

Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

Zhang, C;Sale, PWG;Doronila, AI;Clark, GJ;Livesay, C;Tang, C

Title:

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

Date:

2014-01-01

Citation:

Zhang, C., Sale, P. W. G., Doronila, A. I., Clark, G. J., Livesay, C. & Tang, C. (2014). Australian native plant species *Carpobrotus rossii* (Haw.) Schwantes shows the potential of cadmium phytoremediation. *Environmental Science and Pollution Research*, 21 (16), pp.9843-9851. <https://doi.org/10.1007/s11356-014-2919-3>.

Persistent Link:

<https://hdl.handle.net/11343/216697>

Australian native plant species *Carpobrotus rossii* (Haw.) Schwantes

2 shows the potential of cadmium phytoremediation

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

6

Chengjun Zhang • Peter W.G. Sale • Gary J Clark • Caitlin Livesay • Caixian Tang(✉)

8 Centre for AgriBioscience, La Trobe University, Melbourne Campus, Bundoora, Victoria
3086, Australia

10 email: C.Tang@latrobe.edu.au, Tel.: +61 3 9032 7416; fax: +61 0 94710224

12 Augustine I. Doronila

School of Chemistry, University of Melbourne, Parkville, VIC 3010, Australia

14

ABSTRACT

16 Many polluted sites are typically characterized by contamination with multiple-heavy metals,
drought, salinity and nutrient deficiencies. Here, an Australian native succulent halophytic
18 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
20 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
22 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
24 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
26 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
28 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
30 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
32 promising candidate for phytoextraction of Cd-polluted soils.

Keywords: Cadmium (Cd) · hyperaccumulator · metal contamination · succulent · tolerance ·

34 zinc (Zn)

1 Introduction

36 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
38 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
40 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
42 (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
44 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
46 (McLaughlin & Henderson 1999). Moreover, many polluted sites are typically characterized by
contamination with multiple-heavy metals, drought, salinity and nutrient deficiencies. Therefore, it is
48 crucial for successful phytoextraction to use plants that could not only accumulate relatively high
amounts of heavy metals but also have other tolerant traits.

50 *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,
52 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
54 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
56 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.

58 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
60 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

62 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
64 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
66 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
68 & 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
70 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
72 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
74 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*
76 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
78 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
80 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
82 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
84 high tolerance to Cd and/or Zn, and that Cd and Zn display synergistic effects in phytoextraction.

2 Materials and methods

86 2.1 Plant and soil materials

Carpobrotus rossii (Aizoaceae) was collected from a rural landfill site (37°36'S, 143°35'E, Snake
88 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
90 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,
92 root systems of cuttings were well developed and the seedlings were transplanted to the experiment
pots.

94 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%
96 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.

98 2.2 Experimental design and treatments

The study consisted of three sets of experiments in fully randomized designs. The first set had seven
100 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,
102 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
104 following composition (mg kg^{-1} soil) 150 KNO_3 , 21 $\text{MgSO}_4 \cdot \text{H}_2\text{O}$, 150 KH_2PO_4 , 236 $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 18
 $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.67 H_3BO_3 , 10.33 $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 1.42 $\text{CuCl}_2 \cdot 5\text{H}_2\text{O}$, 0.15 $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ and 90
106 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°C). This incubation time
108 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
110 incubation.

2.3 Plant growth

112 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
114 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°C , respectively.

116 2.4 Harvest

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

118 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.
120 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
122 bags at 70 °C 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
124 roots. The soils were air-dried and sieved through a 2-mm mesh.

2.5 Soil measurements

126 Concentrations of total Cd and Zn in the initial soil were determined through reverse aqua regia
digestion (concentrated HNO₃:HCl, 3:1, v/v). Concentrations of extractable Zn and Cd in treatment
128 soils were measured according to methods of Ayoub (2003). Briefly, 5 g soil samples were shaken with
50 mL 0.01 M CaCl₂ solution for 2 h, and then centrifuged at 3000 rpm for 10 min, followed by
130 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
132 AX CCD, Australia Pty Ltd.). Rhizosphere soil pH was measured by shaking 5 g soil sample with 25
mL 0.01 M CaCl₂ solution for 12 h then measuring the supernatant, after centrifugation, using a pH
134 meter (Thermo Orion 720, USA).

2.6 Concentrations of Cd and Zn in plants

136 Plant samples were digested according to the procedure developed by Monsanto et al. (2008) with some
modifications. Briefly, 0.5 g ground plant samples were digested with 6 ml of a mixture of
138 concentrated HNO₃ and HClO₄ (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
140 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.

142 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
144 roots.

2.7 Statistical analyses

146 All results were presented as the mean values (\pm SE) obtained from three independent replicates.
Statistical analysis was conducted using SPSS statistics 17.0 software package (SPSS, Chicago, Illinois,
148 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
150 means between treatments at $p = 0.05$.

3 Results

152 3.1 Extractable Cd/Zn concentration and soil pH in rhizosphere

Increasing addition of Cd and Zn increased concentrations of CaCl₂-extractable Zn and Cd in
154 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
156 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
158 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.

160 3.2 Biomass production

The shoot biomass generally decreased with increasing concentration of Cd/Zn addition to soil (Fig. 4),
162 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
164 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.

166 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).

168 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
170 1 and 2). The highest concentration in shoots was 442 $\mu\text{g g}^{-1}$ for Cd occurring at 320 mg kg⁻¹ Cd
addition (Table 1), 4862 $\mu\text{g g}^{-1}$ for Zn observed at 2400 mg kg⁻¹ Zn addition (Table 2). Concentrations
172 of Cd and Zn in plant parts showed different orders: roots > stems > leaves for Cd, but leaves > stems >

174 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.

180 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).

184 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

194 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).

198 4 Discussion

4.1 Cd tolerance

200 This study demonstrated that *Carpobrotus rossii* is highly tolerant to Cd with a critical value in its
shoots of $115 \mu\text{g g}^{-1}$ (based on regression analysis between shoot biomass and shoot Cd concentration)
202 at which the shoot biomass was reduced by 10 %. This critical level is much greater than those found in
many non-hyperaccumulator species ($5\text{-}10 \mu\text{g g}^{-1}$) (White & Brown 2010), which was attributed to
204 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
206 $\mu\text{g g}^{-1}$) (Zhao et al. 2006), *N. caerulea* ($> 5000 \mu\text{g g}^{-1}$) (Roosens et al. 2003), *N. praecox* (> 8000
 $\mu\text{g g}^{-1}$) (Koren et al. 2013), *Arabis paniculata* ($> 6000 \mu\text{g g}^{-1}$) (Tang et al. 2009a) and *S. alfredii* ($>$
208 $8000 \mu\text{g g}^{-1}$) (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
210 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).

212 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.
214 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.
216 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
218 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
220 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
222 treatment alone (Fig. 4B).

4.2 Zn tolerance

224 By comparison with Cd tolerance, *C. rossii* is moderately tolerant to Zn with a critical level of $1300 \mu\text{g}$
 g^{-1} based on regression analysis between shoot biomass and shoot Zn concentration. This critical level
226 is greater than that in most species ($300\text{-}600 \mu\text{g g}^{-1}$) (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
228 $\mu\text{g g}^{-1}$ 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
230 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
232 2).

Compared to Zn treatment alone, Cd addition slightly decreased Zn tolerance at low levels of
234 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.*
236 *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
238 levels, which could not inhibit or even stimulate plant growth.

4.3 Cd phytoextraction potential

240 *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
242 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
244 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
246 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
248 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,
250 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).
252 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
254 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
256 recorded in *N. caerulea* 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)

258 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*
260 *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
262 considered as a Cd hyperaccumulator.

Compared to Cd treatment alone, Zn addition significantly ($p < 0.05$) increased shoot Cd uptake per
264 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
266 were observed with other hyperaccumulators *S. alfredii* (Yang et al. 2004) and *P. griffithii* Hook (Qiu et
al. 2011).

268 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
270 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
272 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.

274 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
276 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
278 biomass occurred at the highest level of Cd+Zn compared to equivalent Cd treatment alone (Fig. 4A).

4.4 Zn phytoextraction potential

280 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
282 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 $3000 \mu g g^{-1}$
284 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
286 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).

288 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
290 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
292 levels of Cd in the soil.

The addition of Cd addition did not affect shoot Zn concentration nor extractable Zn concentration
294 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
296 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
298 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*
300 (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).

302 **5 Conclusions**

Carpobrotus rossii is able to hyperaccumulate Cd and is more tolerant to Zn than most species. In
304 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
306 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
308 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
310 and Zn might be related partly to the complexation of Cd with Cl⁻ and/or SO₄²⁻, but further work is
needed to investigate their relationships in plant uptake and accumulation.

312 **Acknowledgements**

We thank Dr. Trevor Edwards for identification of *Carpobrotus rossii* and Mr. Rob Evans for assistance
314 in the experiment. We are grateful to an anonymous reviewer for constructive and detailed comments

on the manuscript. This research was supported by an Australian Research Council *Linkage Project*
316 (LP100100800).

318 **References**

- 320 Assuncao AGL, Bleeker P, ten Bookum WM, Vooijs R, Schat H (2008) Intraspecific variation of metal
preference patterns for hyperaccumulation in *Thlaspi caerulescens*: evidence from binary
metal exposures. *Plant Soil* 303: 289-299
- 322 Ayoub AS, McGaw BA, Shand CA, Midwood AJ (2003) Phytoavailability of Cd and Zn in soil
estimated by stable isotope exchange and chemical extraction. *Plant Soil* 252: 291-300
- 324 Baker AJM, Reeves RD, Hajar ASM (1994) Heavy metal accumulation and tolerance in British
populations of the metallophyte *Thlaspi caerulescens* J. & C. Presl (Brassicaceae). *New*
326 *Phytol* 127: 61-68
- Broadley MR, White PJ, Hammond JP, Zelko I, Lux A (2007) Zinc in plants. *New Phytol* 173: 677-702
- 328 Brown SL, Chaney RL, Angle JS, Baker AJM (1995) Zinc and cadmium uptake by hyperaccumulator
Thlaspi caerulescens grown in nutrient solution. *Soil Sci Soc Am J* 59: 125-133
- 330 Cataldo DA, Garland TR, Wildung RE (1983) Cadmium uptake kinetics in intact soybean plants. *Plant*
Physiol 73: 844-848
- 332 Chaney RL, Malik M, Li YM, Brown SL, Brewer EP, Angle JS, Baker AJM (1997) Phytoremediation
of soil metals. *Curr Opin Biotechnol* 8:279-284
- 334 Chiang HC, Lo JC, Yeh KC (2006) Genes associated with heavy metal tolerance and accumulation in
Zn/Cd hyperaccumulator *Arabidopsis halleri*: A genomic survey with cDNA microarray.
336 *Environ Sci Technol* 40:6792-6798
- Craciun AR, Courbot M, Bourgis F, Salis P, Saumitou-Laprade P, Verbruggen N (2006) Comparative
338 cDNA-AFLP analysis of Cd-tolerant and -sensitive genotypes derived from crosses between
the Cd hyperaccumulator *Arabidopsis halleri* and *Arabidopsis lyrata* ssp. *petraea*. *J Exp Bot*
340 57: 2967-2983
- Cunningham S, Berti W (1993) Remediation of contaminated soils with green plants: an overview. In
342 *Vitro Cell Dev Biol* 29:207-212
- Forbes EA, Posner AM, Quirk JP (1976) Specific adsorption of divalent Cd, Co, Cu, Pb, and Zn on
344 goethite. *J Soil Sci* 27: 154-166
- Geraghty DP, Ahuja KDK, Pittaway J, Shing C, Jacobson GA, Jager N, Jurkovic S, Narkowicz C,
346 Saunders CI, Ball M, Pinkard A, Vennavaram RR, Adams MJ (2011) In vitro antioxidant,
antiplatelet and anti-inflammatory activity of *Carpobrotus rossii* (pigface) extract. *J*
348 *Ethnopharmacol* 134: 97-103
- Green CE, Chaney RL, Bouwkamp J (2003) Interactions between cadmium uptake and phytotoxic
350 levels of zinc in hard red spring wheat. *J Plant Nutr* 26: 417-430
- Haghiri F (1974) Plant uptake of cadmium as influenced by cation exchange capacity, organic matter,
352 zinc, and soil temperature. *J Environ Qual* 3: 180-183
- Hassan Z, Aarts MGM (2011) Opportunities and feasibilities for biotechnological improvement of Zn,
354 Cd or Ni tolerance and accumulation in plants. *Environ Exp Bot* 72: 53-63
- Hawf LR, Schmid WE (1967) Uptake and translocation of zinc by intact plants. *Plant Soil* 27: 249-260
- 356 Hogan GD, Rauser WE (1981) Role of copper-binding, absorption, and translocation in copper
tolerance of *Agrostis gigantea* Roth. *J Exp Bot* 32: 27-36

- 358 Honma Y, Hirata H (1978) Noticeable increase in cadmium absorption by zinc-deficient rice plants.
Soil Sci Plant Nutr 24: 295-297
- 360 Hooda PS, Alloway BJ (1993) Effects of time and temperature on the bioavailability of Cd and Pb from
sludge-amended soils. J Soil Sci 44: 97-110
- 362 Hu PJ, Qiu RL, Senthilkumar P, Jiang D, Chen ZW, Tang YT, Liu FJ (2009) Tolerance, accumulation
and distribution of zinc and cadmium in hyperaccumulator *Potentilla griffithii*. Environ Exp
364 Bot 66: 317-325
- Koren Š, Arčon I, Kump P, Nečemer M, Vogel-Mikuš K (2013) Influence of CdCl₂ and CdSO₄
366 supplementation on Cd distribution and ligand environment in leaves of the Cd
hyperaccumulator *Noccaea (Thlaspi) praecox*. Plant Soil 370: 125-148
- 368 Kramer U (2010) Metal hyperaccumulation in plants. Annual Review of Plant Biology 61: 517-534
- Kupper H, Kochian LV (2010) Transcriptional regulation of metal transport genes and mineral nutrition
370 during acclimatization to cadmium and zinc in the Cd/Zn hyperaccumulator, *Thlaspi*
caerulescens (Ganges population). New phytol 185: 114-29
- 372 Liu Z, He X, Chen W, Yuan F, Yan K, Tao D (2009) Accumulation and tolerance characteristics of
cadmium in a potential hyperaccumulator--*Lonicera japonica* Thunb. J Hazard Mater 169:
374 170-175
- Lombi E, Zhao FJ, Dunham SJ, McGrath SP (2000) Cadmium accumulation in populations of *Thlaspi*
376 *caerulescens* and *Thlaspi goesingense*. New Phytol 145: 11-20
- Long XX, Yang XE, Ni WZ, Ye ZQ, He ZL, Calvert DV, Stoffella JP (2003) Assessing zinc thresholds
378 for phytotoxicity and potential dietary toxicity in selected vegetable crops. Commun Soil Sci
Plant Anly 34: 1421-1434
- 380 McGrath S, Sidoli C, Baker A, Reeves R (1993) The potential for the use of metal-accumulating plants
for the in situ decontamination of metal-polluted soils. In: Eijssackers, H.J.P., Hamers, T.(Eds.),
382 Integrated Soil and Sediment Research: A Basis for Proper Prediction. Kluwer, Dordrecht, pp.
673-676.
- 384 McLaughlin MJ, Lambrechts RM, Smolders E, Smart MK (1998) Effects of sulfate on cadmium uptake
by Swiss chard: II. Effects due to sulfate addition to soil. Plant Soil 202: 217-222
- 386 McLaughlin MJ, Henderson R (1999) Effect of zinc and copper on cadmium uptake by *Thlaspi*
caerulescens and *Cardaminopsis halleri*. In Proceedings of the 5th International Conference
388 on the Bio-geochemistry of Trace Elements, Vienna. 11-15 July 1999. Int. Soc. for Trace
Element Res., Vienna. pp. 886-887
- 390 Mohammad A, Moheman A (2010) The effects of cadmium and zinc interactions on the accumulation
and tissue distribution of cadmium and zinc in tomato (*Lycopersicon esculentum* Mill.). Arch
392 Agron Soil Sci 56: 551-561
- Monsant AC, Tang C, Baker AJ (2008) The effect of nitrogen form on rhizosphere soil pH and zinc
394 phytoextraction by *Thlaspi caerulescens*. Chemosphere 73: 635-642
- Papazoglou EG (2011) Responses of *Cynara cardunculus* L to single and combined cadmium and
396 nickel treatment conditions. Ecotoxicol Environ Saf 74: 195-202
- Papoyan A, Pineros M, Kochian LV (2007) Plant Cd²⁺ and Zn²⁺ status effects on root and shoot heavy
398 metal accumulation in *Thlaspi caerulescens*. New Phytol 175: 51-58
- Podar D, Ramsey MH, Hutchings MJ (2004) Effect of cadmium, zinc and substrate heterogeneity on
400 yield, shoot metal concentration and metal uptake by *Brassica juncea*: implications for human
health risk assessment and phytoremediation. New Phytol 163: 313-324

- 402 Pongrac P, Vogel-Mikuš K, Vavpetič P, Tratnik J, Regvar M, Simčič J, Grlj N, Pelicon P (2010) Cd
induced redistribution of elements within leaves of the Cd/Zn hyperaccumulator *Thlaspi*
404 *praecox* as revealed by micro-PIXE. Nuclear Instruments and Methods in Physics Research
Section B: Beam Interactions with Materials and Atoms 268: 2205-2210
- 406 Qiu RL, Thangavel P, Hu PJ, Senthilkumar P, Ying RR, Tang YT (2011) Interaction of cadmium and
zinc on accumulation and sub-cellular distribution in leaves of hyperaccumulator *Potentilla*
408 *griffithii*. J Hazard Mater 186: 1425-30
- Reeves RD, Brooks RR (1983) Hyperaccumulation of lead and zinc by two metallophytes from a
410 mining area of central Europe. Environ Pollut 31: 277-285
- Roosens N, Verbruggen N, Meerts P, Ximenez-Embun P, Smith JAC (2003) Natural variation in
412 cadmium tolerance and its relationship to metal hyperaccumulation for seven populations of
Thlaspi caerulescens from western Europe. Plant Cell Environ 26: 1657-1672
- 414 Root RA, Miller RJ, Koeppel DE (1975) Uptake of cadmium—its toxicity, and effect on the iron ratio in
hydroponically grown corn. J Environ Qual 4: 473-476
- 416 Salt DE, Smith RD, Raskin I (1998) Phytoremediation. Annu Rev Plant Phys 49: 643-668
- Sanaeiostovar A, Khoshgoftarmanesh AH, Shariatmadari H, Afyuni M, Schulin R (2012) Combined
418 effect of zinc and cadmium levels on root antioxidative responses in three different
zinc-efficient wheat genotypes. J Agron Crop Sci 198: 276-285
- 420 Smilde KW, Vanluit B, Vandriel W (1992) The extraction by soil and absorption by plants of applied
zinc and cadmium. Plant Soil 143: 233-238
- 422 Smith GC, Brennan EG (1983) Cadmium-zinc interaction in tomato plants. Phytopathology 73:
879-882
- 424 Smolders E, Lambregts RM, McLaughlin MJ, Tiller KG (1998) Effect of soil solution chloride on
cadmium availability to Swiss chard. J Environ Qual 27: 426-431
- 426 Tang YT, Qiu RL, Zeng XW, Ying RR, Yu FM, Zhou XY (2009a) Lead, zinc, cadmium
hyperaccumulation and growth stimulation in *Arabis paniculata* Franch. Environ Exp Bot 66:
428 126-134
- Tang YT, Qiu RL, Zeng XW, Fang XH, Yu FM, Zhou XY, Wu YD (2009b) Zn and Cd
430 hyperaccumulating characteristics of *Picris divaricata* Vant. Int J Environ Pollut 38: 26-38
- Turner MA (1973) Effect of cadmium treatment on cadmium and zinc uptake by selected vegetable
432 species. J Environ Qual 2: 118-119
- Ueno D, Iwashita T, Zhao FJ, Ma JF (2008) Characterization of Cd translocation and identification of
434 the Cd form in xylem sap of the Cd-hyperaccumulator *Arabidopsis halleri*. Plant Cell Physiol
49: 540-548
- 436 White PJ, Brown PH (2010) Plant nutrition for sustainable development and global health. Ann Bot
105: 1073-1080
- 438 Wojcik M, Vangronsveld J, Tukiendorf A (2005) Cadmium tolerance in *Thlaspi caerulescens* I. Growth
parameters, metal accumulation and phytochelatin synthesis in response to cadmium. Environ
440 Exp Bot 53: 151-161
- Yang XE, Long XX, Ye HB, ZLHe, Calvert DV, Stoffella PJ (2004) Cadmium tolerance and
442 hyperaccumulation in a new Zn-hyperaccumulating plant species (*Sedum alfredii* Hance).
Plant Soil 259: 181-189
- 444 Ye BH, Yang Y, He B, Long XX, Shi WY (2003) Growth response and metal accumulation of *Sedum*
alfredii to Cd/Zn complex polluted ion levels. Acta Bot Sin 45: 1030-1036

- 446 Ying RR, Qiu RL, Tang YT, Hu PJ, Qiu H, Chen HR, Shi TH, Morel JL (2010) Cadmium tolerance of
carbon assimilation enzymes and chloroplast in Zn/Cd hyperaccumulator *Picris divaricata*. J
448 Plant Physiol 167: 81-87
- Zha HG, Jiang RF, Zhao FJ, Vooijs R, Schat H, Barker JHA, McGrath SP (2004) Co-segregation
450 analysis of cadmium and zinc accumulation in *Thlaspi caerulescens* interecotypic crosses.
New Phytol 163: 299-312
- 452 Zhang GP, Fukami M, Sekimoto H (2002) Influence of cadmium on mineral concentrations and yield
components in wheat genotypes differing in Cd tolerance at seedling stage. Field Crop Res 77:
454 93-98
- Zhao FJ, Lombi E, Breedon T, McGrath SP (2000) Zinc hyperaccumulation and cellular distribution in
456 *Arabidopsis halleri*. Plant Cell Environ 23: 507-514
- Zhao FJ, Jiang RF, Dunham SJ, McGrath SP (2006) Cadmium uptake, translocation and tolerance in
458 the hyperaccumulator *Arabidopsis halleri*. New Phytol 172: 646-654
- Zhu QS, Liu J, Li KQ, Xu JK, Liang JS, Lu XL, Yang JC (2003) Interaction of Cd and five mineral
460 nutrients for uptake and accumulation in different rice cultivars and genotypes. Field Crop Res
83: 271-281

462

464

466 **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
468 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 ($\mu\text{g g}^{-1}$)				Cd distribution(% total)			TF
Cd (mg kg^{-1})	Zn (mg kg^{-1})	Leaves	Stems	Whole shoot	Roots	Leaves	Stems	Roots	
0	0	0.4a	1a	0.5a	1a	46cd	41cd	13a	0.49a
20	0	67b	132b	87b	193b	46cd	43d	12a	0.45a
40	0	76bc	166b	107c	227b	45bcd	43d	12a	0.45a
80	0	82bc	166b	115d	507c	39ab	37bcd	25b	0.23a
160	0	150d	359d	213f	627d	37a	35bcd	28b	0.34a
240	0	233e	576f	320g	761e	39ab	30b	30b	0.41a
320	0	354g	720f	442h	1370f	37a	20a	43c	0.32a
20	150	85c	167b	111cd	213b	49d	38bcd	13a	0.56a
40	300	125d	241c	150e	538c	40abc	35bcd	24b	0.28a
80	600	260f	509e	323g	699d	58e	30b	12a	0.45a

470 **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
 472 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 ($\mu\text{g g}^{-1}$)				Zn distribution(% total)			TF
Cd (mg kg^{-1})	Zn (mg kg^{-1})	Leaves	Stems	Whole shoot	Roots	Leaves	Stems	Roots	
0	0	203a	111a	204a	66a	68a	30a	2a	3.09d
0	150	760b	328b	615b	342b	78a	20a	2a	1.81bc
0	300	1210c	606c	1021c	567c	77a	21a	2a	1.81bc
0	600	1979d	1136d	1728d	1235e	76a	21a	3a	1.42a
0	1200	4465e	4096e	4372e	3040f	77a	18a	6b	1.44a
0	1800	4360e	4867f	4474ef	3906g	78a	15a	7b	1.15a
0	2400	4870e	5072f	4862f	4593h	77a	15a	8b	1.06a
20	150	648b	364b	558b	337b	69a	28a	3a	1.66ac
40	300	893c	484c	807c	584c	77a	18a	5b	1.37a
80	600	1921d	1495d	1813d	799d	77a	18a	5b	2.29c

474

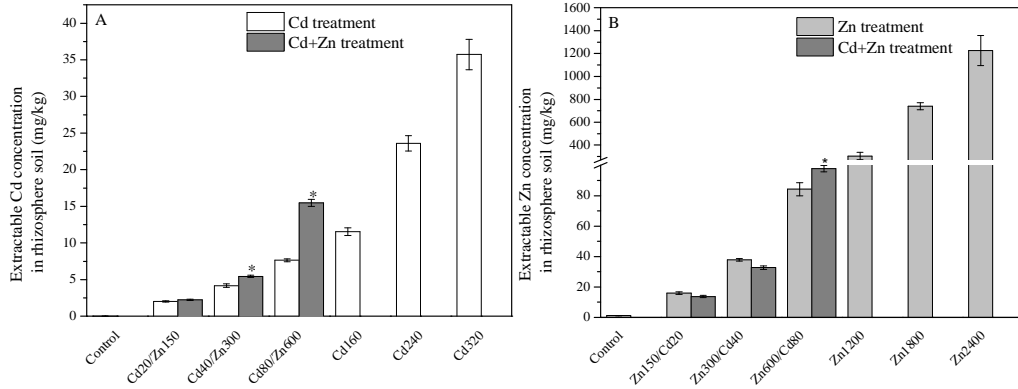
476



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

480

482

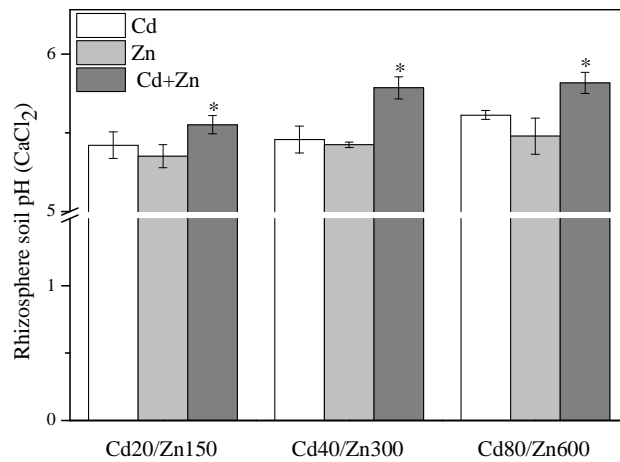


484

Figure 2. Concentrations of extractable (0.01 M CaCl₂) 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 ($p = 0.05$).

488

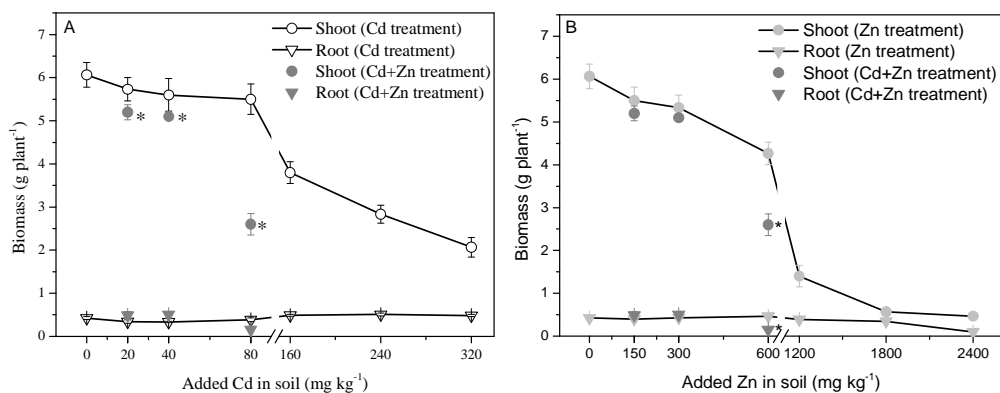
490



492

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 ($p = 0.05$).

496



500

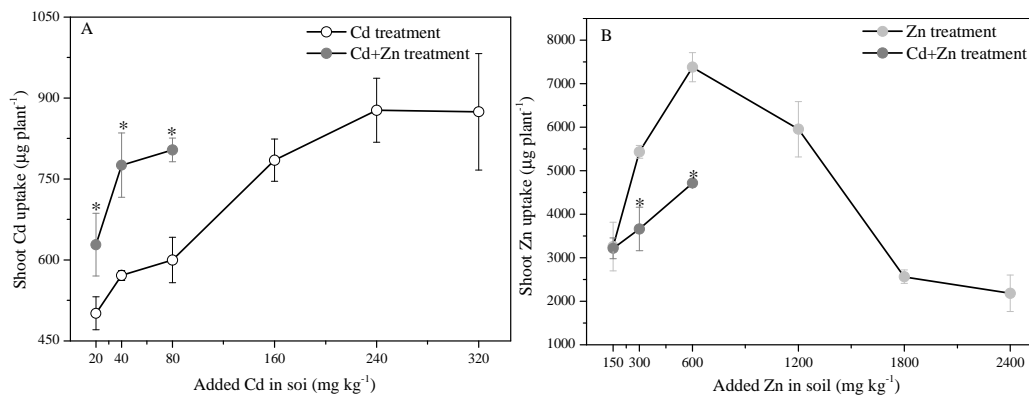
Figure 4. Effects of Cd (A) and Zn (B) addition on dry weights of shoots and roots of *Carpobrotus rossii*. Values are mean and standard errors (n=3). * indicates the significant difference between Cd+Zn treatments and corresponding Cd or Zn treatments alone ($p = 0.05$). Root biomass was significantly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

502

504

506

508



510

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 ($p = 0.05$).

512