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Cadmium uptake by *Carpobrotus rossii* (Haw.) Schwantes under different saline conditions

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Abstract: Plants used for phytoextraction of heavy metals from contaminated soils with high levels of salinity should be able to accumulate heavy metals and also be tolerant to salinity. Australian native halophyte species *Carpobrotus rossii* has recently been shown to tolerate and accumulate multiple heavy metals, especially cadmium (Cd). This study examined the effects of salt type and concentration on phytoextraction of Cd in *C. rossii*. Plants were grown in a contaminated soil for 63 days. The addition of salts increased plant growth and enhanced the accumulation of Cd in shoots up to 162 mg kg^{-1} which almost doubled the Cd concentration (87 mg kg^{-1}) in plants without salt addition. The increased Cd accumulation was ascribed mainly to increased ionic strength in soils due to the addition of salts and resultantly increased the mobility of Cd. In comparison, the addition of Cl^- resulted in 8 - 60% increase in Cd accumulation in shoots than the addition of SO_4^{2-} and NO_3^-. The findings suggest that *C. rossii* is a promising candidate in phytoextraction of Cd-polluted soils with high salinity levels.

Keywords: Cd translocation, chloride, halophyte, phytoextraction, succulent, sulphate

Introduction

Plants that can accumulate extremely high concentrations of heavy metals in their shoots (namely hyperaccumulators) (Brooks et al. 1977), or have high biomass production and relatively high accumulation of heavy metals (e.g. some crops) have been extensively studied for their phytoextraction capability in contaminated soils (Broadhurst et al. 2015, Nanda-Kumar et al. 1995). These plants are generally glycophytes which are sensitive to salinity and thus are not useful for the phytoextraction of heavy metals from polluted soils with high salinity (Helal et al. 1996, Lefèvre et al. 2009, Prasad et al. 2006). For example, high salinity strongly inhibits seed germination and the growth of glycophytic hyperaccumulator *Noccaea caerulescens* (Schwartz et al. 2003, Sirguey &Ouvrard 2013). Alternatively, halophytes are well adapted to saline environments and might have the potential to extract heavy metals from saline metal-contaminated soils. For example, it has been shown that the halophyte *Atriplex nummularia* was able to accumulate more than twice the amount of Cu and Pb in its shoots than the glycophyte *Zea mays* when grown in a mine-tailing soil with a high level of salinity (Jordan et al. 2002). Thus, halophytes may be alternative candidates for phytoextraction, if these species produce high biomass and accumulate high concentrations of heavy metals in the shoots.

Cadmium pollution in soil is a serious problem that can be exacerbated by soil salinity. This is because high salinity generally increases Cd bioavailability in soil and Cd accumulation in plants grown on Cd-

contaminated sites (McLaughlin et al. 1996). However, the effect of salinity on Cd behaviour in soil and on the Cd uptake by plants depends on Cd concentrations in soil, the salt type and soil properties. The possible reasons for the increased phytoavailability of Cd include (i) ion exchange by salt cations such as Na^+, K^+ and Ca^{2+} with Cd^{2+}, (ii) the increased Cd diffusion or solubility due to the formation of soluble complexes with anions such as Cl^- and SO_4^{2-}, and (iii) the decrease in soil pH (Acosta et al. 2011). By comparison, the effects of salts on plant Cd uptake are more complicated due to their effects on biomass production and direct or indirect interactions between Cd and other elements/ions during their uptake and transport in plants.

The anions may also be important in the effect of salinity on Cd accumulation by plants. For example, Girling and Peterson (1981) found that type of Cd salt had a large effect on Cd uptake and accumulation in barley (*Hordeum vulgare*), with Cd concentrations in shoots and roots in the order of sulphate > chloride > nitrate > acetate > sulphide. Later studies have also proven that anions like Cl^- and SO_4^{2-} closely related with the uptake and accumulation of Cd in crop plants (de Livera et al. 2011, Gonzalez-Silva et al. 2009, Hassan et al. 2005, Iqbal et al. 2012). However, these studies used crop plants (glycophytes) with a common attempt to decrease Cd accumulation in edible parts. More recently, as far as phytoextraction is concerned, few studies have been conducted using halophytes to investigate the effects of salt type on Cd accumulation. Moreover, contrasting results have been observed in halophytes. The addition of chloride salts decreased Cd concentrations in shoots of *Sesuvium portulacastrum* (Ghnaya et al. 2007), *Atriplex halimus* (Lefèvre et al. 2009) and *Kosteletzkya virginica* (Han et al. 2012), while NaCl increased both Cd translocation from roots to shoots and Cd content in shoots of *S. portulacastrum* and *Spartina alterniflora* (Chai et al. 2013, Ghnaya et al. 2007), indicating that the mechanisms of halophytes to extract heavy metals differ from those of glycophytes. Additionally, Chai *et a*l. (2013) found that the responses of Cd was concentration-dependent; increasing NaCl addition increased Cd concentration in the shoots of halophyte *S. alterniflora* grown in vermiculite irrigated with nutrient solution containing 1 mM Cd but decreasing it at 3 mM Cd.

Carpobrotus rossii (Haw.) Schwantes is a halophyte that is tolerant of saline conditions (Geraghty et al. 2011, Pirie et al. 2013). Our previous studies showed that *C. rossii* was tolerant of a mixture of the heavy metals including Cd, Cr, Cu, Mn, Ni, Pb and Zn (Zhang et al. 2015) and was able to hyperaccumulate Cd in its shoots (Zhang et al. 2014). Thus, *C. rossii* appears to be a promising species for the phytoextraction of Cd from Cd-polluted soils that are saline.

The objectives of this study were to compare the effects of different salts on the growth response and the accumulation of Cd by *C. rossii* in the presence of added Cd. We hypothesised that 1) salt addition would stimulate the growth and Cd uptake of *C. rossii* because of its halophytic nature and its ability to hyperaccumulate Cd, and 2) the addition of Cl^- and SO_4^{2-} anions would increase Cd accumulation in this species more than the addition of NO_3^- anions.

Materials and methods

Plant and soil materials

Uniform cuttings of *Carpobrotus rossii* (Aizoaceae) were used for propagation in plastic nursery cells filled with the same soil used for the experiment. A silt loam soil was collected from the topsoil (0 - 25 cm) in the farm of La Trobe University. The soil had 0.076 dS m^{-1} electrical conductivity, pH 5.41 (1:5 soil:0.01M $CaCl_2$) and contained 2.75 mg kg^{-1} total N, 44 mg kg^{-1} Colwell P, 126 mg kg^{-1} Colwell K and 0.55 mg kg^{-1} Cd (Zhang et al. 2014).

Experiment one: Effects of salt type on Cd uptake

The previous study showed that the addition of Cd at 20 - 80 mg kg^{-1} level did not affect the growth of *C. rossii* (Zhang et al. 2014) while NO_3^- has been shown to have less effects on Cd bioavailability in soil than Cl^- and SO_4^{2-} (McLaughlin et al. 1996). Thus 20 mg Cd kg^{-1} was chosen as the Cd treatment level and added as $Cd(NO_3)_2$ in this study.

There were totally thirteen treatments (including control and Cd alone), each with four replicates (Table 1). Three cations (Na^+, K^+ and Ca^{2+}) and three anions (Cl^-, NO_3^- and SO_4^{2-}) were chosen and their combinations formed nine treatments of salts. Two higher levels of NaCl and Na_2SO_4 were also included because Cl^- and SO_4^{2-} are more common anions in saline soils (including polluted soils) than NO_3^- in order to investigate the salt tolerance of plants in the presence of Cd.

Basal nutrients were added to the soil in the following composition (mg kg^{-1}): 150 KH_2PO_4, 21 $MgSO_4 \cdot 7H_2O$, 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 N to avoid possible deficiencies of nutrients. The urea was used to balance N addition between the treatments except for nitrate salts. The Cd and salts were added to the corresponding treatments, and mixed thoroughly with the soil together with basal nutrients.

After incubation of the treatment soils for two weeks, two uniform seedlings were planted into each plastic pot containing 1.5 kg soil. Water content in the soil was maintained at 80% of field capacity. After 40 days of growth in a glasshouse, plants were harvested. The rhizosphere soils were collected according to Wenzel et al. (2003). The soils were air-dried and sieved through a 2-mm mesh. Plants were separated into shoots and roots and were thoroughly washed in a solution of 1% Dextran (polysaccharide) for 2 min to remove surface dust contamination and then rinsed with deionized water 3 times (Weggler-Beaton et al. 2000). All plant parts were oven-dried at 70 ºC for 72 h, weighed and then ground into powder with a stainless steel mill (ZM200 Retsch Technology GmbH).

Experiment two: Comparison of NaCl and $NaNO_3$ effects on Cd uptake

In Experiment one, the treatment effect on biomass production might complicate the comparison of the effects of chloride and nitrate on Cd uptake although the Cd concentration in shoots was greater in the Cl^- treatments than in the NO_3^- treatments. Thus, the effects of higher levels (30 mmol kg^{-1}) of NaCl and $NaNO_3$ were further studied. Six treatments were designed as shown in Table 2.

In order to balance N addition between the treatments, 110 mg N kg^{-1} was applied as urea each time to each pot in the 3rd, 5th and 7th week except for the $NaNO_3$ treatments. After 63 days of growth, plants were harvested.

Measurements

The rhizosphere pH was measured using a pH meter (Thermo Orion 720, USA) after shaking 5 g soil sample with 25 mL 0.01 M $CaCl_2$ solution for 1 h. The concentration of water-soluble Cd in rhizosphere soil was determined. Briefly, soil samples (5 g) were shaken with 50 mL deionized water for 2 h at 22 ºC, and then centrifuged at 3000 *rpm* for 10 min, and the supernatants were then filtered through Whatman No. 1 (125 mm) filter paper. The filtrates were stored at 4 °C until analysis with inductively coupled plasma optical emission spectrometry (ICP-OES) (PerkinElmer, USA).

Concentrations of Cd and Zn in plant samples were determined using ICP-OES after digestion with a mixture of concentrated HNO_3 and $HClO_4$ (4:1 v/v).

To assess the translocation of Cd 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.

Statistical analysis

All results were presented as the mean values (±SE) obtained from four independent replicates. Statistical analysis was conducted using SPSS statistics 17.0 software package (SPSS, Chicago, Illinois, USA). Fisher LSD test was used to compare means between treatments at $p = 0.05$.

Results

Effects of salt type on plant growth (Exp. 1)

Compared to the control, root biomass was not affected by low levels of salts except for a significant increase in the Cd+K_2SO_4 treatment, but shoot biomass was significantly ($p = 0.041$) increased in all salt treatments though lesser extent for sulphate salts (Fig. 1). Both root and shoot biomass were significantly increased by the medium levels of NaCl (15 mmol kg^{-1}) and Na_2SO_4 (7.5 mmol kg^{-1}) (Fig. 1). The plants grown in the Cd-alone treatment also produced 22% higher shoot biomass compared with the control.

Effects of salt type on Cd accumulation in shoots (Exp. 1)

Compared to the Cd-alone treatment, the addition of $Ca(NO_3)_2$ at 2.5 - 5 mmol kg^{-1} decreased Cd concentration in shoots by 15% and the addition of KCl, K_2SO_4 and $CaSO_4$ increased the Cd concentration by 16%, 19% and 23%, respectively. Other salt treatments did not affect the Cd concentration in the shoots (Fig. 2). The accumulation of Cd in shoots per plant was increased only by the addition of K_2SO_4 and $CaSO_4$.

The addition of 15 mmol kg^{-1} NaCl increased Cd concentration by 34% and accumulation in shoots by 61%, while Na_2SO_4 addition increased Cd accumulation in shoots by 31% but did not affect Cd concentration (Fig. 2).

Effects of cations and anions on biomass (Exp. 1)

To compare the effects of salt cations or anions, the treatments containing same cations or anions were considered as the cation or anion treatment and thus the corresponding parameters were averaged on the basis of 12 replicates for each cation or anion.

The shoot biomass was significantly increased in all cation treatments compared with the control (Table 3). However, when compared with the Cd-alone treatment, the shoot biomass was not affected by the addition of the cations except the medium level of Na^+ (15 mmol kg^{-1}) with increasing shoot biomass by 18%.

Compared to the Cd-alone treatment, the low levels of anions did not affect the shoot biomass except NO_3^- with a significant increase by 14%, but the medium level of anions (7.5 - 15 mmol kg^{-1}) increased the shoot biomass by 11 - 21%.

Effects of cations and anions on Cd accumulation (Exp. 1)

Compared with the Cd-alone treatment, the addition of cations (Na^+, K^+ and Ca^{2+}) at 2.5 - 5 mmol kg^{-1} levels did not affect Cd concentration or accumulation in shoots except for a significant increase in Cd uptake in shoots by K^+. However, the medium level of Na^+ (15 mmol kg^{-1}) increased Cd concentration by 27% and accumulation in shoots by 45% (Table 3).

The low level of Cl^- (5 mmol kg^{-1}) tended to increase Cd concentration in shoots ($p > 0.05$), but increased Cd accumulation by 12% in shoots. NO_3^- tended to decrease Cd concentration and accumulation in shoots

($p > 0.05$) while SO_4^{2-} significantly increased Cd concentration (18%) and Cd accumulation (11%) in shoots (Table 3). The medium levels of Cl^- (15 mmol kg^{-1}) and SO_4^{2-} (7.5 mmol kg^{-1}) increased Cd concentration by 34% and 20%, and Cd accumulation in shoots by 60% and 30%, respectively (Table 3).

Effects of high levels of NaCl and $NaNO_3$ on soil properties (Exp. 2)

Compared to the control and Cd-alone treatment, the additions of NaCl and $NaNO_3$ significantly increased soil electrical conductivity (EC) in the rhizosphere with EC being greater for NaCl than $NaNO_3$ (Fig. 3).

Compared with the control, rhizosphere pH (water) was not affected by the addition of Cd or NaCl, or their combination, but was increased (0.60 unit) by the addition of $NaNO_3$ (Fig. 4).

Compared with the Cd-alone treatment, the addition of NaCl increased the concentration of water-extractable Cd in rhizosphere soil by 79% but the addition of $NaNO_3$ did not (Fig. 4).

Effects of high levels of NaCl and $NaNO_3$ on plant growth (Exp. 2)

The growth performance of *C. rossii* showed some changes when exposed to 30 mmol kg^{-1} NaCl or $NaNO_3$ in the presence (+) or absence (-) of 20 mg kg^{-1} Cd for 63 days. A visible difference in stem colour was observed between the presence and absence of Cd, lighter pink in the treatments of no Cd addition.

There was no difference in root biomass among the treatments (Fig. 5). Compared with the control, shoot biomass was decreased in the plant grown with Cd alone and Cd+$NaNO_3$, but was not affected in the Cd+NaCl treatment. The addition of NaCl or $NaNO_3$ did not affect shoot biomass compared to the control (Fig. 5).

Effects of high levels of NaCl and $NaNO_3$ on Cd accumulation (Exp. 2)

Compared with the Cd-alone treatment, the addition of NaCl increased Cd concentration in shoots by 17%, but the addition of $NaNO_3$ decreased it by 24% (Fig. 6). Thus, Cd concentration was 53% higher in the NaCl treatment than in the $NaNO_3$ treatment. As a result, the addition of NaCl increased Cd accumulation in shoots by 26% but $NaNO_3$ decreased it by 21%. Thus, Cd accumulation in shoots was 60% greater in the NaCl treatment than in the $NaNO_3$ treatment.

Compared to the Cd-alone treatment, Cd concentration in roots decreased in the NaCl treatment, but not affected in the $NaNO_3$ treatment (Fig. 7a). The Cd translocation factor (TF, the ratio of Cd concentrations in shoots to the concentration in roots) was affected by salinity, increased (by 43%) by NaCl but decreased (by 26%) by $NaNO_3$ (Fig. 7b).

Discussion

Effects of added salts on the growth of *C. rossii*

The addition of salts at 5 mmol kg^{-1} with added Cd stimulated plant growth of halophyte *C. rossii* in this study (Fig. 1). Further increases in concentration of added salts to 15 mmol kg^{-1}, at least for NaCl and Na_2SO_4, resulted in a further stimulation of shoot growth (Fig. 1). However, the growth stimulation disappeared when salt addition (NaCl or $NaNO_3$) increased to 30 mmol kg^{-1} (Fig. 5) with added Cd in the second experiment, with the high salt treatments having similar shoot weights to the control plants. In this second experiment, the added Cd reduced the shoot growth of *C. rossii*, which was most likely associated with the high Cd concentration of 140 mg kg^{-1} in the shoots (Fig. 4). Adding 30 mmol kg^{-1} of NaCl to the Cd treatment was able to alleviate the growth suppression, such that shoot biomass did not differ significantly from that of the control.

This growth stimulation of *C. rossii* is not surprising, given that such stimulations are common in halophytes (Lefèvre et al. 2009, Zurayk et al. 2001). Similarly, the lack of suppression by Cd with added salts is consistent with the results of Ghnaya et al. (2007). Ghnaya et al. (2007) observed that in the presence of Cd (50 - 100 μM), salinity (NaCl, 100 - 400 mM) increased the growth of halophytic Cd hyperaccumulator *Sesuvium portulacastrum*, which is from the same family (Aizoaceae) as *C. rossii*. In contrast, salinity in the presence of Cd has shown to decrease the growth of glycophytes like Cd hyperaccumulator *N. caerulescens* (Sirguey & Ouvrard 2013) and non-hyperaccumulator wheat (Muhling & Lauchli 2003). These responses highlight the potential value of *C. rossii* as a plant for Cd phytoremediation in saline environments.

Effects of added salts on Cd accumulation

The addition of salts increased the accumulation of Cd in the shoots of *C. rossii* in this study (Figs. 2 and 7). One of the reasons for this increased accumulation was that salt addition increased Cd phytoavailability in the soil. This is clearly evident in Figure 4 that the addition of 30 mmol kg^{-1} of NaCl increased the concentration of H_2O-extractable Cd in the rhizosphere soil by 80%, from 0.038 to 0.086 mg kg^{-1} (Fig. 4). This effect can be attributed in part to the decreased soil pH (Fig. 4) and the increased EC (an indication of ionic strength) (Fig. 3). Additionally, the competition of salt-derived cations with soil binding sites might be in part responsible for the increased solubility of Cd in soil (Lores & Pennock 1998). The increased solubility in soil by the addition of salts was also observed in other studies (Acosta et al. 2011, Ondrasek et al. 2012, Ozkutlu & Turan 2013). A further reason for the increased accumulation of Cd was related to the increased plant growth stimulated by salt addition, given that the accumulation of Cd is a product of the concentration of Cd in shoots and the shoot biomass. However, the increase at the high levels of salts (NaCl) was attributed only to the enhanced concentration of Cd in shoots (Fig. 6) since the high salinity did not affect shoot biomass compared to the Cd-alone treatment or the control (Fig. 5).

The marked effects of salinity on increasing Cd accumulation were also reported by Ghnaya et al. (2007) in the halophyte *S. portulacastrum* in solution culture. In another study, Zurayk et al. (2001) observed that Cd accumulation was generally enhanced by low salinity level (9 dS m^{-1}) but not by high salinity level (18 dS m^{-1}) in four salt-tolerant non-hyperaccumulators [barley (*Hordeum vulgare* L.), purslane (*Portulaca oleracea* L.), *Inula crithmoides* L., and *Plantago coronopus* L.]. Thus, these findings suggest that the effects of salinity on Cd accumulation by different species are related mainly to their salt tolerance and their Cd tolerance, irrespective of hyperaccumulators or non-accumulators.

Effects of ion species on Cd accumulation

Cadmium accumulation in the shoots of *C. rossii* in this study was affected by the cation species of the added salts. In this respect, the K^+ ions resulted in significantly more Cd accumulation in the shoots than the Na^+ ions (by 9%) and the Ca^{2+} ions (by 12%). The differences resulted from smaller increases in shoot biomass and Cd concentration in the shoots with the added Na^+ and Ca^{2+} salts (Table 3). Ca^{2+} and Na^+ also slightly increased shoot biomass and Cd concentration in shoots, but lesser extent than did K^+ (Table 3). It is difficult to explain why these differences in Cd accumulation occurred between the cations. It is likely that the different cations of salts like K^+, Na^+ and Ca^{2+} have different effects on Cd uptake in different species. Kim *et al.* (2002) reported that Cd uptake of rice (*Oryza sativa* L.) roots was decreased by Ca^{2+} but not by K^+. However, K^+ addition decreased Cd concentrations in oat (*Avena stiva*) and lettuce (*Lactuca sativa*) (John

1976), but increased Cd concentration in spring wheat (*Triticum aestivum*) (Zhao et al. 2004). Thus it appears that the effect of cations on Cd accumulation is plant-specific.

Cadmium accumulation in the shoots of *C. rossii* was also affected by the anion species of the added salts. The key finding here was the general increase in Cd accumulation with the added Cl^- ions, compared to the SO_4^{2-} and the NO_3^- anions. At the low levels of added salt (5 mmol kg^{-1}), the Cd accumulation with the Cl^- and SO_4^{2-} anions was significantly higher than with the NO_3^- anions (Table 3) while at the moderate levels of salt (15 mmol kg^{-1}) the Cl^- anions resulted in a 23% higher Cd uptake in the shoots than with the SO_4^{2-} ions. At the highest level of added salt (30 mmol kg^{-1}), the Cl^- anions resulted in a 60% increase in shoot Cd accumulation over the accumulation with the NO_3^- anions (Fig. 6), due largely to the higher Cd concentration in the shoots with the Cl^- anions. The common cation in this case was the Na^+ ion. Similar results were also observed in other studies, irrespective of growth medium, growth conditions and species (halophyte or glycophyte) (Lopez-Chuken &Young 2005, McLaughlin et al. 1998, Muhling &Lauchli 2003, Oporto et al. 2009, Weggler-Beaton et al. 2000). However, there were exceptions. Koren et al. (2013) observed no difference in the concentration and accumulation of Cd between Cl^- and SO_4^{2-} treatments (100 - 300 μM) in shoots of Cd-hyperaccumulator *N. praecox* grown in nutrient solution. Lefèvre et al. (2009) showed that Cd concentrations in leaves and roots of halophyte *Atriplex halimus* L. were lower in Cl^- (50 mM) than in NO_3^- (50 mM) treatment. The inconsistent results might be attributed to the high levels of EDTA used in the latter two experiments, 66 vs. 157 μM, which have higher affinity to Cd^{2+} than Cl^-.

There are several properties in the soil solution that contributed to the higher Cd accumulation with 30 mmol kg^{-1} of NaCl, compared with the same amount of added $NaNO_3$ (Fig. 6). The first reason was that the soil pH was 0.6 units lower with the NaCl treatment (Fig. 4) which might have resulted from the form of N supplied to the plants. Urea often decreases soil pH due to the nitrification of the ammonium N after hydrolysis (Ayanaba & KangUrea 1976), whereas NO_3^- application raises soil pH due to the effect of excess uptake of anions over cations (Monsant et al. 2008, Xie et al. 2009). The second reason was that the ionic strength (as indicated by EC) was higher (79%) in the NaCl+Cd treatment than that in the $NaNO_3$+Cd treatment (Fig. 3). The mobility of heavy metals is often increased with increasing ionic strength in soils (Elzinga & Sparks 2002) because of the decreased net negative charges on the surface of soil colloids (Bolan et al. 1986). Furthermore, urea addition could increase ionic strength due to urea hydrolysis followed by nitrification in soil (Mitchell et al. 2000).

The higher accumulation of Cd with Cl^- anions in this study could be related to the formation of soluble complexes of Cd with Cl^- anions. Many studies have shown how Cl^- increases the mobility of Cd in soil more than SO_4^{2-} and NO_3^- anions due to stronger complexation of Cd^{2+} with Cl^-. This could result in more Cd desorption from binding sites of soils (Acosta et al. 2011), especially organic Cd complexes (Gabrijel et al.2009). Additionally, Cl^- may facilitate translocation of Cd from roots to shoots, which was evidenced by the higher Cd concentration in shoots (Fig. 6) and lower Cd concentration in roots (Fig. 7a), and as a result higher translocation factor compared to the $NaNO_3$ and Cd-alone treatments (Fig. 7b). Increased Cd translocation from roots to shoots by Cl^- was also observed with Cd in *Leucaena leucocephala* (Helal et al. 1999) and *Sesuvium portulacastrum* (Ghnaya et al. 2007). Although it has been suggested that the intact complexes of Cd^{2+} and Cl^- are taken up directly by roots (Ghnaya et al. 2007), there is no direct evidence to support this suggestion. Recent studies have indicated that NaCl changes the abundance of mRNA coding for heavy metal transporters (Xu et al. 2011) or modify Cd speciation in plant tissues (Wali et al. 2015), but further work is needed to look into the relevant mechanisms.

Conclusions

The present study revealed that salt addition stimulated the growth of *C. rossii* and increased its Cd accumulation. The increased Cd accumulation was ascribed to increased ionic strength which could enhance the solubility of Cd in soil. There was lack of supportive evidence to explain the marginal difference in Cd accumulation between cations, probably due to the insensitivity of halophyte *C. rossii* to low salinity. In comparison, Cl^- enhanced Cd accumulation more than SO_4^{2-} and NO_3^-, which might be related to stronger affinity of Cd^{2+} with Cl^-. Additionally, the increased translocation of Cd from roots to shoots by Cl^- addition might be in part responsible for a high accumulation but further work is needed to investigate the reason. The findings here suggest that halophyte *C. rossii* could be a promising candidate for phytoextraction of Cd-polluted soils with high salinity levels.

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Table 1 Experiment one: Concentrations of added salts in the soils.

No	Treatment	$Cd(NO_3)_2$	Salt addition	
		(mg Cd kg^{-1})	(mmol kg^{-1})	(mg kg^{-1})
1	Control	0	0	0
2	Cd	20	0	0
3	NaCl +Cd	20	5.0	292
4	KCl +Cd	20	5.0	373
5	$CaCl_2$+Cd	20	2.5	277
6	Na_2SO_4+Cd	20	2.5	355
7	K_2SO_4+Cd	20	2.5	436
8	$CaSO_4$+Cd	20	2.5	340
9	$NaNO_3$+Cd	20	5.0	425
10	KNO_3+Cd	20	5.0	506
11	$Ca(NO_3)_2$+Cd	20	2.5	410
12	NaCl+Cd	20	15.0	877
13	Na_2SO_4+Cd	20	7.5	1065

Table 2 Experiment two: Concentrations of added salts in soil.

No	Treatment	$Cd(NO_3)_2$	Salt addition	
		(mg Cd kg^{-1})	(mmol kg^{-1})	(mg kg^{-1})
1	Control	0	0	0
2	Cd	20	0	0
3	NaCl	0	30	1753
4	$NaNO_3$	0	30	2550
5	NaCl +Cd	20	30	1753
6	$NaNO_3$+Cd	20	30	2550

Table 3 Experiment one: Effects of cations and anions on shoot biomass, Cd concentration and accumulation in shoots of *Carpobrotus rossii* exposed to various salts in the presence or absence of 20 mg Cd kg^{-1} [added as $Cd(NO_3)_2$] for 40 days. Different letters within a column indicate the significant difference among treatments ($p = 0.05$).

Treatment		Shoot biomass	Shoot Cd concentration	Shoot Cd accumulation
Salt	Added concentration (mmol kg^{-1})	(g $plant^{-1}$)	(mg kg^{-1})	(μg $plant^{-1}$)
Control	-	2.5(0.04)^{a}	0.2(0.01)^{a}	5(0.2)^{a}
Cd	-	2.8(0.11)^{bc}	74(4)^{bc}	211(11)^{b}
Cations				
Na^++Cd	5	2.9(0.12)^{bc}	81(3)^{cd}	226(12)^{bc}
K^++Cd	5	3.0(0.12)^{cd}	83(2)^{cd}	247(5)^{cd}
Ca^{2+}+Cd	2.5	2.9(0.08)^{c}	76(4)^{bc}	220(9)^{bc}
Na^++Cd	15	3.3(0.09)^{ef}	94(3)^{e}	306(15)^{ef}
Anions				
Cl^-+Cd	5	3.0(0.11)^{cd}	80(2)^{cd}	237(9)^{bc}
SO_4^{2-}+Cd	2.5	2.6(0.06)^{ab}	87(3)^{d}	234(8)^{bc}
NO_3^-+Cd	5	3.2(0.09)^{def}	69(3)^{b}	219(11)^{b}
Cl^-+Cd	15	3.4(0.07)^{f}	99(5)^{e}	338(12)^{f}
SO_4^{2-}+Cd	7.5	3.1(0.08)^{cde}	89(2)^{de}	275(0.4)^{de}

Note: the values were the averages calculated from the same cation or anion treatments (e.g. for Na^+: NaCl, $NaNO_3$ and Na_2SO_4). Data in brackets are standard errors.

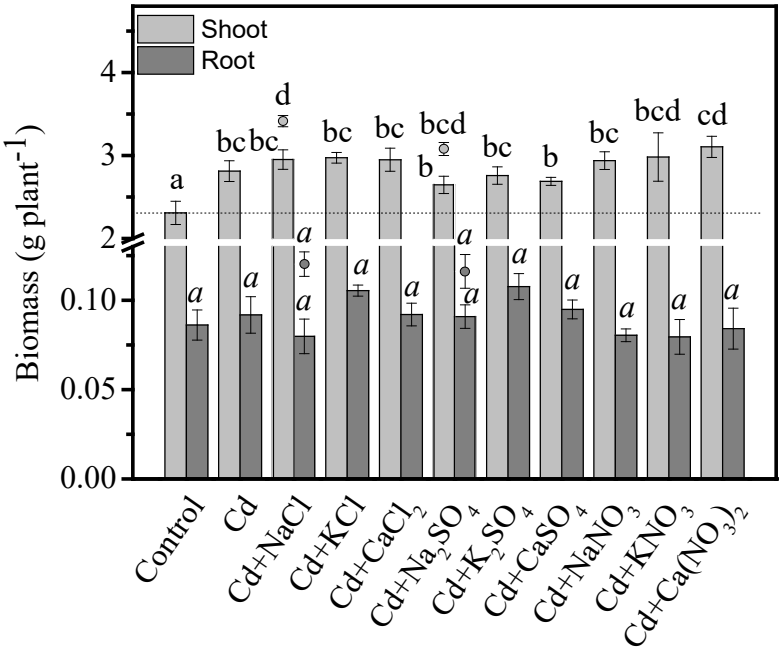

Fig. 1 Experiment one: Biomass of shoots and roots of *Carpobrotus rossii* exposed to different salts at the presence of 20 mg kg^{-1} Cd [added as $Cd(NO_3)_2$] for 40 days. The values are the mean of four replicates and the vertical error bars are ± standard errors. Different letters above the error bars indicate the significant difference among treatments (p = 0.05), italic lowercase for root biomass. The small cycles are for the medium NaCl (15 mmol kg^{-1}) or Na_2SO_4 (7.5 mmol kg^{-1}) treatment. The dot line is the value of the control.

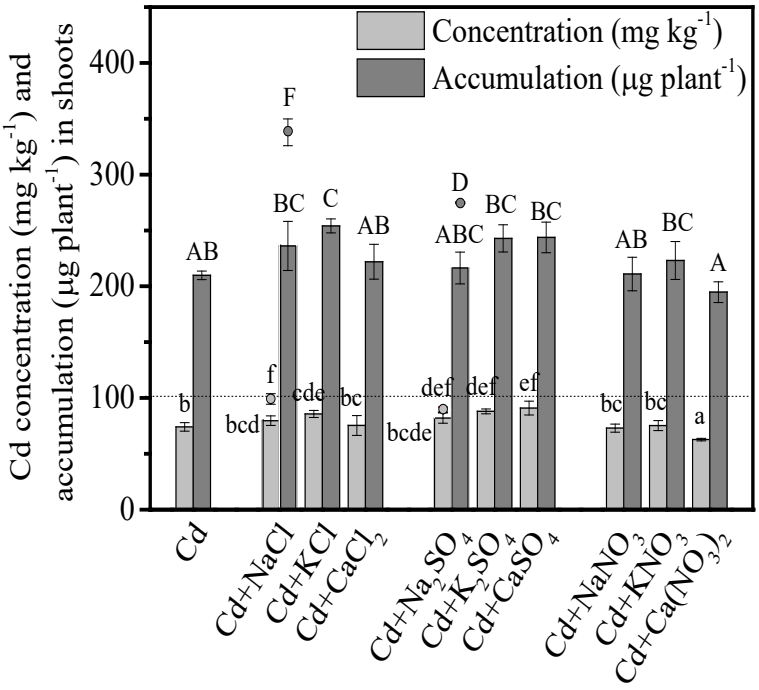

Fig. 2 Experiment one: The concentration (mg kg^{-1}) and accumulation (μg $plant^{-1}$) of Cd in shoots of *Carpobrotus rossii* exposed to nine salts at the presence of 20 mg Cd kg^{-1} [added as $Cd(NO_3)_2$] for 40 days. The values are means of four replicates and vertical error bars are ± standard errors. Different letters above the error bars indicate the significant difference among treatments (p = 0.05) for concentration (lower case) or accumulation (upper case). The small cycles are for medium level of NaCl (15 mmol kg^{-1}) or Na_2SO_4 (7.5 mmol kg^{-1}) treatment. The dot line was based on 100 mg Cd kg^{-1} in shoots which is the threshold concentration of Cd hyperaccumulators.

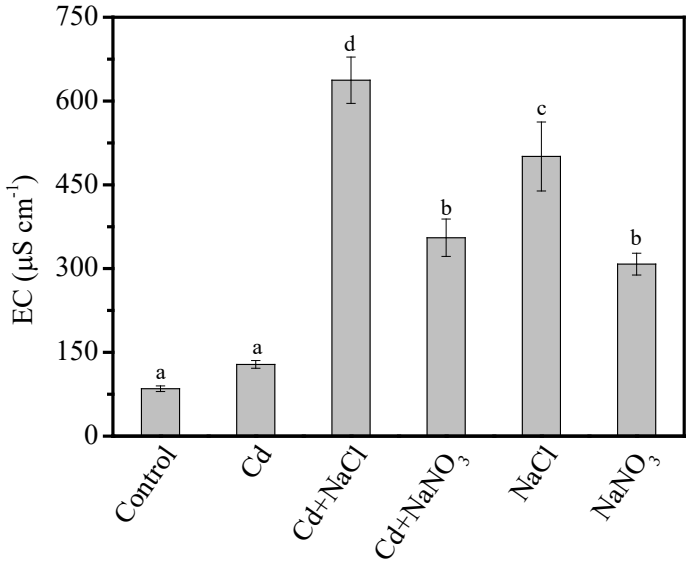

Fig. 3 Experiment two: Changes in electrical conductivity (EC) (1:5 water) of rhizosphere soil of *Carpobrotus rossii* exposed to 30 mmol kg^{-1} NaCl or $NaNO_3$ in the presence or absence of 20 mg Cd kg^{-1} [added as $Cd(NO_3)_2$] for 63 days. The values are the mean of four replicates and the vertical error bars are ± standard errors. Different letters above the error bars indicate the significant difference among treatments (p = 0.05).

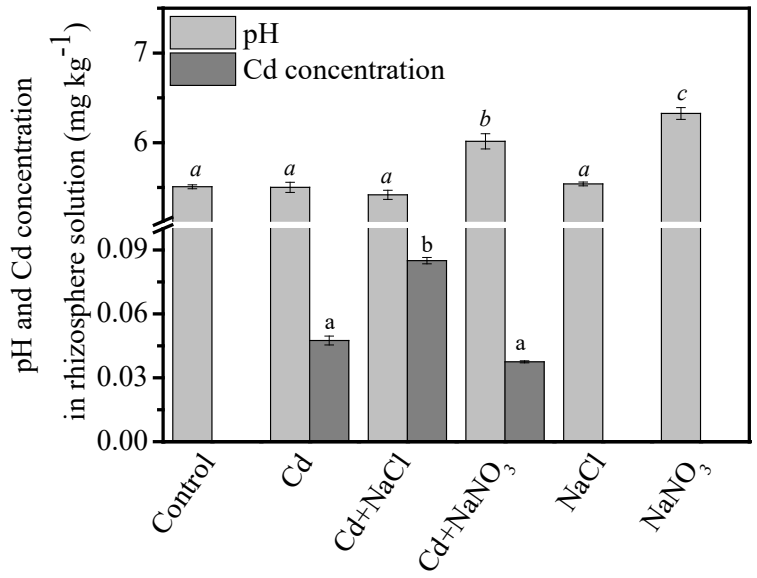

Fig. 4 Experiment two: Changes in rhizosphere soil pH (water) and concentrations of water-extractable Cd in rhizosphere soil of *Carpobrotus rossii* exposed for 63 days to 30 mmol kg^{-1} NaCl or $NaNO_3$ with or without the addition of 20 mg Cd kg^{-1} [added as $Cd(NO_3)_2$]. The values are the mean of four replicates and the vertical error bars are ± standard errors. Different letters above the error bars indicate the significant difference among treatments (p = 0.05) for rhizosphere pH (italic) and Cd concentration.

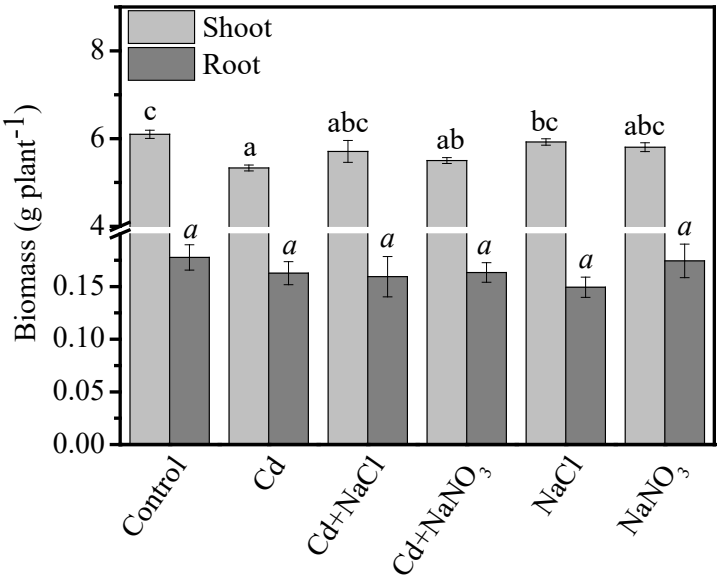

Fig. 5 Experiment two: Biomass of shoots and roots of *Carpobrotus rossii* exposed to 30 mmol kg^{-1} NaCl or $NaNO_3$ with or without the addition of 20 mg Cd kg^{-1} [added as $Cd(NO_3)_2$] for 63 days. The values are the mean of four replicates and the vertical error bars are ± standard errors. Different letters above the error bars within a plant tissue indicate the significant difference among the treatments (p = 0.05).

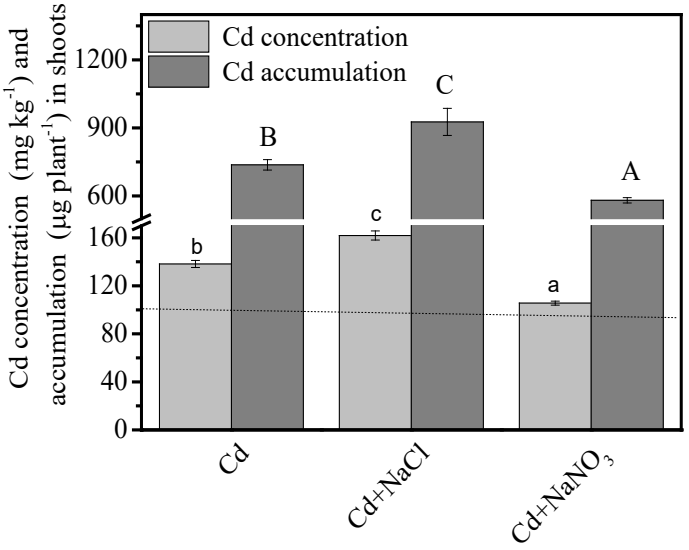

Fig. 6 Experiment two: The concentration and accumulation of Cd in shoots of *Carpobrotus rossii* exposed to 30 mmol kg^{-1} NaCl or $NaNO_3$ with the addition of 20 mg Cd kg^{-1} [added as $Cd(NO_3)_2$] for 63 days. The values are the mean of four replicates and the vertical error bars are ± standard errors. Different letters above the error bars indicate the significant difference among treatments (p = 0.05) for concentration (lower case) or accumulation (upper case). The dot line was based on 100 mg Cd kg^{-1} in shoots which is the threshold concentration of Cd hyperaccumulators.

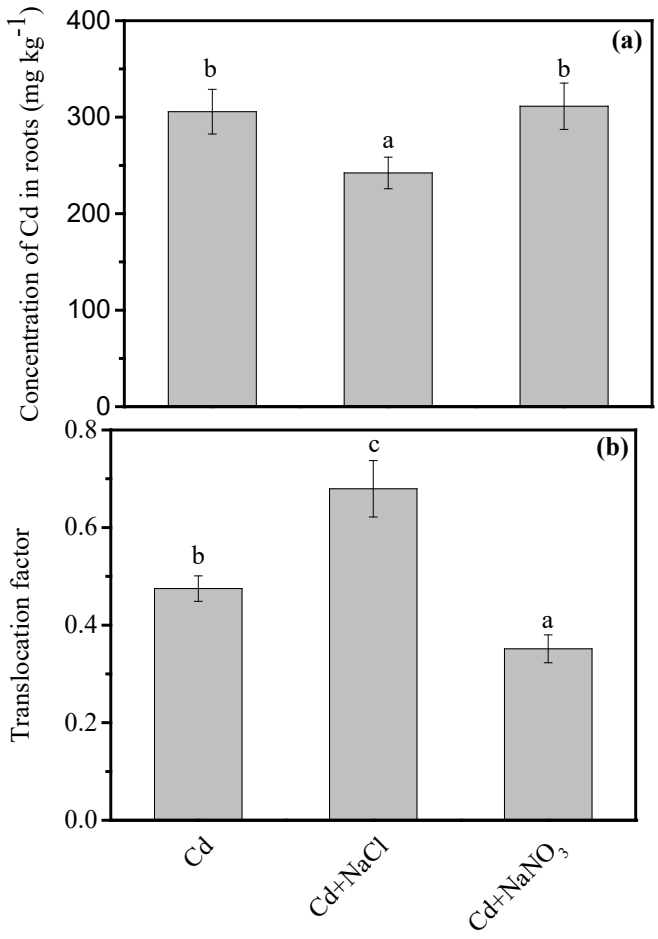

Fig. 7 Experiment two: Cd concentration in roots **(a)** and Cd translocation factor (TF) **(b)** of *Carpobrotus rossii* exposed to 20 mg Cd kg^{-1} [added as $Cd(NO_3)_2$] for 63 days. The TF is the ratio of Cd concentration in shoots to the concentration in roots. The values are the mean of four replicates and the vertical error bars are ± standard errors. Different letters above the error bars indicate the significant difference among treatments ($p = 0.05$).