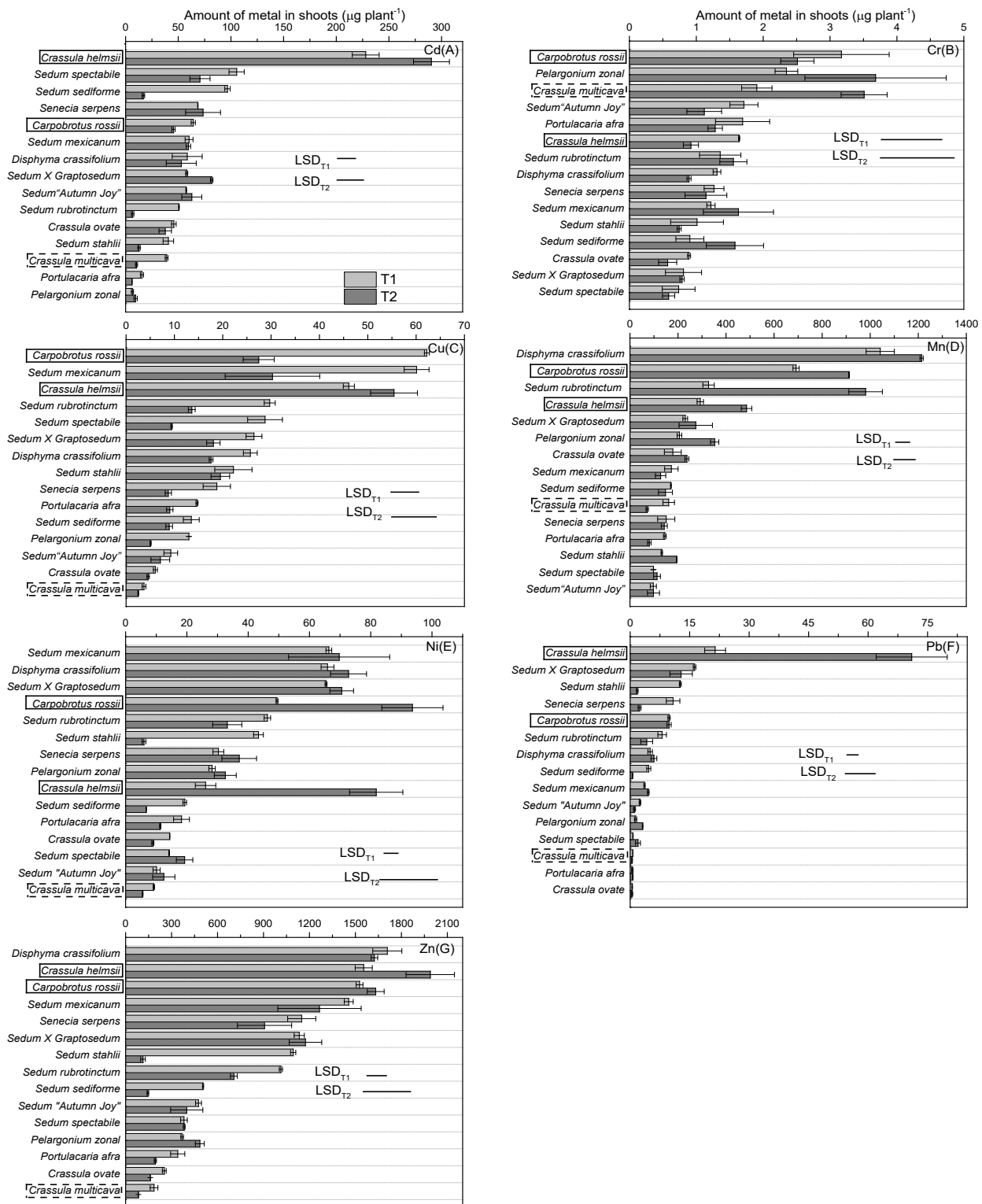
**Fig. 3** Concentrations of Cd (A), Cr (B), Cu (C), Mn (D), Ni (E), Pb (F) and Zn (G) in the roots of succulent species and *Pelargonium zonal* exposed to low (T1) and high (T2) concentrations of multiple heavy metals. Error bars represent  $\pm$  SE ( $n = 4$ ). Note: The roots of *Crassula helmsii* were too fine to be collected and the roots of *Sedum stahlii* in T2 treatment were too small to be collected. The species highlighted in a solid-line rectangle had the lowest reduction in RGR at T1 compared to at T0, and the species highlighted in a dash-line rectangle had the highest reduction in RGR at T1.



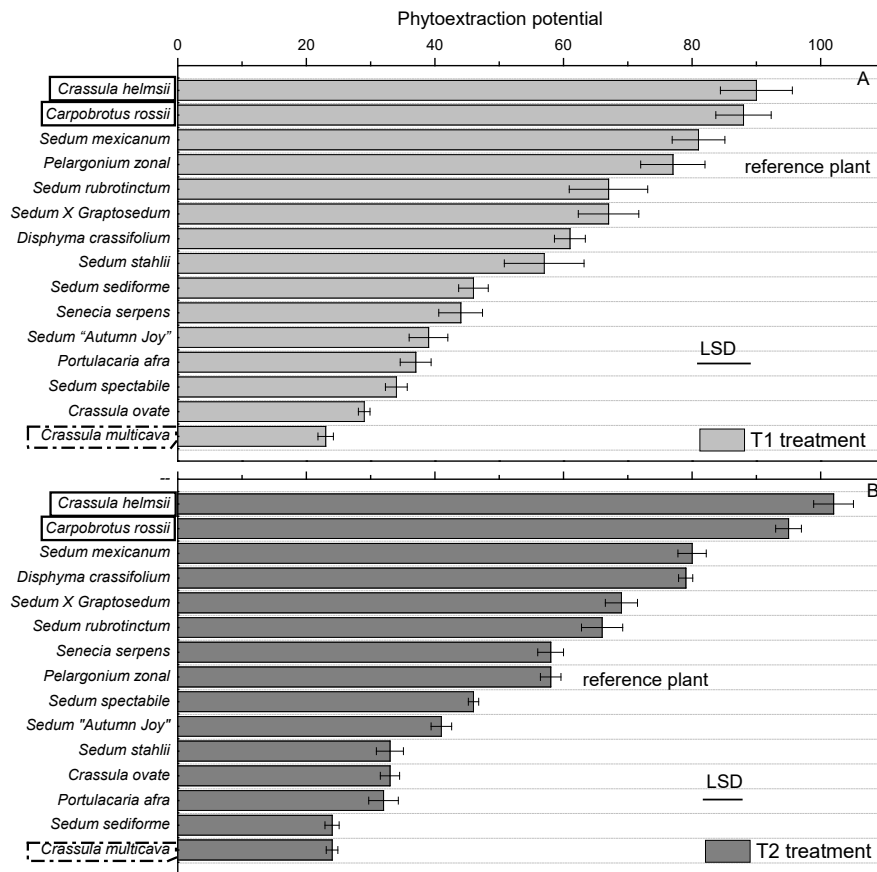
**Fig. 4** Bioaccumulation factor of Cd (A), Cr (B), Cu (C), Mn (D), Ni (E), Pb (F) and Zn (G) of 14 succulent species and *Pelargonium zonal* exposed for 84 days to low (T1) and high (T2) concentrations of multiple heavy metals. Error bars are  $\pm$ SE (n=4). Bioaccumulation factor = metal concentration in shoots/ total metal concentration in soil (Baker et al. 1994). The species were ordered based on their concentrations at low level (T1). The species highlighted in a solid-line rectangle had the lowest reduction in RGR at T1 compared to at T0, and the species highlighted in a dash-line rectangle had the highest reduction in RGR at T1.



**Fig. 5** Translocation factor (TF) of Cd (A), Cr (B), Cu (C), Mn (D), Ni (E), Pb (F) and Zn (G) of 13 succulent species and the reference plant *Pelargonium zonal* exposed for 84 days to low (T1) and high (T2) concentrations of multiple heavy metals. Error bars represent  $\pm$ SE (n=4). TF = metal concentration in shoots/metal concentration in roots (Baker et al. 1994). The roots of *Crassula helmsii* were too tiny to be collected and the roots of *Sedum stahlia* in T2 treatment were too rare to be collected. The species were ordered based on their concentrations at low level (T1). The species highlighted in a solid-line rectangle had the lowest reduction in RGR at T1 compared to at T0, and the species highlighted in a dash-line rectangle had the highest reduction in RGR at T1.



**Fig. 6** Amounts of Cd (A), Cr (B), Cu (C), Mn (D), Ni (E), Pb (F) and Zn (G) in the shoots of 14 succulent species and *Pelargonium zonal* exposed for 84 days to low (T1) and high (T2) concentrations of multiple heavy metals. Error bars represent  $\pm$  SE ( $n = 4$ ). Amounts of heavy metals in shoots ( $\mu\text{g plant}^{-1}$ ) = heavy metal concentration in shoot  $\times$  shoot RGR  $\times$  experimental period. The species highlighted in a solid-line rectangle had the lowest reduction in RGR at T1 compared to at T0, and the species highlighted in a dash-line rectangle had the highest reduction in RGR at T1.



**Fig. 7** Phytoextraction potential of 14 succulent species and *Pelargonium zonal* exposed for 84 days to low (T1) (A) and high (T2) (B) concentrations of multiple heavy metals. Error bars represent  $\pm$ SE (n = 4). The species highlighted in a solid-line rectangle had the lowest reduction in RGR at T1 compared to at T0, and the species highlighted in a dash-line rectangle had the highest reduction in RGR at T1.