Amaranthus tricolor has the potential for phytoremediation of Cd-contaminated soils

Toshihiro Watanabe, Yasutoshi Murata, Mitsuru Osaki

Research Faculty of Agriculture, Hokkaido University, Hokkaido, Japan

Address correspondence to Toshihiro Watanabe: Research Faculty of Agriculture, Hokkaido University, Kita-9, Nishi-9, Kita-ku, Sapporo, Hokkaido, 060-8589, Japan

Abstract: Phytoremediation is a developing technology that uses plants to cleanup pollutants in soils. To adopt this technology to cadmium (Cd)-contaminated soils efficiently, a Cd hyperaccumulator with high growth rate and large biomass is required. In the present study, we selected Caryophyllales as a potential clade that might include Cd hyperaccumulators because this clade had a high mean concentration of zinc, which was a same group element as Cd. Three species in Caryophyllales and three species in different clades were grown with Cd. Among them, *Amaranthus tricolor* showed high accumulating ability for Cd under both water culture and soil culture conditions, whereas *Brassica juncea*, a known Cd hyperaccumulator, accumulated high concentration of Cd in shoots only under water culture conditions. This result suggests that *A. tricolor* has Cd-solubilizing ability in rhizosphere. Since *A. tricolor* has large biomass and high growth rate, this species could be useful for phytoremediation of Cd-contaminated fields.

Keywords: *Amaranthus tricolor*, cadmium, Caryophyllales, phytoremediation

INTRODUCTION

Cadmium (Cd) contamination in soils is a significant worldwide environmental problem. The crops cultivated in such contaminated soils often contain significant levels of Cd that can impair human health. There are several methods for remediating Cd-contaminated soils, including soil washing, soil dressing, and Cd fixation in soils (Mulligan, Yong, and Gibbs 2001). However, the remediation of large areas of agricultural land by these technologies is not feasible economically. In contrast, phytoremediation is the use of plant-based systems to remediate contaminated soils. Phytoremediation involves the use of green plants to remove pollutants from the environment (Salt, Smith, and Raskin 1999). Plants that can accumulate and tolerate high concentration of pollutants in their shoots are candidates for the phytoremediation (Blaylock and Huang 2000).

When growing in heavy-metal-contaminated soils, plants need several systems to archive hyperaccumulation of heavy metals, including 1) solubilization ability in rhizosphere, 2) membrane transport, 3) transport from roots to shoots, and 4) internal tolerance. Until now, several Cd hyperaccumulators have been found, including *Thlaspi caerulescens* (Pence et al. 2000), *Sedum alfredi* (Yang et al. 2004), *Arabidopsis halleri* (Bert et al. 2003), and *Athyrium yokoscense* (Nishizono, Suzuki, and Ishii 1987). However, the most of these species are not suitable for phytoremediation because of their small biomass and/or slow growth rate.

Pteris vittata is well known as a hyperaccumulator of arsenic (As) (Ma et al. 2001). Meharg (2003) determined As concentration in fronds of various fern species and showed that high As accumulation ability was definitely found in the genus *Pteris* in order Pteridales. Thus, it has been elucidated that plant mineral accumulation is affected by evolutionary factors (Jansen et al. 2002; Watanabe et al. 2007). Although Cd hyperaccumulators have often been found in Brassicaceae, phylogenetic variation in Cd accumulation has not been studied. In the present study, we estimated the clade, in which unknown Cd hyperaccumulators could occur, by analyzing an existing database (Watanabe et al. 2007). Several plant species in the clade with appropriate biomass and growth rate for phytoremediation was selected, and their Cd accumulation abilities were assessed under hydroponic and soil conditions.

MATERIALS AND METHODS

Selection of candidates for screening

To perform the screening efficiently, possible plant clade for phytoremediation was determined by analyzing an existing database. A database, which is published by Watanabe et al. (2007) as supplementary information on the Web (http://www.blackwell-synergy.com/doi/suppl/10.1111/j.1469-8137.2007.02078.x/suppl_file/NPH2078sm_TableS1.XLS), was used for the analysis. This database includes concentration of various minerals in leaves obtained from over 2,000 samples, representing 670 species and 138 families of terrestrial plants mainly in Japan. In case of Cd, however, the number of samples in the database was not enough for phylogenetic analysis. Therefore, concentration of Zn, which was a same group element as Cd and is considered to share the same transporting system as Cd (Hart et al. 2002), was used for the analysis. The median was used to expect the clade with high Zn accumulation potential. The average was not used because the outliers affected the value greatly.

Cd accumulation in various plant species in water culture experiment

Three species in Caryophyllales; *Amaranthus tricolor* L., *Rumex acetosella* L., and *Polygonum sachalinense* L., and three species in different clades; *Brassica juncea* (L.) Czern. (Eurosids II), *Melastoma malabathricum* L. (Myrtales), and *Pteris cretica* L. (Polypodiophyta) were grown in a nutrient solution with Cd. *B. juncea* was used as a positive control because it was reported that this plant accumulated high concentration of Cd in shoots when grown in hydroponic culture (Sankaran and Ebbs 2008). *M. malabathricum* and *P. cretica*, Al and As accumulators, respectively (Watanabe et al. 1998; Zhao, Dunham, and McGrath 2002), were also tested. All experiments were carried out in a greenhouse at Hokkaido University (13-15 h photoperiod and a day/night temperature of 20-28/18-22 ^{o}C). The seeds of *B. juncea, A. tricolor, R. acetosella*, and *P. sachalinense* were surface-sterilized with sodium hypochlorite for 10 min, washed with deionized water, and germinated on the mixture of vermiculite and perlite (1:1, v/v) or dried *Sphagnum* moss with moderate moisture. Uniform cuttings of *M. malabathricum* were prepared from *M. malabathricum* plants grown in a standard nutrient solution that was aerated constantly for more than one year. Plants of *P. cretica* were purchased from a garden shop. Plants of all species were transplanted to 40-L containers containing a standard nutrient solution at pH 5.5 and precultured for 30 days. The solutions were continuously aerated. The standard nutrient solution contained 2.14 mM N (NH_4NO_3), 32 μM P ($NaH_2PO_4 \cdot 2H_2O$), 0.77 mM K (K_2SO_4 : KCl =

1:1), 1.25 mM Ca ($CaCl_2 \cdot 2H_2O$), 0.82 mM Mg ($MgSO_4 \cdot 7H_2O$), 35.8 µM Fe ($FeSO_4 \cdot 7H_2O$), 9.1 µM Mn ($MnSO_4 \cdot 4H_2O$), 46.3 µM B (H_3BO_3), 3.1 µM Zn ($ZnSO_4 \cdot 7H_2O$), 0.16 µM Cu ($CuSO_4 \cdot 5H_2O$), and 0.05 µM Mo ($(NH_4)_6Mo_7O_{24} \cdot 4H_2O$); total SO_4 = 1.06 mM.

After the preculture, the plants were transplanted to 1.5-L pots containing the treatment solution that was continuously aerated (2 plants per pot). The treatment solution contained the standard nutrient solution with or without 10 µM Cd ($CdCl_2$). The pH of the solutions was adjusted to 5.5 with NaOH or HCl every day, and the solutions were renewed every week. The plants were sampled 2 weeks after the treatment started. The plants were washed with deionized water and then separated into roots and shoots. The samples were dried in a forced-air oven at 80 ^{o}C for 72 h, weighed and ground. After that 100 mg of the ground samples were digested by H_2SO_4-H_2O_2, Cd and Zn concentrations were determined by an inductively coupled plasma emission spectrophotometry (ICPS-7000, Shimadzu, Kyoto, Japan).

Cd accumulation in various plant species in soil culture experiment

B. juncea, *A. tricolor*, and *P. sachalinense* were grown in Cd-contaminated soils. Cd-contaminated soils were prepared by adding appropriate amounts of $CdCl_2$ to the field soils in Hokkaido University (brown lowland soil, pH(H_2O) = 6.5). The soils were incubated in a greenhouse for 6 months with occasional watering. Finally, the contaminated soils contained 10 mg kg^{-1} Cd (0.1 M HCl extractable). Non-contaminated field soils were also prepared for the control treatment. The soils were passed through a 2 mm sieve before use. Fifteen seeds of a plant species were surface-sterilized with sodium hypochlorite for 10 min, washed with deionized water, and sown in each pot. After germination, plants were thinned to 4 plants per pot. The plants were grown for 30 days in a greenhouse of Hokkaido University. After the treatment, the shoot of each plant was cut and washed with deionized water. Then, the shoots were dried at 80 ^{o}C for 72 h, weighed, and ground. Cd and Zn concentrations in the ground samples were determined as described above.

Statistics

The results were analyzed with analysis of variance and Tukey's multiple comparison test, or with Student's *t*-test ($P < 0.05$).

RESULTS AND DISCUSSION

Water culture experiment

Caryophyllales was selected as a possible clade which includes Cd accumulators because the median concentration of Zn, a same group element of Cd, was the highest in this clade (Fig. 1). High accumulation of metal elements in Caryophyllales has been found so far: aluminum in *Fagopyrum esculentum* (Ma et al. 1997), some heavy metals in *Polygonum thunbergii* (Kim et al. 2003). In the present study, three plant species in Caryophyllales, *A. tricolor*, *R. acetosella*, and *P. sachalinense,* were selected as candidates for screening and grown in a nutrient solution with Cd. Ten µM of Cd did not significantly inhibit the growth of all plant species used in the hydroponic experiment (Fig. 2). Although it did not reach the level of *B. juncea*, a positive control, Cd concentration in shoots of *A. tricolor* and *R. acetosella* were >100 mg kg^{-1} and is significantly higher than that in *P. sachalinense*, *M. malabathricum*, and *P. cretica* (Fig. 3). Cd accumulation in roots showed the same trend as in shoots, except for *P. cretica* (Fig. 3).

It is considered that organic acid in *M. malabathricum* and phytochelatin in *P. cretica* have significant roles in detoxification and/or translocation of Al and As in plant, respectively (Watanabe and Osaki 2002; Dong et al. 2005). As these compounds can make complex with Cd and be involved in its detoxification and/or translocation as well (Callahan et al. 2006), hyperaccumulation of Cd in *M. malabathricum* and *P. cretica* had been expected. However, these species did not have high Cd accumulating abilities. The lacks of membrane transport system for Cd and Cd-induced synthesis of these compounds may be related to this phenomenon. In fact, the synthesis of citrate, a ligand for Al translocation, might be specifically induced by only Al stress in *M. malabathricum* (Watanabe, Jansen, and Osaki 2006).

It has been suggested that Cd shares the same transport system with Zn (Hart et al. 2002). Actually, Zn concentration in shoots tended to be decreased by the Cd application, except for *P. cretica* (Fig. 4). In contrast, there seems no antagonistic relationship between Cd and Zn in *P. cretica*, suggesting that ferns have different mechanism(s) of Cd or Zn accumulation from angiosperms.

Soil culture experiment

Among plant species used in the water culture experiment, *A. tricolor* showed relatively high concentration of Cd in its shoots (Fig. 3). Therefore, Cd accumulating ability of *A. tricolor* was compared with that in *B. juncea* and *P. sachalinense* under soil culture conditions (10 mg Cd kg^{-1}, 0.1 M HCl extraction). Growth of *A. tricolor* was enhanced by the Cd application (Fig. 5). This may be due to the Cd-induced changes in physicochemical characteristics of soils. It has been reported that *B. juncea* does not have high Cd accumulating ability when grown in Cd-contaminated soils (Podar, Ramsey, and Hutchings 2004). Our study also showed low Cd accumulation in shoots of *B. juncea* as well as *P. sachalinense* under soil culture conditions (Fig. 6). In contrast, *A. tricolor* accumulated much higher concentration of Cd in shoots in comparison with *B. juncea* and *P. sachalinense* (Fig. 6). This indicates that *A. tricolor* has Cd-solubilizing ability in rhizosphere. Root exudates, such as organic acids, may be involved in this ability (Mench and Martin, 1991). Although *B. juncea* can efficiently absorb "soluble Cd", this species cannot be used for phytoremediation because of lacking Cd-solubilizing ability in rhizosphere.

There are several reports studying trace element accumulation in *Amaranthus* sp. *A. blitoides* accumulated high concentration of Zn and Pb in shoots when grown in artificial Zn/Pb-contaminated soils (Río-Celestio et al. 2006). Moreover, mineral analysis of shoots of 99 plant species growing in trace-element-contaminated fields showed the highest concentration of Pb, Zn, Cu, and As in *A. blitoides* (Río et al. 2002). The present study also indicated high concentration of Zn in shoots of *A. tricolor* grown in soil culture whereas it was significantly reduced by the Cd application (Fig. 6). These results suggest that plants in genus *Amaranthus* have high accumulating ability for various trace elements when growing in soils.

CONCLUSIONS

In this study, we expected that plant with high Cd accumulating ability occurred in Caryophyllales, according to the analysis of an existing mineral concentration database. Among the several Caryophyllales species tested in this study, *A. tricolor* accumulated high concentration of Cd under both water culture and soil culture conditions, whereas *B. juncea* showed high accumulating ability only under water culture conditions. This result indicates that *A. tricolor* has specific mechanisms to solubilize Cd adsorbed to soil particles. Since plants in genus *Amaranthus* often have high growth rate and large biomass, these species could be useful for phytoremediation of Cd-contaminated soils.

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FIGURE LEGENDS

Fig. 1. Median Zn concentration in leaves of different plant clades. The tree is based on recent phylogenetic insights. A plant mineral concentration database which has been developed by

Watanabe et al. (2007) is used for the analysis.

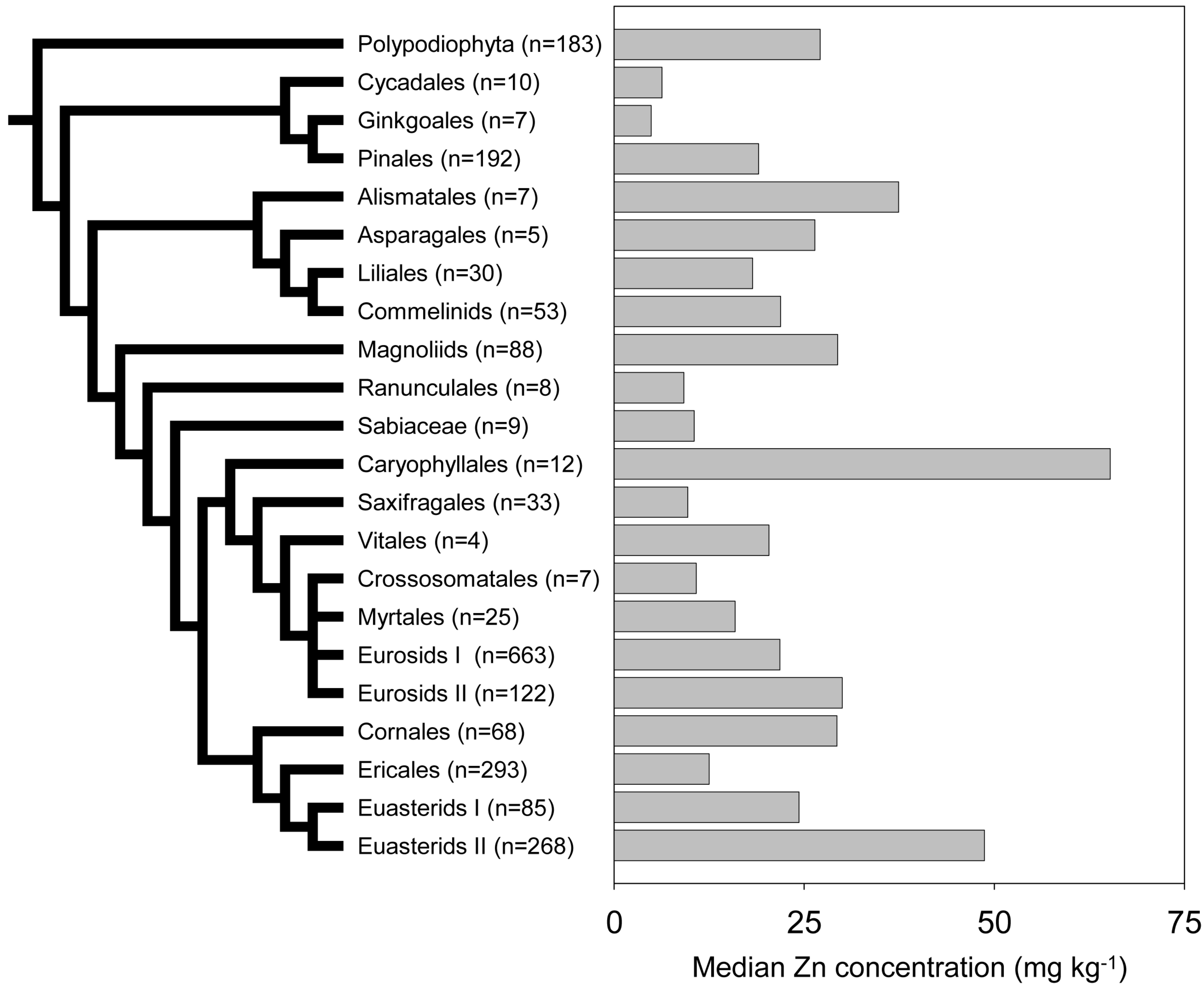
Fig. 2. Effect of Cd on the growth of plants, treated with or without 10 μM Cd in water culture experiment. Data are means of dry weight of three replicates (±SE). *, significantly different from the -Cd treatment (*P* <0.05, Student's *t*-test).

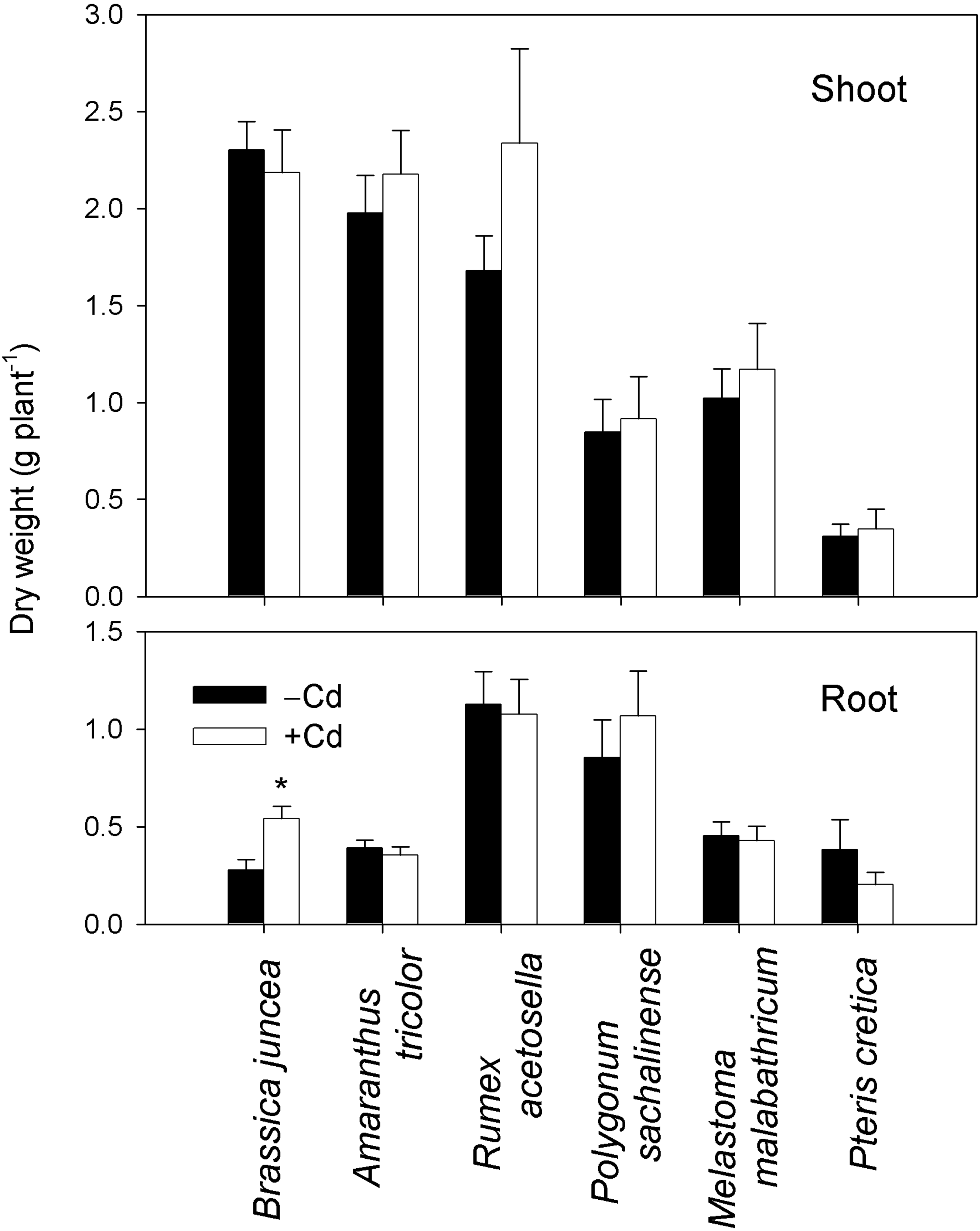
Fig. 3. Cd concentration in shoots and roots with the +Cd treatment in water culture experiment. Data are means of three replicates (±SE). Different letters in each organ indicate significant differences at $P < 0.05$.

Fig. 4. Zn concentration in shoots and roots with the treatment in water culture experiment. Data are means of three replicates (±SE). *, significantly different from the -Cd treatment (*P* <0.05, Student's *t*-test).

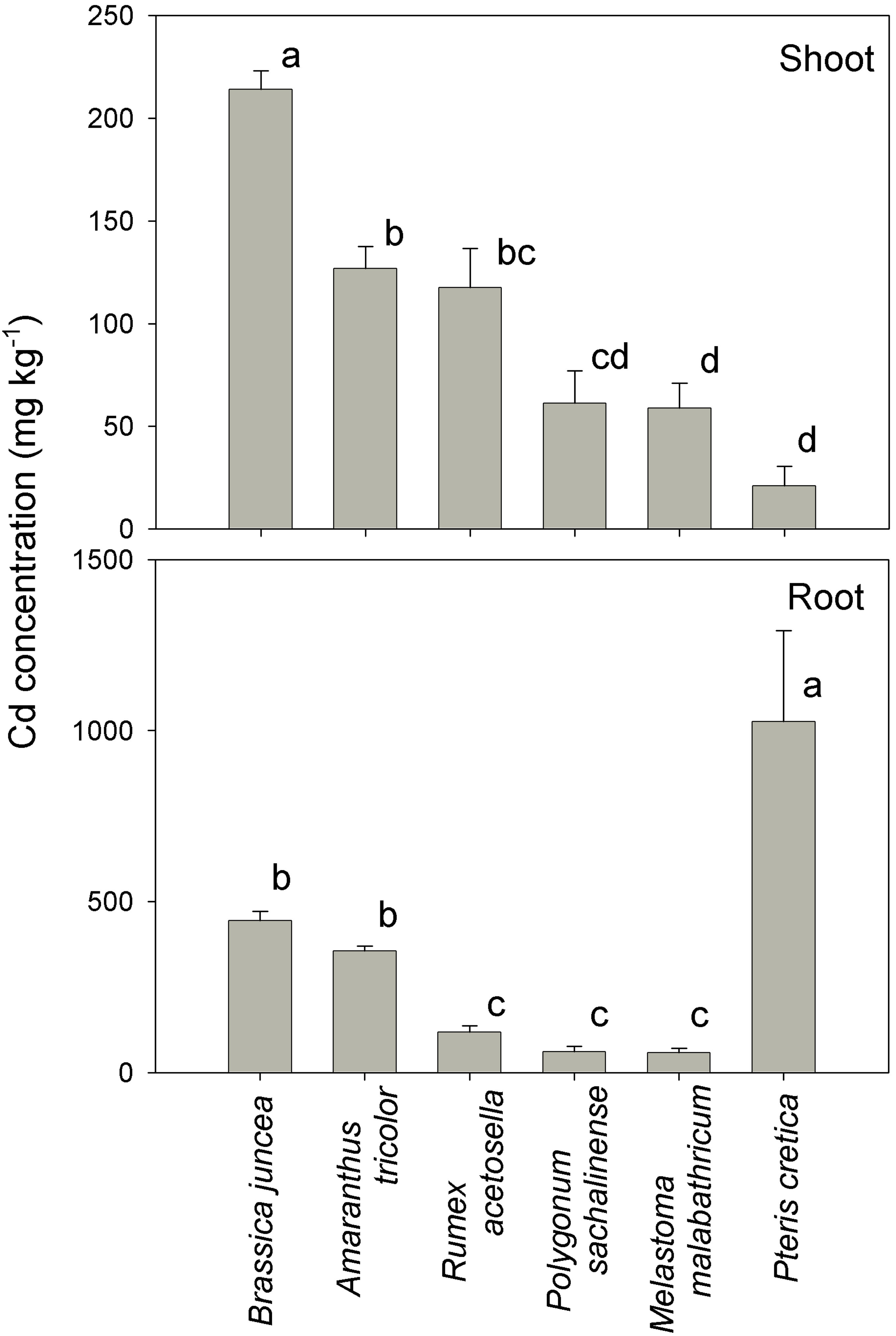
Fig. 5. Dry weight of shoots of plant after the cultivation in Cd-contaminated or non-contaminated soils. Data are means of three replicates (±SE). *, significantly different from the -Cd treatment (*P* <0.05, Student's *t*-test).

Fig. 6. Cd and Zn concentrations in shoots of plant grown in Cd-contaminated or non-contaminated soils. Only data in the +Cd treatment is shown in case of Cd concentration. Data are means of three replicates (±SE). Different letters in each organ indicate significant differences at $P < 0.05$. *, significantly different from the -Cd treatment ($P < 0.05$, Student's *t*-test).

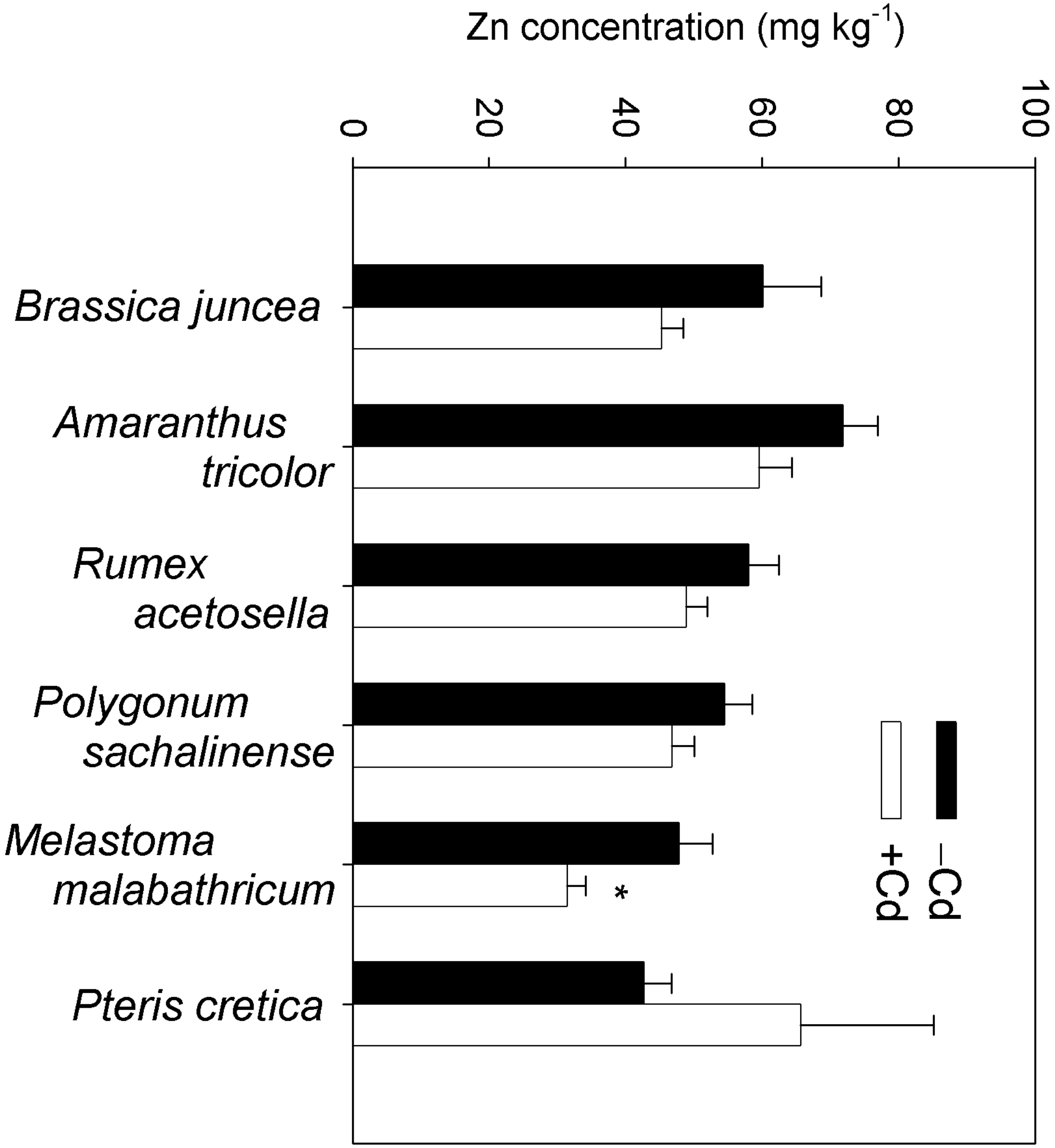

Polypodiophyta (n=183)
Cycadales (n=10)
Ginkgoales (n=7)
Pinales (n=192)
Alismatales (n=7)
Asparagales (n=5)
Liliales (n=30)
Commelinids (n=53)
Magnoliids (n=88)
Ranunculales (n=8)
Sabiaceae (n=9)
Caryophyllales (n=12)
Saxifragales (n=33)
Vitales (n=4)
Crossosomatales (n=7)
Myrtales (n=25)
Eurosids I (n=663)
Eurosids II (n=122)
Cornales (n=68)
Ericales (n=293)
Euasterids I (n=85)
Euasterids II (n=268)
0
25
50
75
Median Zn concentration (mg kg-1)



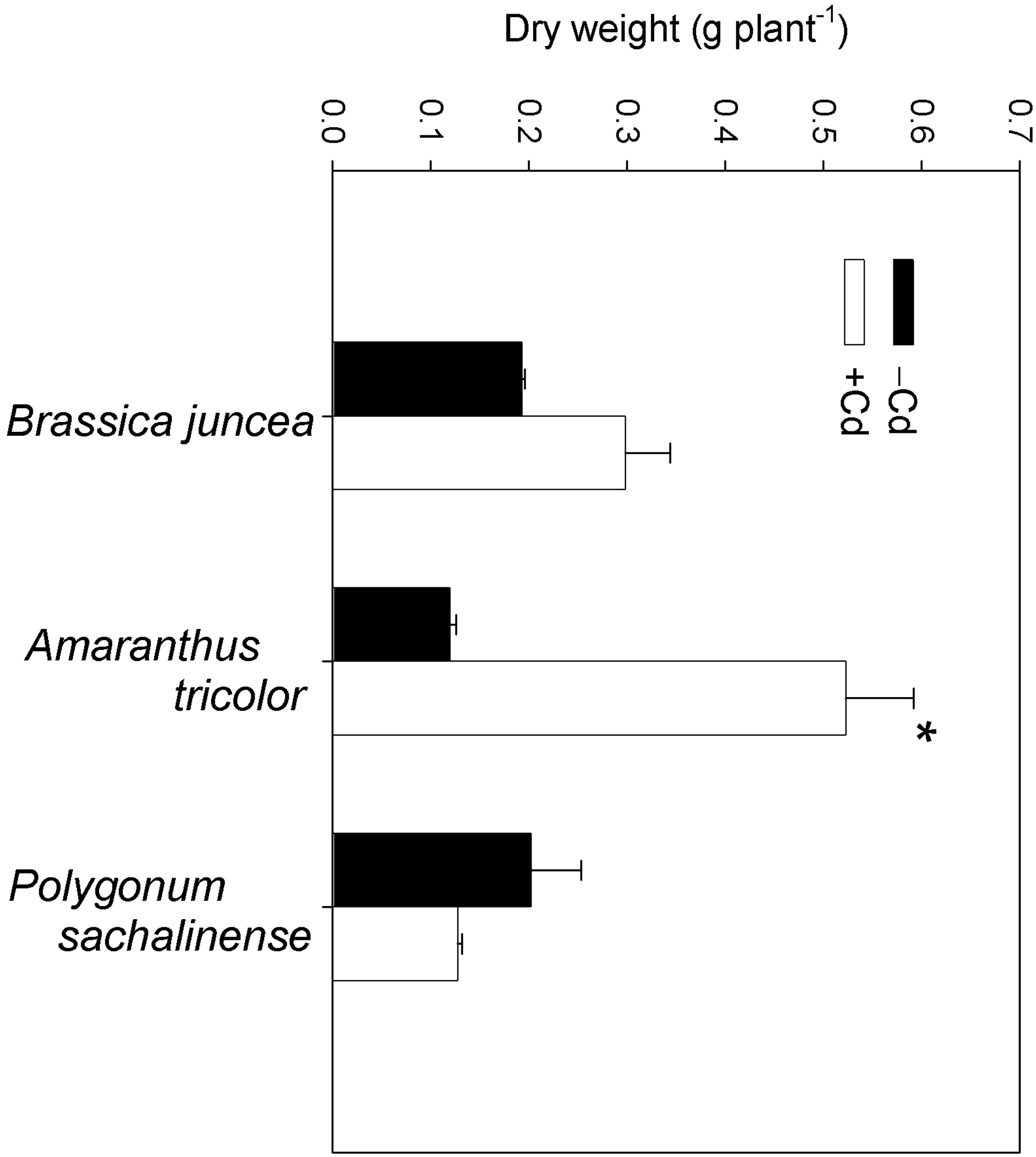
Shoot
Root
−Cd
+Cd
*
Dry weight (g plant-1)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1.5
1.0
0.5
0.0
Brassica juncea
Amaranthus tricolor
Rumex acetosella
Polygonum sachalinense
Melastoma malabathricum
Pteris cretica



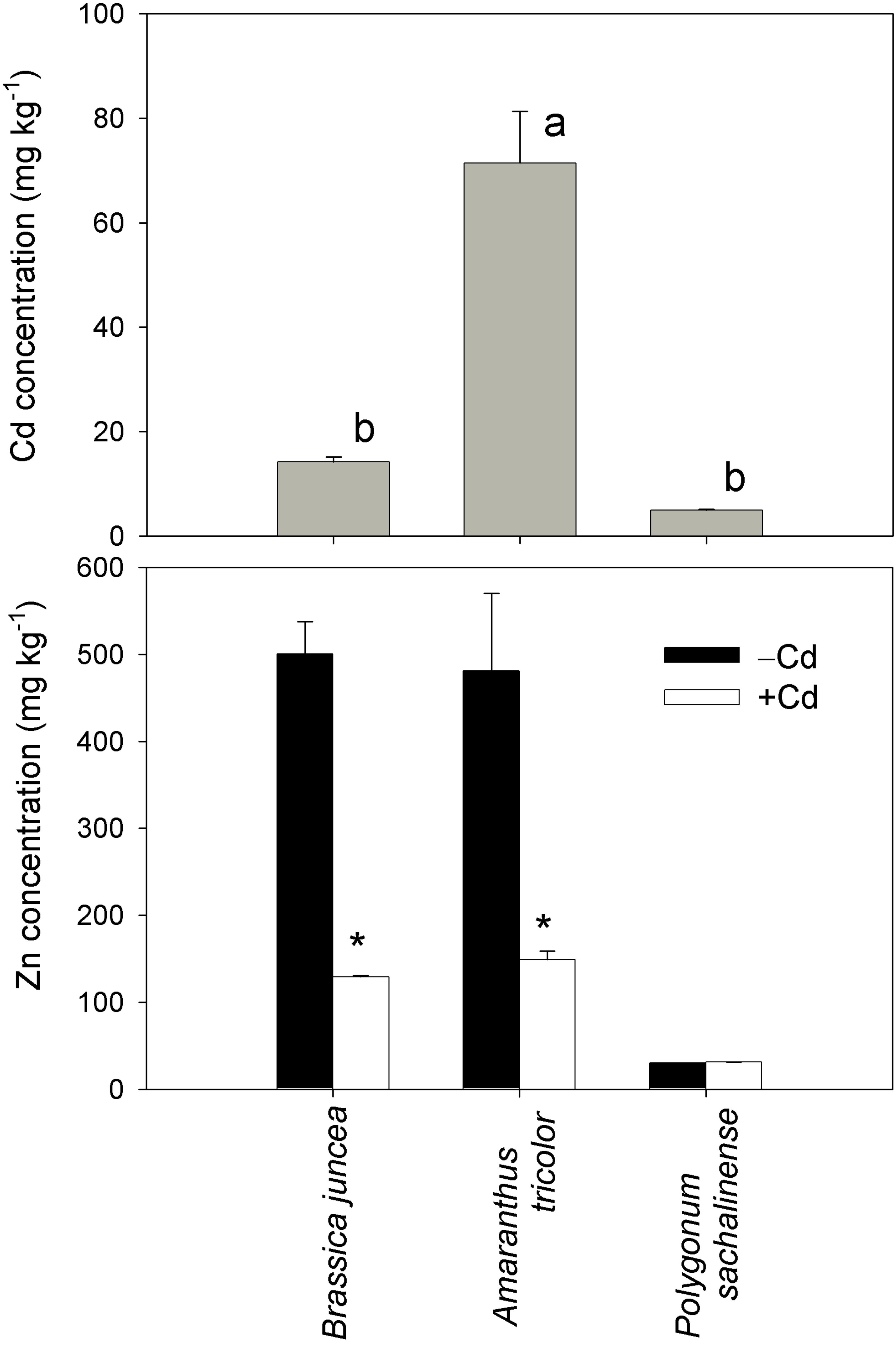
Shoot
Root
Cd concentration (mg kg-1)
250
200
150
100
50
0
1500
1000
500
0
a
b
bc
cd
d
d
b
b
c
c
c
a
Brassica juncea
Amaranthus tricolor
Rumex acetosella
Polygonum sachalinense
Melastoma malabathricum
Pteris cretica



Zn concentration (mg kg-1)
0
20
40
60
80
100
Brassica juncea
Amaranthus tricolor
Rumex acetosella
Polygonum sachalinense
Melastoma malabathricum
Pteris cretica
−Cd
+Cd
*



Dry weight (g plant-1)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
–Cd
+Cd
Brassica juncea
Amaranthus tricolor
Polygonum sachalinense
*



Cd concentration (mg kg^{-1})
100
80
60
40
20
0
a
b
b
Zn concentration (mg kg^{-1})
600
500
400
300
200
100
0
–Cd
+Cd
*
*
Brassica juncea
Amaranthus tricolor
Polygonum sachalinense