Australian native plant species *Carpobrotus rossii* (Haw.) Schwantes shows the potential of cadmium phytoremediation

Chengjun Zhang • Peter W.G. Sale • Augustine I. Doronila • Gary J Clark • Caitlin Livesay • Caixian Tang

Chengjun Zhang • Peter W.G. Sale • Gary J Clark • Caitlin Livesay • Caixian Tang(✉)
Centre for AgriBioscience, La Trobe University, Melbourne Campus, Bundoora, Victoria 3086, Australia
email: C.Tang@ latrobe.edu.au, Tel.: +61 3 9032 7416; fax: +61 0 94710224

Augustine I. Doronila
School of Chemistry, University of Melbourne, Parkville, VIC 3010, Australia

ABSTRACT

Many polluted sites are typically characterized by contamination with multiple-heavy metals, drought, salinity and nutrient deficiencies. Here, an Australian native succulent halophytic plant species, *Carpobrotus rossii* (Haw.) Schwantes (Aizoaceae) was investigated to assess its tolerance and phytoextraction potential of Cd, Zn and the combination of Cd and Zn, when plants were grown in soils spiked with various concentrations of Cd (20-320 mg kg^{-1} Cd), Zn (150-2400 mg kg^{-1} Zn) or Cd+Zn (20+150, 40+300, 80+600 mg kg^{-1}). The concentration of Cd in plant parts followed the order of roots > stems > leaves, resulting in Cd translocation factor (TF, concentration ratio of shoots to roots) less than one. In contrast, the concentration of Zn was in order of leaves > stems > roots, with Zn TF greater than one. However, the amount of Cd and Zn were distributed more in leaves than in stems or roots, which was attributed to higher biomass of leaves than stems or roots. The critical value that causes 10% shoot biomass reduction was 115 µg g^{-1} for Cd and 1300 µg g^{-1} for Zn. The shoot Cd uptake per plant increased with increasing Cd addition while shoot Zn uptake peaked at 600 mg kg^{-1} Zn addition. The combined addition of Cd and Zn reduced biomass production more than Cd or Zn alone and significantly increased Cd concentration, but did not affect Zn concentration in plant parts. The results suggest that *C. rossii* is able to hyperaccumulate Cd and can be a promising candidate for phytoextraction of Cd-polluted soils.

Keywords: Cadmium (Cd) · hyperaccumualtor · metal contamination ·succulent· tolerance · zinc (Zn)

1 Introduction

Phytoremediation that uses plants to clean up polluted soils/waters (Cunningham &Berti 1993) is generally considered as a cost-effective and environment-friendly technique (Salt et al. 1998). As one of important phytoremediation approaches, phytoextraction utilizes some plants to take up heavy metals from contaminated soils or waters, and translocate them into shoots which are then harvested to get heavy metals recycled and soil/water cleaned through further processing methods (Salt et al. 1998). Although some plant species (defined as hyperaccumulators) can accumulate extraordinarily high (10-100 times) concentrations of heavy metals in shoots than do most plants, they are often not suitable for practical application to phytoextraction due to their specificity to a particular heavy metal and low biomass production (Hassan &Aarts 2011). For example, *Noccaea caerulescens* hyperaccumulates Cd and Zn (Brown et al. 1995) and is tolerant to Ni and Pb (Baker et al. 1994), but is sensitive to Cu (McLaughlin &Henderson 1999). Moreover, many polluted sites are typically characterized by contamination with multiple-heavy metals, drought, salinity and nutrient deficiencies. Therefore, it is crucial for successful phytoextraction to use plants that could not only accumulate relatively high amounts of heavy metals but also have other tolerant traits.

Carpobrotus rossii (Haw.) Schwantes (Aizoaceae) is an Australian native halophytic succulent plant species, and may be a promising plant for phytoextraction. When exposed to the combination of Cd, Cr, Cu, Mn, Ni, Pb and Zn with concentrations of 20, 20, 74, 200, 30, 300 and 300 mg kg^{-1}, respectively, it showed higher multi-metal tolerance and greater shoot biomass production, but also exhibited higher phytoextraction potential of these seven heavy metals compared with 14 other succulent species (CJ Zhang, unpublished data). This species is grown for reclamation of coastal sand dunes in southern Australian and Tasmania due to its dense groundcover and high salt tolerance (Geraghty et al. 2011), and may be adapted to growing in polluted sites with high salinity and under dry conditions.

The toxic heavy metal Cd is often present in soils together with Zn. Both elements have chemical similarities, which results in interactions in soils and plants. Some species have already been identified as co-hyperaccumulators of Cd and Zn. These species include *N. caerulescens* (McGrath et al. 1993), *Arabidopsis halleri* (Zhao et al. 2006, Zhao et al. 2000), and *Sedum alfredii* (Yang et al. 2004), which

may further suggest such interactions between Cd and Zn in plants.

Mutual influences of Cd and Zn have been studied in a number of plants species. So far, three modes of Cd-Zn mutual influences have been reported: antagonism, synergism and no effect, depending on plant species (Turner 1973), genotype (Sanaeiostovar et al. 2012, Zhang et al. 2002) or ecotype (Zha et al. 2004), growth stage (Zhu et al. 2003), plant tissues (root, stem and leaf) (Smith &Brennan 1983, Ye et al. 2003), contamination levels of Cd and Zn used in experiments (Honma &Hirata 1978, Smith &Brennan 1983) and soil types (Smilde et al. 1992). The antagonistic effects have been attributed to both metals competing for transporters or uptake processes (Cataldo et al. 1983) or interfering with the expression of transporter gene (Kupper &Kochian 2010), which has been documented mainly with non-hyperaccumulators like wheat and soybean (Green et al. 2003, Papoyan et al. 2007). The synergisms of Cd and Zn in plants have been suggested due to high expression of transporters stimulated by one metal (Papoyan et al. 2007), which were observed in hyperaccumulators like *N. caerulescens* (Papoyan et al. 2007), *S. afredii* (Yang et al. 2004) and *Potentilla griffithii* Hook (Qiu et al. 2011). However, synergism was also found in non-hyperaccumulators like oats (*Avena sativa* L.) (Haghiri 1974). Thus, interactions of Cd and Zn are complicated in plants, and further studies on various plants are necessary to clarify the nature of their interactions. As a promising candidate for phytoextraction, little is known about Cd-Zn mutual influences on tolerance and accumulation in *C. rossii*. Hence, an understanding of these interactions is essential for the optimization of the phytoextraction of these heavy metals from contaminated soils.

The aims of the present study were: (i) to assess the tolerance level of *C. rossii* to Cd and Zn alone or in combination; (ii) to investigate distribution patterns of Cd and Zn in plant parts with an attempt to characterize tolerant traits. We hypothesized that *C. rossii* is a Cd or Zn hyperaccumulator and has a high tolerance to Cd and/or Zn, and that Cd and Zn display synergistic effects in phytoextraction.

2 Materials and methods

2.1 Plant and soil materials

Carpobrotus rossii (Aizoaceae) was collected from a rural landfill site (37°36′S, 143°35′E, Snake Valley, Shire of Pyrenees) in Victoria, Australia (Fig. 1). Uniform cuttings (two nodes per cutting) were used for propagation in plastic nursery cells (5×5×8 cm) filled with the same soil used for the experiment. The soil was fertilized with Osmocote (N 15.3%, P 3.56%, K 12.6%, Scotts Australia Pty

Ltd) at 10 g kg^{-1}, and was irrigated with tap-water using an auto-watering sprayer. After one month, root systems of cuttings were well developed and the seedlings were transplanted to the experiment pots.

A silt loam soil was collected from the topsoil (0-25 cm) in the university farm, air-dried and passed through a 2-mm sieve. The initial soil contained 21.3% clay, 54.5% silt, 24.1% sand, 2.4% organic C, 0.076 dS m^{-1} electrical conductivity, pH 5.41 (1:5 soil:0.01M $CaCl_2$), 2.75 mg kg^{-1} total N, 44 mg kg^{-1} Colwell P, 126 mg kg^{-1} Colwell K, 0.55 mg kg^{-1} Cd and 119 mg kg^{-1} Zn.

2.2 Experimental design and treatments

The study consisted of three sets of experiments in fully randomized designs. The first set had seven levels of added $CdCl_2$ ranging from 0 to 320 mg kg^{-1}. The second set had seven levels of added $ZnSO_4$ ranging from 0 to 2400 mg kg^{-1}. The third set had three combinations of Cd and Zn, namely 20+150, 40+300 and 80+600 mg kg^{-1}, respectively. Soil (1.5 kg) was weighed into each plastic bag, and spiked with Cd and/or Zn at the designed rates. The basal nutrients were added as a solution to each bag in the following composition (mg kg^{-1} soil) 150 KNO_3, 21 $MgSO_4 \cdot H_2O$, 150 KH_2PO_4, 236 $CaCl_2 \cdot 2H_2O$, 18 $MnCl_2 \cdot 4H_2O$, 0.67 H_3BO_3, 10.33 $ZnSO_4 \cdot 7H_2O$, 1.42 $CuCl_2 \cdot 5H_2O$, 0.15 $Na_2MoO_4 \cdot 2H_2O$ and 90 NH_4NO_3. After thoroughly mixing the treatment solutions and basal nutrients, soils were watered to 80% of field capacity and incubated for 2 weeks in a constant temperature (25℃). This incubation time was based on our preliminary experiment on incubation time, and was also used by Hooda and Alloway (1993). The soils were re-mixed daily by hand-shaking the bag for 3 min during the incubation.

2.3 Plant growth

After incubation, the treated soils were transferred into plastic pots lined with plastic bags to avoid leaching loss of chemicals. Two uniform seedlings were transplanted into each pot. The pots were irrigated with distilled water to maintain 80% of field capacity every two days. The plants were grown in a glasshouse with minimum and maximum temperatures of 19 and 33℃, respectively.

2.4 Harvest

Plants were harvested 70 days after transplanting. Shoots were separated from the belowground parts 2

cm above the soil surface. They were rinsed with running tap water and then distilled water, and then soaked in 0.01 M HCl for 5 s (Papazoglou 2011), and again rinsed in distilled water to remove dust. Leaves and stems were separated. After removing the soil particles clinging to the surface, the roots were subject to the same washing procedure as the shoots. All plant parts were oven-dried in paper bags at 70 ℃ for 72 h, weighed and then ground into powder with a stainless steel mill (ZM200 Retsch Technology GmbH). Rhizosphere soil was collected by shaking off gently the soil adhering to roots. The soils were air-dried and sieved through a 2-mm mesh.

2.5 Soil measurements

Concentrations of total Cd and Zn in the initial soil were determined through reverse aqua regia digestion (concentrated HNO_3:HCl, 3:1, v/v). Concentrations of extractable Zn and Cd in treatment soils were measured according to methods of Ayoub (2003). Briefly, 5 g soil samples were shaken with 50 mL 0.01 M $CaCl_2$ solution for 2 h, and then centrifuged at 3000 rpm for 10 min, followed by filtering the supernatant through Whatman No. 1 (125 mm) filter paper. The filtrates were analyzed for Cd and Zn with inductively coupled plasma optical emission spectrometry (ICP-OES) (Varian Vista AX CCD, Australia Pty Ltd.). Rhizosphere soil pH was measured by shaking 5 g soil sample with 25 mL 0.01 M $CaCl_2$ solution for 12 h then measuring the supernatant, after centrifugation, using a pH meter (Thermo Orion 720, USA).

2.6 Concentrations of Cd and Zn in plants

Plant samples were digested according to the procedure developed by Monsant et al. (2008) with some modifications. Briefly, 0.5 g ground plant samples were digested with 6 ml of a mixture of concentrated HNO_3 and $HClO_4$ (4:1 v/v) for 24 h. The samples were then diluted to 75 mL using Milli-Q water (18 MΩ cm) for further analysis. Concentrations of Cd and Zn in digests were determined using ICP-OES. For quality control, three reference plant samples and three blanks were included for every batch. All plant tissue concentrations are expressed on a dry weight basis.

To assess the translocation of a metal from roots to shoots, the translocation factor (TF) (Hogan &Rauser 1981) was calculated as the ratio of metal concentration in shoots to metal concentration in roots.

2.7 Statistical analyses

All results were presented as the mean values (± SE) obtained from three independent replicates. Statistical analysis was conducted using SPSS statistics 17.0 software package (SPSS, Chicago, Illinois, USA). Metal concentrations were transformed logarithmically before ANOVA analysis. The interactions of Cd and Zn were analyzed by two-way ANOVA. Fisher LSD test was used to compare means between treatments at $p = 0.05$.

3 Results

3.1 Extractable Cd/Zn concentration and soil pH in rhizosphere

Increasing addition of Cd and Zn increased concentrations of $CaCl_2$-extractable Zn and Cd in rhizosphere soils, respectively (Fig. 2). The combined addition of Cd and Zn significantly ($p < 0.05$) increased the concentration of extractable Cd in rhizosphere soil (Fig. 2A) but not the concentration of extractable Zn except an increase at the highest level of Cd+Zn (80+600 mg kg^{-1}) (Fig. 2B). Increasing addition of Cd/Zn had no effect ($p > 0.05$) on rhizosphere soil pH (Fig. 3). The combined addition of Cd and Zn significantly ($p < 0.05$) increased the rhizosphere pH when compared to Cd or Zn treatment alone at their equivalent levels.

3.2 Biomass production

The shoot biomass generally decreased with increasing concentration of Cd/Zn addition to soil (Fig. 4), and compared with the control, no significant reduction in shoot biomass occurred when Cd addition was 80 mg kg^{-1} or less (Fig. 4A), or Zn addition up to 300 mg kg^{-1} (Fig. 4B). The root biomass was much lower than shoot biomass, and was not affected significantly by Cd or Zn addition except for significant decreases when Zn addition was 1800 mg kg^{-1} or more.

The combined addition of Cd and Zn inhibited biomass production more than the addition of Cd or Zn alone at their equivalent levels, especially at the highest level of Cd+Zn (80+600 mg kg^{-1}) (Fig. 4).

3.3 Accumulation of Cd and Zn in plant parts

The concentration of Cd or Zn in plant parts increased with increasing Cd or Zn addition to soil (Tables 1 and 2). The highest concentration in shoots was 442 µg g^{-1} for Cd occurring at 320 mg kg^{-1} Cd addition (Table 1), 4862 µg g^{-1} for Zn observed at 2400 mg kg^{-1} Zn addition (Table 2). Concentrations of Cd and Zn in plant parts showed different orders: roots > stems > leaves for Cd, but leaves > stems >

roots for Zn except no significant difference between stems and leaves from 1800 to 2400 mg kg^{-1} Zn addition (Tables 1 and 2).

The concentrations of Cd and Zn in plants showed different responses to the combined addition of Cd and Zn (Tables 1 and 2). Compared to Cd or Zn treatment alone, the combined addition significantly ($p < 0.05$) increased Cd concentration in roots and shoots at the two highest levels of Cd+Zn addition, but did not change Zn concentration in plants, except for decreased Zn concentration in roots by Cd addition at the highest level of Cd+Zn treatment.

3.4 Distribution and translocation of Cd/Zn in plants

With increasing Cd addition, Cd distribution (% total) showed a decreasing trend in leaves and stems but an increasing trend in roots (Table 1). Zinc addition enhanced Zn distribution (% total) in roots but decreased Zn distribution in stems and no significant change in leaves (Table 2).

The combined addition of Cd+Zn tended to increase Cd distribution in leaves and Zn distribution in roots, indicating Zn addition enhanced Cd translocation from stems and roots to leaves while Cd addition suppressed Zn translocation from roots to leaves.

The translocation factor (TF) was less than one for Cd but greater than one for Zn (Table 1), indicative of low Cd translocation ability and high Zn translocation ability from roots to shoots. There was no significant difference in the Cd TF in Cd or Cd+Zn treatments. Zn TF showed a decreasing trend in Zn treatments alone, indicating more Zn distribution in roots with increasing Zn addition. The addition of Cd tended to decrease Zn translocation from roots to shoots at 20 and 40 mg kg^{-1} Cd addition levels, but increased Zn translocation at 80 mg kg^{-1} Cd addition level (Table 2).

3.5 Phytoextraction potential

Shoot Cd uptake per plant had a plateau-curve response, increasing with increasing Cd addition and reaching the maximum at 240 mg kg^{-1} Cd addition (Fig. 5A). However, in the Zn alone treatments, the Zn uptake showed a bell-shaped pattern, peaking at 600 mg kg^{-1} Zn addition (Fig. 5B). The combined addition of Cd+Zn increased shoot Cd content but decreased Zn (Fig. 5).

4 Discussion

4.1 Cd tolerance

This study demonstrated that *Carpobrotus rossii* is highly tolerant to Cd with a critical value in its shoots of 115 µg g^{-1} (based on regression analysis between shoot biomass and shoot Cd concentration) at which the shoot biomass was reduced by 10 %. This critical level is much greater than those found in many non-hyperaccumulator species (5-10 µg g^{-1}) (White & Brown 2010), which was attributed to lower concentration of Cd in photosynthetic leaves than that in non-photosynthetic tissues, stems and roots (Table 1). However, this critical value is lower than typical hyperaccumulators like *A. halleri* (228 µg g^{-1}) (Zhao et al. 2006), *N. caerulescens* (> 5000 µg g^{-1}) (Roosens et al. 2003), *N. praecox* (> 8000 µg g^{-1}) (Koren et al. 2013), *Arabis paniculata* (> 6000 µg g^{-1}) (Tang et al. 2009a) and *S. alfredii* (> 8000 µg g^{-1}) (Yang et al. 2004). The lower Cd critical value of *C. rossii* may be related partly to its thick succulent leaves and thus much lower specific leaf area, compared to leafy herbaceous hyperaccumulators with higher specific leaf area. These broad leaf plants have Cd distribution which is often higher in epidermis cells than in mesophyll cells (Pongrac et al. 2010).

In this experiment, Zn addition caused significant reduction in shoot biomass when compared to Cd treatment alone (Fig. 4A), indicating that Cd tolerance of this species was decreased by Zn addition. The response of *C. rossii* was consistent with that of Cd-Zn hyperaccumulator *P. griffithii* showing a significant decreased Cd tolerance by Zn addition at the high level of their combination (Qiu et al. 2011). In this present study, the decreased Cd tolerance might be attributed partly to the increased Cd concentration in shoots compared to Cd treatment alone, especially at the highest level of combination of Cd and Zn (Fig. 4A and Table 1). Additionally, increased shoot Zn concentration by Zn addition, together with the increased distribution of Cd in the leaves (Table 1), might also have contributed to the decrease in shoot biomass, in comparison to the Cd only treatment. In contrast, the addition of 80 mg kg^{-1} Cd with increasing levels of Zn had less effect on the shoot biomass, in comparison to the Zn treatment alone (Fig. 4B).

4.2 Zn tolerance

By comparison with Cd tolerance, *C. rossii* is moderately tolerant to Zn with a critical level of 1300 µg g^{-1} based on regression analysis between shoot biomass and shoot Zn concentration. This critical level is greater than that in most species (300-600 µg g^{-1}) (Long et al. 2003) and possibly greater than that of *B. juncea* which showed > 20% and > 80% reduction in shoot biomass at approximately 500 and 1500 µg g^{-1} in shoots, respectively, when grown in a loam-based compost spiked with ZnO for 35 days

(Podar et al. 2004). Additionally, our preliminary experiments also showed that *C. rossii* was more tolerant than *B. juncea* to the mixtures of Cd, Cr, Cu, Mn, Ni, Pb and Zn. Furthermore, photosynthetic tissues, leaves, had higher concentrations of Zn than non-photosynthetic tissues, stems and roots (Table 2).

Compared to Zn treatment alone, Cd addition slightly decreased Zn tolerance at low levels of combination of Zn and Cd, but significantly ($p < 0.05$) decreased Zn tolerance at the highest level of their combination (Fig. 4B). A similar response was also reported in the Cd-Zn hyperaccumulator *P. griffithii* although its growth was stimulated by low levels of Zn or Cd treatment alone (Qiu et al. 2011). These findings suggest that the combination of Cd and Zn is more phytotoxic even at their respective levels, which could not inhibit or even stimulate plant growth.

4.3 Cd phytoextraction potential

Carpobrotus rossii in the present experiment had a extensive fine root system and was observed with higher root Cd concentration than shoots (Table 1), which might partly contribute to high metal accumulation in shoots due to a large absorptive surface area. Some Cd hyperaccumulators like *A. halleri* also have a strong root uptake system (Ueno et al. 2008), with higher Cd concentrations in roots than shoots (Zhao et al. 2006). High metal accumulation in shoots in the present experiment was also confirmed by increased shoot Cd uptake per plant with increasing Cd addition although shoot biomass was inhibited significantly at Cd addition level at and above 80 mg kg^{-1}.

It is interesting to note that though the TF < 1 (Table 1), shoot Cd critical value of *C. rossii* was greater than 100 µg Cd g^{-1}, the threshold value for Cd hyperaccumulators (Chaney et al. 1997), showing high accumulation of Cd in shoots of this species. According to previous studies, hyperaccumulators were defined based on at least three criteria. First, a hyperaccumulator should have a metal concentration in shoots or leaves ≥ the critical or threshold value (10% reduction in biomass). In the case of Cd, this critical level is ≥ 100 µg g^{-1}. Second, a hyperaccumulator has a bioaccumulation factor (BF, the ratio of metal concentration in shoots to that in medium) greater than one. Third, a species defined as a hyperaccumulator has a translocation factor (TF) greater than one. In fact, TF values may be related to experiment conditions. For example, TF values less than one were also recorded in *N. caerulescens* in its Ganges and Prayon ecotypes in a solution culture (Lombi et al. 2000, Wojcik et al. 2005). More recently, substantial high critical shoot concentrations (e.g. > 100 µg g^{-1})

with BF > 1 but TF < 1 have widely been accepted as a measure to define plants as hyperaccumulators, e.g. *A. halleri* (Chiang et al. 2006, Craciun et al. 2006, Kramer 2010, Zhao et al. 2006), *Arabis paniculata* (Tang et al. 2009a), *Lonicera japonica* (Liu et al. 2009), *Potentilla griffithii* (Hu et al. 2009) and *Picris divaricate* (Tang et al. 2009b, Ying et al. 2010). Therefore, *C. rossii* in this study could be considered as a Cd hyperaccumulator.

Compared to Cd treatment alone, Zn addition significantly ($p < 0.05$) increased shoot Cd uptake per plant (Fig. 5A) although it decreased shoot biomass (Fig. 4B), indicating that Cd phytoextraction ability was improved by Zn addition through increasing Cd concentration in shoots. Similar results were observed with other hyperaccumulators *S. alfredii* (Yang et al. 2004) and *P. griffithii* Hook (Qiu et al. 2011).

The increased Cd concentration in shoots in this experiment might be attributed mainly to the enhanced extractable Cd concentration in rhizosphere soil by Zn addition (Fig. 2A), possibly due to the displacement of Cd^{2+} by Zn^{2+} from cation exchange sites in soil (Forbes et al. 1976) and/or complexation of Cd with Cl^- and/or SO_4^{2-}, thus enhancing uptake (McLaughlin et al. 1998, Smolders et al. 1998), since rhizosphere soil pH was significantly increased by Zn addition (Fig. 3) and thus was unlikely to be a cause of the enhanced extractable Cd concentration by Zn addition.

Additionally, it is noticeable that at the highest level of Cd+Zn, Zn addition increased shoot Cd concentration by approximate 200% (Table 1). This cannot be caused mainly by the 100% increase in extractable Cd concentration (Fig. 2A), and thus biomass effect (diluting and concentrating) (Haghiri 1974) might also be responsible for the increased shoot Cd concentration since 50% reduction of shoot biomass occurred at the highest level of Cd+Zn compared to equivalent Cd treatment alone (Fig. 4A).

4.4 Zn phytoextraction potential

In this experiment, *C. rossii* had TF values of Zn greater than one in all treatments (Table 2) and had a higher critical value of 1300 $\mu g\ g^{-1}$ than most species, indicating that it could have high Zn translocation from roots to shoots and higher accumulation than most species. However, in Zn hyperaccumulators, plants could accumulate over 10 000 $\mu g\ g^{-1}$ (Reeves &Brooks 1983) or 3000 $\mu g\ g^{-1}$ in shoots (Broadley et al. 2007). Thus, we consider that *C. rossii* can be classified as a Zn accumulator. But unlike the Cd case (Fig. 5A), shoot Zn uptake per plant decreased significantly when Zn addition was greater than 600 mg kg^{-1} (Fig. 5B), indicating that this species may have a limited phytoextraction

ability for contaminated sites with high Zn levels (e.g. > 600 mg kg^{-1}).

Although Cd addition did not affect shoot Zn concentration (Table 2) compared with equivalent level of Zn treatment alone, shoot Zn uptake per plant was decreased significantly ($p < 0.05$) except for the lowest level of combination of Cd and Zn (20+150), suggesting that Zn phytoextraction ability was inhibited by Cd addition and thus this species was not suitable for phytoextraction of Zn with high levels of Cd in the soil.

The addition of Cd addition did not affect shoot Zn concentration nor extractable Zn concentration in soil except for a slight increase at the highest level of Cd+Zn (Fig. 2B). The responses of Zn in plants to Cd addition here is consistent with those of some hyperaccumulators, but opposite to responses of most non-hyperaccumulation crop plants showing inhibitory effect (Cataldo et al. 1983, Hawf &Schmid 1967, Mohammad &Moheman 2010, Root et al. 1975). The inhibitory effect is due to sharing some common transport sites and resulting in competition between Cd and Zn. In the case of hyperaccumulators, no effect of Cd addition on Zn accumulation in shoots was observed with *A. halleri* (Zhao et al. 2006) and high-Zn tolerant Prayon ecotypes of *N. caerulescens* (Assuncao et al. 2008, Papoyan et al. 2007, Roosens et al. 2003).

5 Conclusions

Carpobrotus rossii is able to hyperaccumulate Cd and is more tolerant to Zn than most species. In combination with its easy-growing, salt and drought tolerant traits, this species could be a promising candidate for phytoextraction of Cd-polluted soils, especially in drought prone areas and soils with high salinity. Further studies are needed to look into the responses of Cd phytoextraction in this species under high salinity and/or drought. The interactions of Cd and Zn showed concentration-dependent responses, antagonism at low levels but one-sided synergism at high levels, enhanced Cd but not affected Zn concentration in plants. The enhanced Cd concentration by the combined addition of Cd and Zn might be related partly to the complexation of Cd with Cl^- and/or SO_4^{2-}, but further work is needed to investigate their relationships in plant uptake and accumulation.

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Table 1. Concentrations and distribution of Cd in plant parts and the concentration ratio of Cd in shoot to roots (translocation factor, TF) of *Carpobrotus rossii* in response to Cd and Zn additions. The data of concentrations were analyzed after $\log_{10}$ transformation. The data in the same column followed by a common letter are not significantly different at $p = 0.05$.

Treatment		Cd concentration (μg g^{-1})				Cd distribution(% total)			TF
Cd (mg kg^{-1})	Zn (mg kg^{-1})	Leaves	Stems	Whole shoot	Roots	Leaves	Stems	Roots	
0	0	0.4*a*	1*a*	0.5*a*	1*a*	46*cd*	41*cd*	13*a*	0.49*a*
20	0	67*b*	132*b*	87*b*	193*b*	46*cd*	43*d*	12*a*	0.45*a*
40	0	76*bc*	166*b*	107*c*	227*b*	45*bcd*	43*d*	12*a*	0.45*a*
80	0	82*bc*	166*b*	115*d*	507*c*	39*ab*	37*bcd*	25*b*	0.23*a*
160	0	150*d*	359*d*	213*f*	627*d*	37*a*	35*bcd*	28*b*	0.34*a*
240	0	233*e*	576*f*	320*g*	761*e*	39*ab*	30*b*	30*b*	0.41*a*
320	0	354*g*	720*f*	442*h*	1370*f*	37*a*	20*a*	43*c*	0.32*a*
20	150	85*c*	167*b*	111*cd*	213*b*	49*d*	38*bcd*	13*a*	0.56*a*
40	300	125*d*	241*c*	150*e*	538*c*	40*abc*	35*bcd*	24*b*	0.28*a*
80	600	260*f*	509*e*	323*g*	699*d*	58*e*	30*b*	12*a*	0.45*a*

Table 2. Concentration and distribution of Zn in plant parts and the concentration ratio of Zn in shoot to roots (translocation factor, TF) of *Carpobrotus rossii* in response to Cd and Zn additions. The data of concentrations were analyzed after $\log_{10}$ transformation. The data in the same column followed by a common letter are not significantly different at $p = 0.05$.

Treatment		Zn concentration (μg g^{-1})				Zn distribution(% total)			TF
Cd (mg kg^{-1})	Zn (mg kg^{-1})	Leaves	Stems	Whole shoot	Roots	Leaves	Stems	Roots	
0	0	203*a*	111*a*	204*a*	66*a*	68*a*	30*a*	2*a*	3.09*d*
0	150	760*b*	328*b*	615*b*	342*b*	78*a*	20*a*	2*a*	1.81*bc*
0	300	1210*c*	606*c*	1021*c*	567*c*	77*a*	21*a*	2*a*	1.81*bc*
0	600	1979*d*	1136*d*	1728*d*	1235*e*	76*a*	21*a*	3*a*	1.42*a*
0	1200	4465*e*	4096*e*	4372*e*	3040*f*	77*a*	18*a*	6*b*	1.44*a*
0	1800	4360*e*	4867*f*	4474*ef*	3906*g*	78*a*	15*a*	7*b*	1.15*a*
0	2400	4870*e*	5072*f*	4862*f*	4593*h*	77*a*	15*a*	8*b*	1.06*a*
20	150	648*b*	364*b*	558*b*	337*b*	69*a*	28*a*	3*a*	1.66*ac*
40	300	893*c*	484*c*	807*c*	584*c*	77*a*	18*a*	5*b*	1.37*a*
80	600	1921*d*	1495*d*	1813*d*	799*d*	77*a*	18*a*	5*b*	2.29*c*

Figure 1. *Carpobrotus rossii* growing at a landfill site during the dry season.

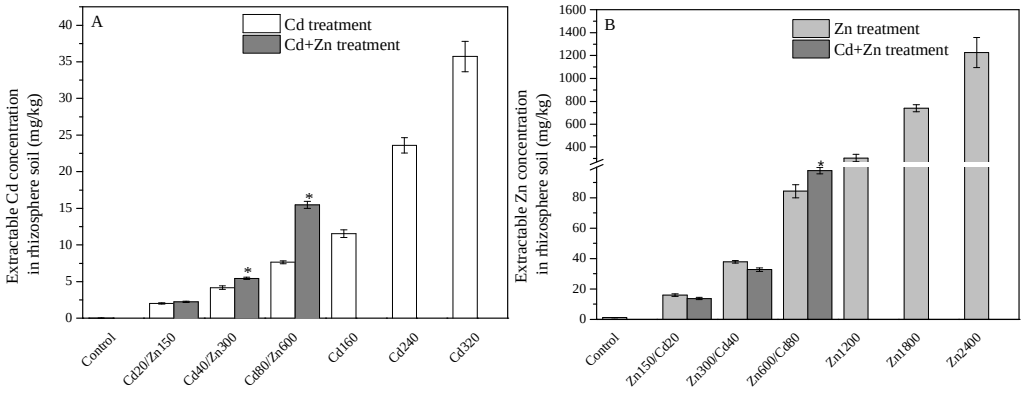

Figure 2. Concentrations of extractable (0.01 M $CaCl_2$) Cd (A) and Zn (B) in rhizosphere soil of *Carpobrotus rossii* exposed to Cd, Zn and Cd+Zn treatments with additions of 0-320 mg kg^{-1} Cd and 0-2400 mg kg^{-1} Zn for 70 days. The values are mean of three replicates and vertical bars are standard errors. * indicates the significant difference between Cd+Zn treatments and corresponding Cd or Zn treatments alone (p = 0.05).

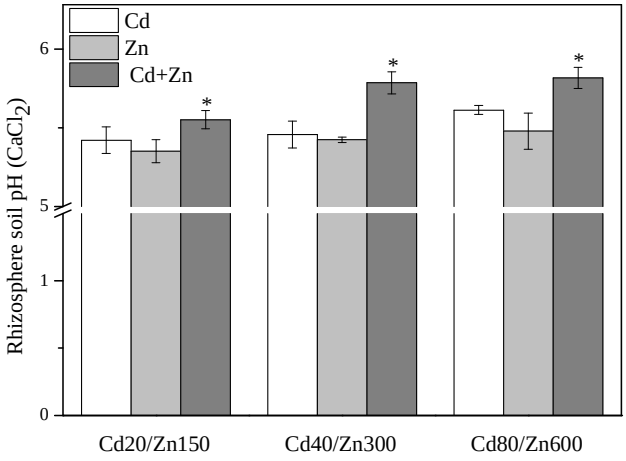

Figure 3. Rhizosphere soil pH of *Carpobrotus rossii* exposed to Cd, Zn and Cd+Zn treatments. The values are mean of three replicates and vertical bars are standard errors. * indicates the significant difference between Cd+Zn treatments and corresponding Cd or Zn treatments alone (p = 0.05).

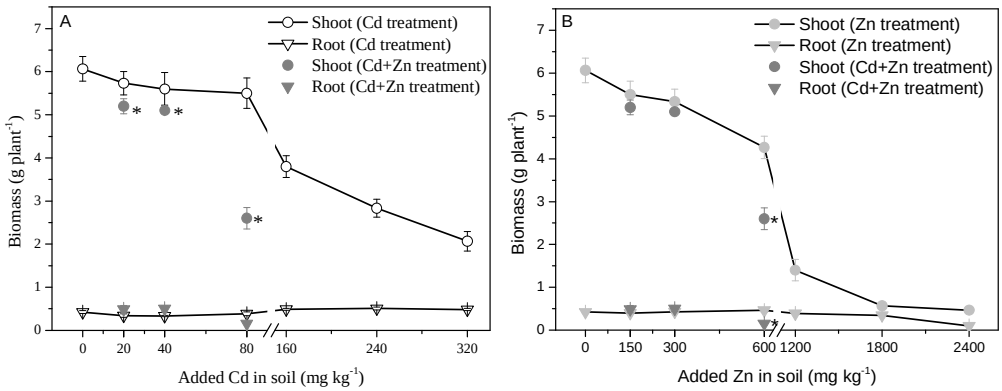

Figure 4. Effects of Cd (A) and Zn (B) addition on dry weights of shoots and roots of *Carpobrotus rossii*. Values are mean and standard errors (n=3). * indicates the significant difference between Cd+Zn treatments and corresponding Cd or Zn treatments alone (p = 0.05). Root biomass was significantly lower at 1800 and 2400 mg Zn kg^{-1} than other Zn treatments (p < 0.05).

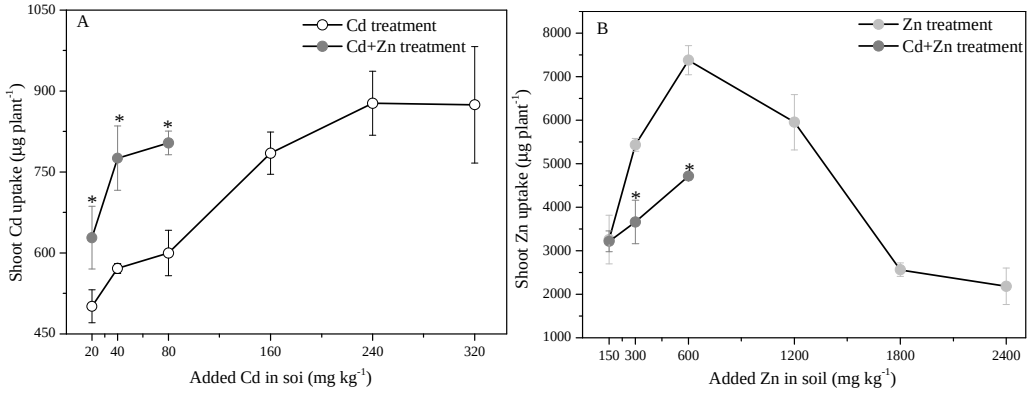

Figure 5. Total uptake (µg plant^{-1}) of Cd (A) and Zn (B) in shoots of *Carpobrotus rossii* exposed to various Cd and Zn treatments. Values are means ± standard errors (n = 3). * indicates the significant difference between Cd+Zn treatments and corresponding Cd or Zn treatments alone (p = 0.05).

Author/s:
Zhang, C; Sale, PWG; Doronila, AI; Clark, GJ; Livesay, C; Tang, C

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