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RESEARCH ARTICLE

Cadmium accumulation and subcellular distribution in populations of *Hylotelephium spectabile* (Boreau) H. Ohba

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Abstract

A pot experiment was conducted among six populations of *Hylotelephium spectabile* (Boreau) H. Ohba: four from Jiangsu province, one from Shandong province, and one from Shanxi province, China, to investigate the variation of Cd accumulation and subcellular distribution of this species (a newly reported Cd high accumulator). Under five different real Cd-contaminated soils (Cd: 0.93–97.97 mg/kg), results showed considerable differences in Cd concentration in (a) leaf (1.09–50.7 mg/kg), (b) stem (0.61–13.0 mg/kg), and (c) root (1.55–24.5 mg/kg) among the populations. Analysis of subcellular Cd distribution indicated that Cd accumulated in the leaves of *H. spectabile* was mainly in the cellular debris (44.1 to 53.5%), followed by heat-stable protein (HSP, 20.9 to 29.0%), Cd-rich granules (MRG, 9.9 to 19.5%), heat-denatured protein (6.0 to 8.5%), and organelle fractions (3.1 to 7.4%). The populations of *H. spectabile* with more Cd partitioned to cellular debris and biological detoxified metal (HSP + MRG) fractions have greater capacity to accumulate Cd, indicating the probable intrinsic mechanism to accumulate Cd. Therefore, *H. spectabile* has the considerable potential of phytoremediation for Cd-contaminated soils, but screening suitable populations according to soil Cd concentrations is necessary before used for phytoremediation of Cd-contaminated soils.

Keywords *Hylotelephium spectabile* (Boreau) H. Ohba · Phytoremediation · Cadmium · Subcellular distribution

Introduction

Cadmium is one of the most hazardous and widely distributed contaminants in soil, which originates from industrial and agricultural activates, such as mining, metallurgy, electroplating, sewage irrigation, and the misuse of chemical fertilizers and pesticides (Sun et al. 2009). It cannot only reduce the yield of crops but may also pose hazard to human health due to the non-biodegradable character of Cd and its sequential bioaccumulation through the food chain, particularly inducing some fatal diseases, such as the "itai-itai disease" (Ji et al. 2011; Nie et al. 2016). The Bulletin of National Survey of Soil Pollution showed that 7% of soil in China is contaminated by Cd. Of the mentioned soil percentage, 85.7% of which is mainly mild and moderate Cd-contaminated soil (Ministry of Environmental Protection of the People's Republic of China, Ministry of Land and Resources of People's Republic of China 2014). Therefore, the cleanup of Cd-contaminated soils, especially farmland soil, is emergent and imperative and has aroused public concern in China (Wang et al. 2009).

Phytoremediation is widely considered as an economical, effective, and environmentally friendly strategy, as the conventional soil remediation technologies used to entail large costs for the remediation of heavy metal-contaminated soil (Ji et al. 2011; Nie et al. 2016). In the previous studies, *Hylotelephium spectabile* (Boreau) H. Ohba, formerly called *Sedum spectabile* Boreau, which is a perennial flower under Crassulaceae and widely used as an attractive landscaping material, was found to be a potential plant for sustainable phytoremediation in Cd-contaminated soil. High concentrations of Cd (15–20 mg/kg) could be accumulated in the shoot of *H. spectabile* when planted in mild Cd-contaminated (Cd: 2.2 mg/kg) real farmland soil under pot and field conditions (Guo et al. 2017). In addition, obvious differences were observed in Cd accumulation among different populations of *H. spectabile* under hydroponic conditions (Guo et al. 2018). As a widely cultivated landscape plant, *H. spectabile* is dis-

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tributed in North China and South China and has formed different genetic structures of plant populations. However, geographic isolation is well known to be one of the main factors for the formation of different genetic structures of plant populations (Yamada and Maki 2012; Chung et al. 2016). Therefore, different populations of *H. spectabile* possess significant differences in their ability of Cd accumulation, and in-depth interspecies studies needed.

The investigation on subcellular partitioning of heavy metals in plants may shed light on the mechanism of the plant to prevent toxic effects from heavy metals on the subcellular level (Wallace et al. 2003; Qiao et al. 2015). Subcellular partitioning was first used in the study of marine diatoms and bivalves under metal stress (Wallace et al. 2003; Wang and Rainbow 2006) and then optimized to explore the underlying mechanism of tolerance and toxicity of heavy metal accumulators (Zhang et al. 2015), which indicated that the differences in Cd tolerance and toxicity between two different castor cultivars (*Ricinus communis* L.) can be explained by subcellular partitioning. However, limited research is available on the subcellular distribution of Cd in *H. spectabile*, especially in different populations of *H. spectabile*, and the intrinsic mechanism of endurance and accumulation of Cd in *H. spectabile* is unclear yet.

The main objectives of this research are as follows: (1) to compare Cd uptake and accumulation of different populations of *H. spectabile* collected from clean sites and (2) to investigate the changes in the characteristics of Cd subcellular distribution of two populations of *H. spectabile* with remarkable ability of Cd translocation grown in moderate and high natural Cd-contaminated soils for exploring internal mechanism of Cd detoxification of different populations of *H. spectabile*.

Materials and methods

Hylotelephium populations and experimental design

A pot experiment was conducted to investigate the Cd accumulation and subcellular distribution of different populations of *H. spectabile* in different soils with varying degrees of Cd concentration. The pot experiment was set in a greenhouse in Beijing from 20 January to 1 March 2017 with an average diurnal temperature of 20 to 25 °C. Six populations of *H. spectabile* with obvious differences in the morphologies (Fig. 1) were screened from different nurseries, namely, four from Jiangsu province (JS), the other two from Shandong (SD), and Shanxi (SX) provinces. Five different soils were collected from the surface layer (0–20 cm) of Mianzhu city (MZ, Sichuan province), Jiyuan city (JY, Henan province), Baoding city (BD, Hebei province), Yangshuo city (YS, Guangxi province), and Qujing city (QJ, Yunnan province). Each soil was air dried, thoroughly mixed, and sieved through a 2-mm coarse sieve. Three samples were obtained from each of the bulk soil for lab analysis. The Cd concentration and physicochemical properties of tested soils are shown in Table 1. All of the tested soils exceeded the risk screening values of Cd (0.3 mg/kg with pH < 7.5 or 0.6 mg/kg with pH > 7.5) for soil contamination of agricultural land according to the "Soil environmental quality Risk control standard for soil contamination of agricultural land (GB15618-2018)."

Polyethylene pots with an inner diameter of 15 cm and height of 15 cm were used for the pot experiment. A total of 1 kg of air-dried and well-mixed soil was placed in each pot. Deionized water was added into the pot to maintain approximately 60% of the water holding capacity.

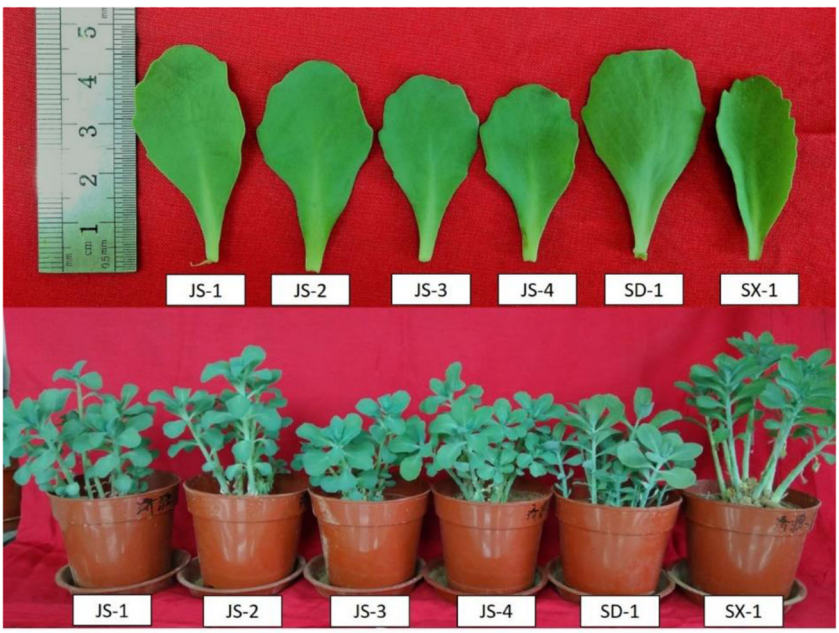

Fig. 1 Morphologies of six populations of *H. spectabile*

Table 1 Basic physicochemical properties and Cd concentrations in the tested soils

Soils	Cd concentration (mg/kg)	pH	Total nitrogen %	Total phosphorus	Total potassium	Organic matter	Available phosphorus (mg/kg)
MZ	0.93	5.5	0.12	0.08	1.49	4.16	29.1
JY	1.97	7.6	0.10	0.05	1.85	1.72	13.4
BD	4.36	8.0	0.13	0.12	1.96	1.98	70.2
YS	26.51	6.3	0.17	0.07	1.86	3.13	10.6
QJ	97.97	6.2	0.13	0.13	2.50	2.49	26.3

A two-factor experiment was designed. One factor was *H. spectabile* with six populations: JS-1, JS-2, JS-3, JS-4, SD-1, and SX-1. Another factor was five soils for trial with different Cd concentrations and physicochemical properties. The ratoon with consistent size and morphology of each population was selected for the pot experiment. Six populations of *H. spectabile* were transplanted into each pot with five different soils. Each pot was transplanted with six plants, and four replicates were set.

Plant sample preparation and chemical analysis

After 40 days of growth, plants were harvested and divided into root, stem, and leaf tissues. All the samples were washed with tap water to remove the surface dust and soil particles, and rinsed thoroughly with ultra-pure water. Then, the weight of roots, stems, and leaves were recorded separately. One part of the plant samples was used to analyze the biomass and Cd concentration; another part of the plant samples was stored in the refrigerator at − 80 °C for determination in the subcellular distribution of Cd. The samples used for determination of biomass and Cd concentration were oven dried at 65 °C to a constant weight and ground to < 0.5 mm. Each tissue of roots, stems, and leaves was weighed separately into a 50-mL flask (0.5 g) and then digested with conc. HNO_3 and $HClO_4$ (5:1; *v*/*v*). The digested samples were diluted to 25 mL with ultra-pure water, and the Cd concentration in digests of samples was determined by inductively coupled plasma mass spectrometry (ICP-MS; Elan DRC-e, Perkin Elmer, USA). Blanks and plant standard reference materials for bush twigs and leaves (GBW-07602) were used for quality control. Data are presented as the means of four replicates with standard errors. The recovery rate of samples ranges from 97.8 to 104.4%.

Subcellular distribution of Cd in *Hylotelephium* population leaves

Two populations of *H. spectabile* with different abilities of Cd translocation were selected to determine subcellular distribution of Cd. Cd subcellular partitioning in the leaves of *H. spectabile* was performed according to the differential centrifugation method (Lavoie et al. 2009; Li et al. 2014). The intracellular Cd was separated into five subcellular fractions as follows: (i) Cd-rich granules, NaOH-resistant, or phosphorus/sulfur-containing compounds (MRG); (ii) cellular debris comprising cell walls, cell membranes, and nuclei; (iii) organelle factions including chloroplasts, mitochondria, and microsomes; (iv) heat-denatured protein (HDP) fractions, e.g., enzymes; (v) heat-stable protein (HSP) fractions, including phytochelatins and metallothioneins. A brief description of the method is as follows: a 0.2-g sample was ground to powder using liquid nitrogen and a mortar and pestle. Then, the sample was placed in 10-mL plastic centrifuge tubes and homogenized in 10.0 mL of buffer solution containing 0.25 M sucrose, 1.0 mM dithioerythritol, and 50 mM Tris-HCl (pH 7.5). The homogenates were centrifuged at 15,000*g* for 15 min. The resulting pellet contained granules and cellular debris and was first re-suspended in 2.0 mL of ultra-pure water and heated for 2 min at 100 °C, digested with 2.0 mL of 1.0 M NaOH at 70 °C for 1 h, and centrifuged at 10,000*g* for 15 min to separate the Cd-rich granules (MRG, pellet) and cellular debris (supernatant). The supernatant of 15,000 g, containing the cytosol and the organelle fractions, was then centrifuged at 100,000*g* for 60 min to sediment organelle components, which was defined as organelle fractions. The 100,000 g supernatant containing the cytosol fraction was heated at 80 °C for 10 min then cooled in ice for an hour and finally centrifuged at 50,000*g* for 15 min, separating the HSP (supernatant) and the HDP (pellet). All centrifugation steps were performed at − 4 °C. All five fractions were digested with 10 mL pure concentrated HNO_3-$HClO_4$ (5:1, *v*/*v*).

The Cd concentration of the five fractions was analyzed using ICP-MS (Elan DRC-e, Perkin Elmer, USA). The Cd associated with organelles and enzymes (HDP) can be defined as a subcellular compartment containing metal-sensitive fractions (MSF), and the Cd within metallothioneins (HSP) and MRG can be presumed as biologically detoxified metal (BDM) (Wallace et al. 2003; Wang and Rainbow 2006).

Phytoextraction efficiency

The bioconcentration factor (BCF) was defined as the ratio of Cd in the leaf or stem tissues of a plant to that in soil and it was calculated as follows:

$$\mathrm{BCFleaf} = \frac{\text{Cd concentration in the leaf (mg/kg)}}{\text{Cd concentration in soil (mg/kg)}}$$

$$\mathrm{BCFstem} = \frac{\text{Cd concentration in the stem (mg/kg)}}{\text{Cd concentration in soil (mg/kg)}}$$

The translocation factor (TF) was used to evaluate the capability of plant to accumulate Cd, which is absorbed by roots to the aerial parts. The TF from stem to leaf (named TF_1) was defined as the ratio of Cd in the leaf to that in the stem, and the TF from root to stem (named TF_2) was defined as the ratio of Cd in the stem to that in root. The index was calculated as follows:

$$\mathrm{TF1} = \frac{\text{Cd concentration in the leaf (mg/kg)}}{\text{Cd concentration in the stem (mg/kg)}}$$

$$\mathrm{TF2} = \frac{\text{Cd concentration in the stem (mg/kg)}}{\text{Cd concentration in the root (mg/kg)}}$$

Statistical analysis

All experimental data in the tables and figures were analyzed using ANOVA testing. Data were presented as mean ± SE, and the difference of means was subjected to LSD test at 0.05 probability level using SPSS statistics 19.

Results

Biomass

The dry biomass of six populations of *H. spectabile* is shown in Table 2. The dry biomass of all populations ranged from 0.05 to 0.25 g/plant under the five soils. The biomasses of six populations slightly decreased with the increase of Cd concentrations in soils, except that of YS. Although the Cd concentration in YS was lower than QJ, the inhibition of growth in YS was greater than QJ. The JS-3 achieved the highest biomass in QJ and was significantly higher than JS-1. Compared with planting in MZ soil, the biomass of JS-4 population increased in JY and BD soils with the increase in Cd concentration in soil and then decreased in YS and QJ soils.

Cd concentration

The Cd concentration in different tissues of six populations of *H. spectabile* is shown in Table 3. The Cd concentrations in different tissues of all populations in MZ were equal to or a bit higher than that in JY, though the soil Cd concentration in JY was double of MZ. Similarly, although the soil Cd concentration in BD was far above JY and MZ, the Cd concentrations in different tissues of all populations in BD were far below those of JY and MZ. Cd concentrations in leaf and stem of all populations achieved the highest value in QJ soil, with the leaf Cd concentration ranging from 15.0 to 50.7 mg/kg and the stem Cd concentration ranging from 7.04 to 13.0 mg/kg.

No significant differences were observed in Cd concentration in leaf, stem, and root among all populations in MZ and YS soil as well as in stem and root Cd concentration in QJ soil. The leaf Cd concentration of JS-4 population in JY soil reached 11.8 mg/kg and was significantly higher than that of other populations ($p < 0.05$), whereas the leaf Cd concentration of JS-3 in QJ soil reached the highest to 50.7 mg/kg and was significantly higher than that of JS-1, JS-4, and SD-1 populations ($p < 0.05$).

Cd uptake

The total quantity of Cd in the shoot of *H. spectabile* is shown in Table 4. All populations had the highest Cd uptake in QJ soil, which ranged from 5.42 to 32.6 μg/pot, but lowest in BD soil, only in the range of 0.97–1.95 μg/pot. No significant difference is shown in Cd uptake of different populations in MZ and YS soils, whereas that of JS-4 in JY soil was more than twice higher than other populations with a significant level ($p < 0.05$). However, the Cd uptake of JS-3 was significantly higher than JS-2 and JS-4 in BD soil and significantly higher than JS-1 and SD-1 in QJ soil ($p < 0.05$).

BCF and TF

The BCF and TF of *H. spectabile* are shown in Table 5. Both BCF_{leaf} and BCF_{stem} of all populations decreased gradually with the increase in Cd concentration in soils. The BCF_{leaf} of JS-4 was significantly higher than other populations in JY soil ($p < 0.05$), whereas that of JS-3 was significantly above other populations in QJ soil ($p < 0.05$). No significant difference is shown in the BCF_{leaf} and BCF_{stem} of all populations in MZ and YS soils as well as in the BCF_{stem} in QJ soil.

The TF_1 of SX-1 was significantly higher than other populations except JS-1 in YS soil ($p < 0.05$), whereas that of JS-3 significantly exceeded other populations in QJ soil ($p < 0.05$). In BD soil, the TF_1 values of JS-3 and SX-1 were significantly higher than JS-4, and TF_2 of SD-1 was significantly higher than JS-1 ($p < 0.05$).

Cd subcellular distribution

Since the notable differences in Cd accumulation capacity between two populations (JS-3 and JS-4) of *H. spectabile* and the quite different accumulation characteristics of the

Table 2 Dry biomasses of six populations of *H. spectabile* growing in five soils contaminated with different Cd concentrations for 40 days (mean ± SE, g/plant, $n = 4$)

Populations	MZ	JY	BD	YS	QJ
JS-1	0.24 ± 0.02 a	0.14 ± 0.05 a	0.07 ± 0.00 a	0.13 ± 0.01 a	0.08 ± 0.01 b
JS-2	0.18 ± 0.03 ab	0.17 ± 0.02 a	0.17 ± 0.02 a	0.05 ± 0.01 b	0.11 ± 0.04 ab
JS-3	0.19 ± 0.01 ab	0.16 ± 0.04 a	0.11 ± 0.04 a	0.08 ± 0.03 ab	0.18 ± 0.04 a
JS-4	0.10 ± 0.01 c	0.14 ± 0.02 a	0.18 ± 0.03 a	0.07 ± 0.04 ab	0.10 ± 0.05 ab
SD-1	0.13 ± 0.01 bc	0.11 ± 0.01 a	0.10 ± 0.01 a	0.08 ± 0.00 ab	0.09 ± 0.00 ab
SX-1	0.25 ± 0.03 a	0.17 ± 0.05 a	0.16 ± 0.02 a	0.12 ± 0.00 a	0.15 ± 0.01 ab

Values denoted with the different letters of the same columns indicate a significant difference at $p < 0.05$ according to LSD test among the six populations grown in the same Cd-contaminated soil

two populations in JY and QJ soils, these two populations in the two soils were selected to investigate the subcellular distribution of Cd, and the results are shown in Fig. 2. The Cd enriched in subcellular fraction of the two populations follows the order of cellular debris > HSP > MRG > HDP > organelle. The cellular debris and HSP fractions of JS-4 accumulated 5.25 and 2.68 mg Cd/kg in JY soil, respectively, which are much higher than those of JS-3 ($p < 0.05$). However, the Cd concentration in all subcellular fractions of JY-3 in QJ soil increased dramatically compared with that in JY soil, and the increase in cellular debris was the most significant from 1.57 to 16.55 mg/kg, followed by HSP and MRG increase from 1.03 and 0.64 mg/kg to 6.47 and 4.16 mg/kg, respectively. Similarly, the Cd concentration in all subcellular fractions of JY-4 also increased in the soil of QJ compared with JY. However, the increment of Cd concentration in cellular and HSP of JS-4 was much lesser than that of JS-3, only from 5.25 and 2.68 mg/kg to 9.53 and 4.85 mg/kg, respectively.

The relative Cd distribution is shown in Fig. 3. Both JS-3 and JS-4 achieved Cd percentage decrement in HSP fraction and increment in organelle and HDP fractions in QJ soil in contrast to JY soil. In addition, the relative Cd distribution in cellular debris of JS-3 increased from 44.06 to 53.47% in QJ soil compared to JY soil, whereas that of JS-4 decreased from 52.45 to 43.94%.

Cd concentrations in the MSF (organelles + HDP) and BDM (HSP + MRG) fraction are shown in Fig. 4. The Cd concentration in BDM and MSF of JS-3 in QJ soil was significantly increased from 1.67 and 0.33 mg/kg to 10.64 and 3.77 mg/kg, respectively, compared with that in JY soil. Similarly, the Cd concentration in BDM and MSF of JS-4 was also increased from 3.67 and 1.09 mg/kg to 9.08 and 3.09 mg/kg, respectively, in QJ soil compared with that in JY soil.

Table 3 Cd concentration in different tissues of six populations of *H. spectabile* growing in five soils contaminated with different Cd concentrations for 40 days (mean ± SE, mg/kg, $n = 4$)

Tissues	Populations	MZ	JY	BD	YS	QJ
Leaf	JS-1	4.92 ± 0.89 a	1.66 ± 0.53 b	1.18 ± 0.52 b	10.5 ± 7.02 a	15.3 ± 1.41 b
	JS-2	5.34 ± 1.40 a	3.56 ± 0.07 b	1.12 ± 0.08 b	6.20 ± 0.04 a	28.8 ± 7.56 a
	JS-3	6.29 ± 3.61 a	4.33 ± 2.71 b	2.05 ± 0.09 a	9.32 ± 2.44 a	50.7 ± 18.9 a
	JS-4	4.26 ± 3.22 a	11.8 ± 0.87 a	1.66 ± 0.47 a	6.83 ± 0.06 a	15.0 ± 12.6 b
	SD-1	2.90 ± 0.96 a	5.71 ± 1.65 b	2.23 ± 0.34 a	14.0 ± 3.95 a	17.8 ± 6.55 b
	SX-1	5.22 ± 1.60 a	3.70 ± 1.01 b	1.09 ± 0.10 b	18.9 ± 9.01 a	22.0 ± 10.8 a
Stem	JS-1	2.82 ± 0.06 a	1.06 ± 0.02 b	0.83 ± 0.22 bc	4.29 ± 2.30 a	7.04 ± 0.14 a
	JS-2	2.88 ± 0.64 a	1.73 ± 0.11 b	0.76 ± 0.07 c	4.24 ± 0.15 a	13.0 ± 2.97 a
	JS-3	3.21 ± 1.36 a	5.45 ± 2.44 a	1.27 ± 0.21 abc	5.06 ± 0.69 a	7.66 ± 0.91 a
	JS-4	3.57 ± 2.44 a	3.73 ± 0.94 ab	1.58 ± 0.44 ab	4.79 ± 0.01 a	7.82 ± 4.35 a
	SD-1	2.90 ± 0.51 a	3.58 ± 0.35 ab	1.68 ± 0.18 a	7.16 ± 1.65 a	8.21 ± 0.60 a
	SX-1	2.38 ± 0.85 a	1.70 ± 0.12 b	0.61 ± 0.12 c	6.65 ± 3.07 a	7.77 ± 2.26 a
Root	JS-1	4.49 ± 0.40 a	3.87 ± 0.25 b	3.54 ± 0.22 ab	20.9 ± 4.34 a	10.9 ± 1.79 a
	JS-2	4.85 ± 0.43 a	5.29 ± 0.17 a	1.80 ± 0.25 c	19.1 ± 2.22 a	16.8 ± 0.98 a
	JS-3	5.22 ± 2.39 a	3.83 ± 0.31 b	2.40 ± 0.09 bc	16.7 ± 1.47 a	11.8 ± 0.63 a
	JS-4	6.84 ± 2.51 a	6.12 ± 0.79 a	3.77 ± 0.32 a	24.5 ± 6.5 a	21.1 ± 5.74 a
	SD-1	3.33 ± 0.56 a	2.97 ± 0.16 b	2.36 ± 0.65 bc	20.7 ± 1.75 a	11.5 ± 2.67 a
	SX-1	3.05 ± 1.11 a	2.89 ± 0.07 b	1.55 ± 0.24 c	18.1 ± 0.24 a	14.7 ± 3.82 a

Values denoted with the different letters of the same columns indicate a significant difference at $p < 0.05$ according to LSD test among the six populations grown in the same Cd-contaminated soil

Table 4 Total quantity of Cd uptake of six populations of *H. spectabile* growing in five soil contaminated with different Cd concentrations for 40 days (mean ± SE, μg/pot, $n = 4$)

Populations	MZ	JY	BD	YS	QJ
JS-1	3.44 ± 1.57 a	0.59 ± 0.14 b	1.40 ± 0.42 ab	6.31 ± 4.30 a	5.42 ± 1.06 b
JS-2	4.38 ± 1.42 a	1.90 ± 0.70 b	1.04 ± 0.13 b	1.61 ± 0.24 a	15.7 ± 9.21 ab
JS-3	4.82 ± 0.48 a	3.37 ± 1.47 b	1.95 ± 0.10 a	3.91 ± 1.90 a	32.6 ± 14.4 a
JS-4	3.01 ± 1.95 a	7.62 ± 1.62 a	0.97 ± 0.36 b	2.28 ± 1.52 a	8.57 ± 7.99 ab
SD-1	1.88 ± 0.45 a	3.08 ± 0.78 b	1.54 ± 0.12 ab	5.24 ± 1.08 a	8.31 ± 2.63 b
SX-1	3.63 ± 0.01 a	2.68 ± 0.36 b	1.39 ± 0.31 ab	10.6 ± 5.48 a	13.9 ± 6.75 ab

Values denoted with the different letters of the same columns indicate a significant difference at $p < 0.05$ according to LSD test among the six populations grown in the same Cd-contaminated soil

Discussion

Cd toxicity and accumulation

In the present study, the intra-species differences of six populations of *H. spectabile* are shown after short-term growing in Cd-contaminated soils from five provinces of China. Cd, as a highly toxic metal pollutant of soils, will inhibit the metabolism and normal growth of plant (Metwally et al. 2005). A characteristic that hyperaccumulator should have is to tolerate high levels of metal and grow normally due to internal biochemical detoxification (Baker and Whiting 2002; Van der Ent et al. 2013). The present work showed that all populations of *H. spectabile* are grown normally and showed no obvious grown inhibition, although a downward tendency of aboveground biomass was observed with the increase in Cd in soils. The result indicated that a constitutive trait existed at species level in *H. spectabile* to tolerate high levels of Cd. However,

Table 5 Bioconcentration factor (BCF) and transfer factor (TF) of six populations of *H. spectabile* growing in five soil contaminated with different Cd concentrations for 40 days (mean ± SE, $n = 4$)

	Populations	MZ	JY	BD	YS	QJ
BCF_{leaf}	JS-1	5.29 ± 0.95 a	0.84 ± 0.27 b	0.27 ± 0.12 b	0.40 ± 0.26 a	0.16 ± 0.01 b
	JS-2	5.74 ± 1.51 a	1.81 ± 0.04 b	0.26 ± 0.02 b	0.23 ± 0.00 a	0.29 ± 0.08 ab
	JS-3	6.76 ± 3.88 a	2.20 ± 1.38 b	0.47 ± 0.02 ab	0.35 ± 0.09 a	0.52 ± 0.19 a
	JS-4	4.58 ± 3.46 a	5.97 ± 0.44 a	0.38 ± 0.11 ab	0.26 ± 0.00 a	0.15 ± 0.13 b
	SD-1	3.11 ± 1.03 a	2.90 ± 0.84 b	0.51 ± 0.08 a	0.53 ± 0.15 a	0.18 ± 0.07 b
	SX-1	5.61 ± 1.72 a	1.88 ± 0.51 b	0.25 ± 0.02 b	0.71 ± 0.34 a	0.22 ± 0.11 ab
BCF_{stem}	JS-1	3.04 ± 0.06 a	0.54 ± 0.01 b	0.19 ± 0.05 bc	0.16 ± 0.09 a	0.07 ± 0.00 a
	JS-2	3.09 ± 0.69 a	0.88 ± 0.06 b	0.17 ± 0.02 c	0.16 ± 0.01 a	0.13 ± 0.03 a
	JS-3	3.45 ± 1.46 a	2.77 ± 1.24 a	0.29 ± 0.05 abc	0.19 ± 0.03 a	0.08 ± 0.01 a
	JS-4	3.84 ± 2.62 a	1.89 ± 0.48 ab	0.36 ± 0.10 ab	0.18 ± 0.00 a	0.08 ± 0.06 a
	SD-1	3.12 ± 0.55 a	1.82 ± 0.18 ab	0.38 ± 0.04 a	0.27 ± 0.06 a	0.08 ± 0.01 a
	SX-1	2.56 ± 0.91 a	0.86 ± 0.06 b	0.14 ± 0.03 c	0.25 ± 0.12 a	0.08 ± 0.02 a
TF_1	JS-1	1.74 ± 0.28 a	1.58 ± 0.53 a	1.36 ± 0.26 ab	2.20 ± 0.46 ab	2.17 ± 0.16 b
	JS-2	1.84 ± 0.08 a	2.07 ± 0.17 a	1.47 ± 0.04 ab	1.47 ± 0.04 c	2.19 ± 0.08 b
	JS-3	2.97 ± 2.38 a	1.27 ± 1.07 a	1.66 ± 0.35 a	1.81 ± 0.24 bc	7.01 ± 3.29 a
	JS-4	1.08 ± 0.16 a	3.44 ± 1.10 a	1.05 ± 0.00 b	1.43 ± 0.01 c	1.72 ± 0.25 b
	SD-1	0.96 ± 0.19 a	1.57 ± 0.36 a	1.32 ± 0.06 ab	1.93 ± 0.09 bc	2.11 ± 0.65 b
	SX-1	2.24 ± 0.12 a	2.14 ± 0.44 a	1.84 ± 0.19 a	2.81 ± 0.06 a	2.65 ± 0.63 ab
TF_2	JS-1	0.63 ± 0.04 a	0.27 ± 0.02 c	0.23 ± 0.05 b	0.19 ± 0.07 a	0.66 ± 0.10 ab
	JS-2	0.61 ± 0.19 a	0.33 ± 0.01 c	0.42 ± 0.02 ab	0.23 ± 0.03 a	0.77 ± 0.13 a
	JS-3	0.63 ± 0.03 a	1.38 ± 0.53 a	0.53 ± 0.07 ab	0.31 ± 0.07 a	0.65 ± 0.04 ab
	JS-4	0.75 ± 0.63 a	0.64 ± 0.24 abc	0.43 ± 0.15 ab	0.21 ± 0.05 a	0.31 ± 0.21 b
	SD-1	0.91 ± 0.24 a	1.21 ± 0.09 a	0.79 ± 0.22 a	0.34 ± 0.05 a	0.76 ± 0.16 a
	SX-1	1.02 ± 0.65 a	0.59 ± 0.03 ab	0.39 ± 0.02 ab	0.37 ± 0.18 a	0.52 ± 0.02 ab

Values denoted with the different letters of the same columns indicate a significant difference at $p < 0.05$ according to LSD test among the six populations grown in the same Cd-contaminated soil

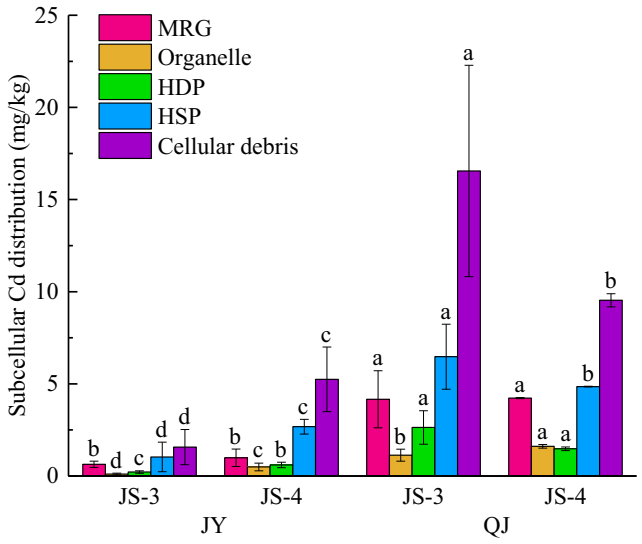

Fig. 2 Cd concentrations in the subcellular portion of JS-3 and JS-4 leaves (mean ± SE, mg/kg, $n = 4$). Different letters on the bars of the same fractions show significant difference at $p < 0.05$ according to LSD test. MRG, Cd-rich granule; HDP, heat-denatured protein; HSP, heat-stable protein

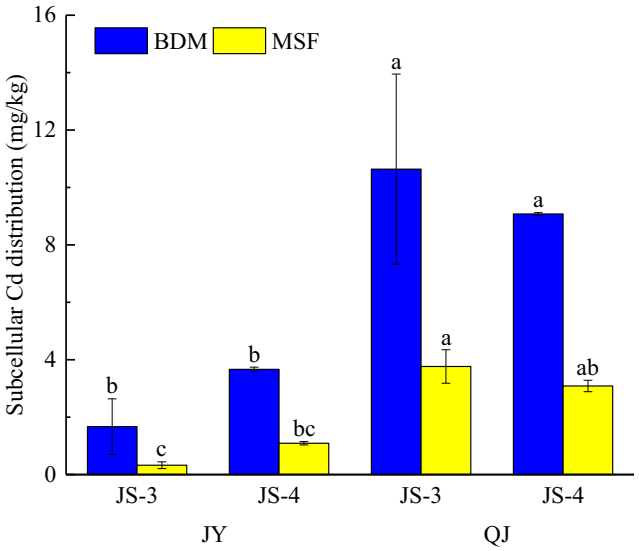

Fig. 4 Cd concentrations in the metal-sensitive fractions (MSF) and the biologically detoxified metal (BDM) fractions of leaves in the subcellular portion of JS-3 and JS-4 leaves (mean ± SE, %, $n = 4$). Different letters on the bars of the same fractions show significant difference at $p < 0.05$ according to LSD test. MRG, Cd-rich granule; HDP, heat-denatured protein; HSP, heat-stable protein

some differences were observed among different populations, for example, the biomass of JS-4 population increased first and then decreased with the increase in Cd concentration in soil. This result agrees with the findings of Deng et al. (2007) that appropriate amount of Cd promotes the growth of *Sedum alfredii* Hance, a population of Cd hyperaccumulator. Some populations of Cd accumulators appear to have the ability to promote growth under low concentrations of Cd but inhibit at high levels of Cd. Moreover, an interesting phenomenon was found that the growth of *H. spectabile* in YS was more severely inhibited than in QJ, although the Cd concentration in YS soil is less than one-third of that in QJ. That may be attributed to the relatively low level of total phosphorus and available phosphorus content in YS soil. On the one hand, the deficiency of P limited the nutrient uptake of *H. spectabile*, and on the other hand, low level of P might lead to a relatively high availability of Cd in soils and cause severe stress on plant growth. It has been well studied that the Cd and phosphorus could be deposited in the form of Cd-phosphate to reduce the Cd availability (Yu and Zhou 2009).

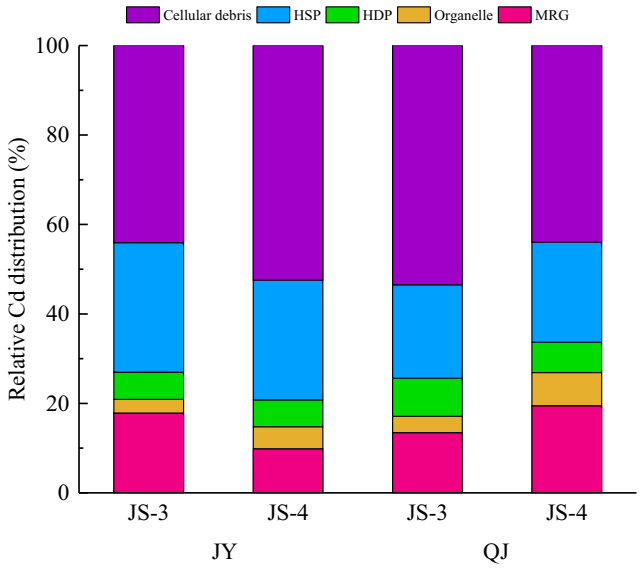

Fig. 3 Relative Cd distribution in the subcellular portion of JS-3 and JS-4 leaves (mean ± SE, %, $n = 4$). Different letters on the bars of the same fractions show significant difference at $p < 0.05$ according to LSD test. MRG, Cd-rich granule; HDP, heat-denatured protein; HSP, heat-stable protein

Generally, the plants capable of accumulating Cd to > 100 mg/kg in the leaves or other organs could be regarded as Cd hyperaccumulators (Baker et al. 1994; Van der Ent et al. 2013). In the present study, the Cd concentration in the leaf and stem of most tested populations of *H. spectabile* increased with the increment of Cd content in soil, and that of JS-3 exceeded 50 mg/kg when planted in QJ soil for 40 days. Although below the threshold concentration of Cd hyperaccumulators, we should note that much of the Cd hyperaccumulators reported in early studies were only based on hydroponic experiments with artificial exogenous addition of Cd (Gao et al. 2010; Yang et al. 2004), whereas the present results are totally subjected to natural conditions. Thus, *H. spectabile* is considered as a potential Cd accumulator that could be used in Cd-contaminated soil.

Metal hyperaccumulation is not always a constitutive trait at species level, and inter- and intra-species variation in heavy metal sensitivity has been previously reported (Metwally et al. 2005; Qiao et al. 2015). Deng et al. (2007) found that different

populations of *S. alfredii* showed a population-specific and metal-specific variation in Zn and Cd accumulation. Lombi et al. (2002) also found an intraspecific variation among four populations in *Thlaspi caerulescens*, unlike the As hyperaccumulator, *Pteris vittata* L., which showed a similar stable As accumulation ability between the two populations of plants collected from contaminated and uncontaminated soils (Chen et al. 2002). In the present study, different populations of *H. spectabile* showed significant difference on the Cd concentration in shoots, especially in leaves. In addition, the Cd concentration in leaves of JS-3 accumulated nearly three times higher than that of JS-4 in JY soil (1.97 mg Cd/kg); however, the result is opposite in QJ soil (97.97 mg Cd/kg) that JS-4 accumulated over three times of Cd than JS-3. This result might suggest that JS-3 may be suitable for severe Cd-contaminated soils but JS-4 suitable for moderate Cd-contaminated soils.

BCF and TF

BCF and TF were commonly used as reference criteria for the evaluation of hyperaccumulators, usually > 1 (Van der Ent et al. 2013). In this study, BCF_{leaf} and BCF_{stem} as well as the TF_1 and TF_2 were analyzed to clarify the accumulation and transport of Cd by *H. spectabile*. Results showed that both BCF_{leaf} and BCF_{stem} declined with the increase in Cd concentration in soil, indicating that high soil Cd concentration can lead to a decrease in BCF (Van der Ent et al. 2013). BCF_{leaf} was higher than BCF_{stem}, and TF_1 was higher than TF_2, indicating that Cd was efficiently transported in the stem of *H. spectabile*, and some mechanisms exist in the leaves to store large amounts of Cd. In JY soil, the BCF_{leaf} of JS-4 was significantly higher than other populations and up to 5.97, which was higher than some reported Cd accumulators, such as *Viola baoshanensis* (2.38) and *Solanum nigrum* L. (2.09) under the condition of similar Cd pollution (Liu et al. 2004; Wei et al. 2005). However, the BCF_{leaf} and TF_1 of JS-3 in QJ soil were significantly higher than other populations, up to 0.52 and 7.01, revealing that the phytoextraction efficiency of *H. spectabile* can remain at a relatively high level in severe Cd-contaminated soils.

Potential of phytoremediation

Cd hyperaccumulators have a suite of evaluation criteria, such as (1) accumulate > 100 mg Cd/kg in the aboveground; (b) the BCF > 1; and (c) TF > 1(Baker and Whiting 2002; Van der Ent et al. 2013). However, the potential for phytoremediation of Cd-contaminated soil mainly depends on the biomass of plant and the amount of Cd in the aboveground (Deng et al. 2007). Previous field trials conducted in JY city showed that *H. spectabile* can remove more than 70 g/ha Cd annually from the soil contaminated with 2.22 mg Cd/kg, far exceeding some reported Cd hyperaccumulators, such as *T. caerulescens* and *Arabidopsis halleri*, showing considerable potential for phytoremediation although strictly speaking it cannot be defined as a Cd hyperaccumulator (Guo et al. 2017; Mcgrath et al. 2006). The result presented in this study (Table 4) showed that the Cd uptake of JS-4 reached 7.62 μg/pot in JY soil, which was significantly higher than that of other populations. However, JS-3 accumulated the most amount of Cd and up to 32.6 μg/pot in QJ soil. Therefore, JS-3 population was more efficient and suitable for phytoremediation of severe Cd-contaminated soils, whereas JS-4 population has the potential for remediation of mild Cd-contaminated soils.

Although the Cd concentration of BD soil is higher than that of MZ and JY soils, *H. spectabile* accumulated rather low levels of Cd in the BD soil and showed a weak phytoremediation efficiency. This result may be explained by the high pH and high phosphorus content. Previous studies showed that high soil pH can promote the formation of Cd-carbonate precipitate and decrease the bioavailability of Cd, thereby reducing the Cd uptake by plants (Eriksson 1989; Oliver et al. 1998). Yu and Zhou (2009) found the Cd could be absorbed as an ion pair of $H_2PO_4^-$ and Cd or be deposited in the form of Cd-phosphate. Not only selecting the suitable populations of *H. spectabile* is necessary in the process of phytoremediation of Cd-contaminated soils, but also the soil physicochemical properties of the contaminated soils should be considered.

Cd subcellular distribution

Cd in different subcellular compartments may exhibit different ecotoxicological significances (Lavoie et al. 2009), and Cd subcellular partition within organisms may reveal some internal mechanisms relating to metal detoxification in the process of Cd accumulation by plants (Hall 2002; Li et al. 2011; Wallace et al. 2003). A number of papers have studied on subcellular distribution of trace elements in plants and the subcellular fractions were usually divided into cell wall, organelles, and soluble fraction which consists mostly of vacuoles (Wang et al. 2008, 2015). More than 40% of Cd was stored in the soluble fraction to the cell wall fraction of *Sedum plumbizincicola* which seems to function as the dominant detoxification mechanism protecting the protoplast from Cd toxicity (Li et al. 2013). However, it is difficult to fully disclose the detoxification mechanisms of Cd in plants only focusing on the distribution of Cd in the soluble fraction, as the soluble fractions including different components exhibit different biotoxicity such as enzymes and metallothioneins. Therefore, the Cd accumulated in leaves of *H. spectabile* in this study were separated into five fractions, which were cellular debris, organelle, Cd-rich granules, heat-stable protein,

and heat-denatured protein, for deeply revealing the Cd detoxification mechanisms of *H. spectabile*.

In the present study, Cd subcellular distribution of leaves of JS-3 and JS-4 is mainly distributed in cellular debris (44.06 to 53.47%, Fig. 3), which mainly contains cell wall and exuviation of cell membrane, and the main component is pectin and phospholipid membrane (Fu et al. 2011). Present studies indicated that Cd associated with cellular debris of JS-4 (5.25 mg/kg) is higher than JS-3 (1.57 mg/kg) in JY soil, while just the opposite in QJ soil (16.55 mg/kg of JS-3 and 9.53 mg/kg of JS-4). The result suggested that the higher proportion of Cd associated with cellular debris, the stronger the ability of Cd accumulation in *H. spectabile*. Once Cd enters the *H. spectabile*, it is bound to the ligands (hydroxyl, carboxyl) on the cell walls (mainly composed of pectins, proteins, and polyoses), and the transmembrane transport into the protoplast is greatly restricted, ensuring the normal metabolism of *H. spectabile* (Koren et al. 2013). This result is consistent with previous research on Cd accumulators, such as ramie (*Boehmeria nivea* (L.) Gaudich.) (Wang et al. 2008), pokeweed (*Phytolacca americana* L.) (Fu et al. 2011), tall fescue (*Festuca arundinacea* Schreb.) (Xu and Wang 2013), and castor (*R. communis*) (Zhang et al. 2015), which confirmed that the cell wall may be an important barrier to separate the protoplast and heavy metal, and Cd combined with pectates, phosphates, oxalates, and residuals contribute to alleviate toxicity to plant (Qiao et al. 2015; Wang and Wang 2009).

When the ligands on cell walls are saturated with Cd, the excess Cd will transmembrane transport into the cell and bond to different partitions: BDM (HSP + MRG) and MSF (organelles + HDP) (Qiao et al. 2015). HSPs, such as phytochelatins (PCs, a family of glutathione-derived peptides) and metallothioneins, were considered to play a key role in the detoxification of Cd (Lavoie et al. 2009; Verkleij et al. 2003). MRG were also considered to alleviate metal toxicity and could be observed in leaves of terrestrial plant as a response to Cd stress (Lavoie et al. 2009; Li et al. 2014). The Cd binding to HSP was the secondary most in the subcellular fractions in this study, followed by MRG, indicating *H. spectabile* possess an effective detoxification mechanism against Cd. The Cd bound to either HSP or MRG of JS-3 was higher than that of JS-4 in JY soil, but the opposite in QJ soil. This result may partially explain why JS-4 accumulates relatively higher Cd in mildly contaminated soils, whereas JS-3 accumulates relatively higher Cd in severe contaminated soils. The result revealed that the possible mechanism of Cd accumulation in *H. spectabile* is to compart Cd to BDM fractions.

MSF (organelles + HDP) used to be the target of metal toxic actions and could be used in predicting metal toxicity to plants (Lavoie et al. 2009; Li et al. 2014; Wang et al. 2008; Wang and Wang 2009). Organelles (such as mitochondria and chloroplasts) and HDP (enzymes) are essential for maintaining the normal metabolism of plants (Lavoie et al. 2009). Once entered enzymes, Cd competes with Zn and displaces this essential microelement from sulfhydryl groups of enzymes, causing loss of enzymatic activities and organelle dysfunction (Deckert 2005). In the present study, both the Cd concentrations and relative distribution in the organelles and HDP of the two populations were higher in QJ soil than in JY soil, indicating a promoting of Cd toxicity to *H. spectabile* as the increase in Cd concentration in soil. In addition, the relative Cd distribution in HSP and MRG were declined though the Cd bond to HSP and MRG of JS-4 was higher than JS-3 in JY soil, similar to JS-3 in QJ soil. With the increase in Cd concentration in soil, the relative Cd distribution of JS-3 and JS-4 decreased in BDM but increased in MSF, which is consistent with the result of Zhang et al. (2015). This result may be because a ceiling of the detoxification capacity of HSP and MRG exists, and when exceeded, the excess Cd might enter the MSF and cause greater toxicity to the organism.

Overall, the majority of accumulated Cd by *H. spectabile* in this study was in the fractions of cellular debris (44.06 to 53.47%) and BDM (34.37 to 46.82%) and thus inferred that the Cd was mainly in the pectate- and protein-integrated forms, which were reported to have less toxicity to plants (Fu et al. 2011; Wang et al. 2008; Xu and Wang 2013).

Conclusion

The present result showed that *H. spectabile* possesses the ability to accumulate high levels of Cd in the leaves in the low, moderate, and high Cd-contaminated soils. However, different populations exhibited significant difference in capacity to accumulate Cd. For example, JS-3 accumulated 50.7 mg Cd/kg in the leaves and was significantly higher than other populations when planted in QJ soils with 97.97 mg Cd/kg, whereas the Cd concentrations of JS-4 in JY soil with 1.97 mg Cd/kg were significantly higher than other populations. The present results also showed that the populations' possession of greater ability to accumulate Cd distribute more Cd to cell debris and BDM fractions, which suggested that Cd bond to cell wall, phytochelatins, and metallothioneins may be the probable internal mechanisms of *H. spectabile* to accumulate Cd. In this study, JS-3 population has the potential to be applied for phytoremediation in severe Cd-contaminated soils, whereas JS-4 population maybe more suitable for moderate Cd-contaminated soils. Overall, *H. spectabile* has the considerable potential of phytoremediation for Cd-contaminated soils, but screening suitable populations according to soil Cd concentrations is necessary.

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