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Research Article

Cadmium and zinc accumulation behaviour of hyperaccumulator *Arabidopsis halleri* ssp. *gemmifera* in the hydroponic system

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Abstract

Arabidopsis halleri ssp. *gemmifera* is classified as Cd and Zn hyperaccumulator plant; however, the disparity accumulation preferences in organs (root, stem and leaves) between cadmium and zinc seems less understandable. Therefore, this study aimed to portray accumulation behaviour toward the presence of Cd and Zn in the hydroponic method employing *A. halleri* ssp. *gemmifera*. The experiment was conducted by applying this plant using 2 and 300 µM of Cd, and 2 and 200 µM Zn, together with 20% Hoagland solution for 7 days, separately. The results showed that Zn in the medium was uptake faster than Cd. Furthermore, increasing Cd/Zn supply at the medium resulted in an increasing accumulation of Cd/Zn in the organs of the plant. In both Cd treatments, the accumulation followed the order of stem>root>leaves, indicating Cd transportation to the upper part has occurred during this period. The same accumulation preference pattern was also reported in the 200 µM Zn supply. However, at 2 µM Zn supply, Zn accumulation was mainly found in the leaves, followed by the root and stem. *A. halleri* ssp. *gemmifera* uptake Zn faster from the medium and translocate rapidly to the leaves at low-level Zn supply. Increasing Zn supply concentration might inhibit the translocation of Zn from stem to leaves. Meanwhile, regardless of Cd supply concentrations, this plant could only translocate Cd to the stem mostly within a short-time exposure period. Therefore, this study concluded that *A. halleri* ssp. *gemmifera* exhibited different accumulation responses when exposed to different Cd and Zn supply concentrations.

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Introduction

Environmental pollution of heavy metals is constantly a worldwide concern. In order to solve this issue, env ironmentally friendly technology is in great demand. A technique that has gained much attention for decades is phytoremediation. This term refers to the removal action of contaminants effectively from the environment by employing plants (Shah and Daverey, 2020). Through the phytoextraction method, these types of plants, called hyperaccumulators, can accumulate heavy metals from the environment in their tissue parts (Patra et al., 2020). There are approximately 450 species of angiosperm have been studied for heavy metal hyperaccumulator plants

(Verbruggen et al., 2009). Specifically, by growing in their native habitat, hyperaccumulator plants accumulate more than 10,000 mg kg^{-1} for Mn or 3,000 mg kg^{-1} for Zn, at least 1,000 mg kg^{-1} for As, Cr, Co, Cu, and Ni, and up to 100 mg kg^{-1} for Cd, Se or Ti in the upper organs for relatively one specimen at the same time when complete its entire life cycle (Li et al., 2018). It is concluded that hyperaccumulator plants can be distinguished from normal plants due to the enormous amount of metals stored in aerial parts without appearing phototoxic signs (Rascio and Navari-Izzo, 2011).

Arabidopsis halleri ssp. *gemmifera* (Matsum.) O'Kane and Al-Shehbaz, originated from East Asia, is well known as a cadmium and zinc hyperaccumulator plant. As cadmium hyperaccumulator plants, *A. halleri* ssp. *gemmifera* accumulated approximately 16,000 mg kg^{-1} dry weight (DW) of cadmium in the leaves through the hydroponic method (Kashem et al., 2007). Moreover, as a zinc hyperaccumulator plant, another sub-species, *Arabidopsis halleri* (L.) O'Kane and Al-Shehbaz can accumulate about 6,000 mg kg^{-1} and up to 32,000 mg kg^{-1} of the shoot (DW) in the soil and hydroponic method, respectively (Zhao et al., 2000; Babst-Kostecka et al., 2018).

The ability of *A. halleri* ssp. *gemmifera* to accumulate a high amount of cadmium and zinc is believed to correlate with their tolerance and detoxification mechanisms. Considered the baseline tolerance mechanism, the hyperaccumulator plants, including *A. halleri* ssp. *gemmifera* concentrated metals accumulation site into specific organs. It is hypothesized that hyperaccumulator plants like *A. halleri* ssp. *gemmefera* might exhibit different responses to metal accumulation in their tissues/organs among different characteristics of metals. For instance, *Sedum alfredii* Hance, confirmed as a Cd/Zn co-accumulator, this plant also has a high tolerance to lead and copper.

In mine soil, *S. alfredii* accumulated cadmium and zinc mostly in the shoot, while lead and copper accumulated mainly in the root (Xv et al., 2020). In *Cleome rutidosperma* DC., cadmium was found to be slightly higher in the shoot, while Pb was accumulated primarily in the root part using a pot experiment (Bhattacharya and Mandal Biswas, 2022). In the case of *A. halleri* ssp. *gemmifera*, cadmium and zinc concentrations were recorded mainly in the shoot of *A. halleri* ssp *gemmifera* after 7 months (1,000 mg kg^{-1} for cadmium and 6,000 mg kg^{-1} for zinc) in the field trial. However, there was a difference between cadmium and zinc bioavailability in the soil, so the specific capacity of plant organs to store different metals cannot be comparable (Zhang et al., 2017). Therefore, this study aimed to elucidate cadmium and zinc accumulation preference in the organs (root, stem and leaves) of *A. halleri* ssp. *gemmifera* using the hydroponic method. It is expected to gain more understanding of tolerance and detoxification responses conducted by hyperaccumulator *A. halleri* ssp. *gemmifera*.

Materials and Methods

Plant material consisted of *A. halleri* ssp. *gemmifera* seed was supplied by Fujita Co., Ltd. The seed was cultivated in grow sponge laid on a water-filled tray in the dark condition. After 2 weeks, the seedling was grown in 20% Hoagland solution with 14 hours of photoperiod inside a phytotron (NK System Bio and Clean LPH-241/411SP, Japan). The condition was maintained with 22 ºC of temperature, 70% of humidity and 20% of irradiance. The cultivation was conducted for 2 months. The nutrient was maintained by replacing the Hoagland solution weekly. Each seedling was then transferred into a 250 mL polyethylene bottle wrapped in aluminium foil for 4 months with the same cultivation condition as the previous stage growth.

Two sets of similar size of 6 months *A. halleri* ssp. *gemmifera* plants were prepared for further experiments. Each set was divided into two groups representing Cd and Zn treatments. For Cd group, set of *A. halleri* ssp. *gemmifera* plants were grown in 20% of Hoagland solution with the addition of 2 and 300 μM of $CdSO_4$ separately. Another group of plants was transferred into 20% of Hoagland solution with the addition of 2 and 200 μM of $ZnSO_4$. All treatments consisted of three replications and were exposed to cadmium/zinc for 0, 1, 2 and 7 days. After each sampling time, the plants were harvested by immersing each plant's roots for 20 min in 20 mM EDTA solution to remove metal surface adsorption (Zhou and Qiu, 2005). The plants were then washed with Milli-Q water and dried with tissues, followed by the separation of roots, stems and leaves of each plant sample. The organs of the plant sample were dried in 70 ºC oven until they reached a constant weight.

The concentrations of cadmium and zinc were determined in the remaining medium in all sampling time, while the organs (root, stem, and leaves) were detected at 7 days exposure time by using Inductively Coupled Plasma Mass Spectrometry (ICP-MS, NexION 300, Perkin Elmer Japan, Japan). The dried solid samples were powdered, and approximately 0.01 g DW was applied to 2 mL 60% HNO_3 and placed into a 130 ºC hot plate for 2 hours. Milli-Q water was added into a digested solution of up to 10 mL to gain a constant volume. For measuring Cd and Zn, the solution sample was added 10 ng mL^{-1} of internal standard indium (In) and then filtered by a Filtstar syringe filter (diameter 25 mm) with hydrophilic nylon membrane (pore size 0.45 μm). Cd in the medium was pre-treated by adding 0.5 mL of 60% HNO_3 and in standard solution before being filtered using the same filter paper as the solid sample. The concentration of cadmium and zinc was calculated from a cadmium and zinc standard curve (R^2 >0.99) (Wiyono et al., 2021).

The bioconcentration factor (BCF) and translocation factor (TF) of Cd in the root, stem, and leaves of A. *halleri* ssp. *gemmifera* was calculated (Yoon et al., 2006). The calculation of BCF and TF is described as follows:

$$\mathrm{BCF} = \frac{\text{Concentration of Cd in plant tissue (root)}}{\text{Concentration of Cd in medium}}$$

$$\mathrm{TF} = \frac{\text{Concentration of Cd in plant tissue (shoot)}}{\text{Concentration of Cd in root}}$$

The data was presented in average (n = 3) with standard error (SE) of means. The difference in Cd accumulation content between root, stem and leaves was analyzed statistically using one-way ANOVA with LSD post hoc test with SPSS 28.0. A significance level of 95% was employed in this study.

Results

The present study targeted to analyze the responses Cd accumulation preference site in *A. halleri* ssp. *gemmifera* after exposure with different concentrations of cadmium and zinc supplies separately and evaluate growth biomass during a short exposure period (7 days). The concentration of cadmium/zinc supply of 2 µM represented the low-level concentration, while 300 and 200 µM of cadmium and zinc were categorized as high-level concentrations. The total biomass of plants treated with 2 and 300 µM of cadmium supply showed 56 and 21% increase for 7 days, respectively, compared to the biomass at the initial period (0 days). In zinc treatment, 132 and 110% of biomass increased for 2 and 200 µM treatments during the same period. This finding indicated that the presence of cadmium seemed to own greater inhibition of growth effect than zinc. The effect of different concentrations in cadmium and zinc supplies on the growth of biomass plants (dry weight) can be seen in Figure 1. Although remarkably increasing biomass was found in all zinc treatments, at high-level zinc treatment, the biomass growth was suppressed after 2 days of exposure.

The ratio of decrement metals (cadmium and zinc) in the medium at different time exposure is presented in Figure 2. A decrement in cadmium (Figure 2A), and zinc (Figure 2B) can clearly be seen between low and high metal supplies. The decrement of these metals was significantly observed at low-level treatment after 7 days. In the case of low-level cadmium treatment, the plants uptake approximately 20, 35 and 65% of the total cadmium available in the medium during the initial period. Meanwhile, at high-level cadmium treatment, the decrease seemed insignificant, less than 10%. Interestingly, during the same period, more than 90% of zinc was uptake by these plants for 7 days in low-level zinc treatment, whereby about 60% of zinc remained in the medium at high-level zinc treatment. A time course analysis on cadmium and zinc in the medium found that the difference in cadmium uptake pattern between low and high treatment was exhibited from the initial period until the end of the exposure period. For zinc treatment, for 0-2 days, there was no significant difference in uptake ability by *A. halleri* ssp. *gemmifera* occurred in both levels of treatment. The difference in uptake behaviour between both treated plants was clearly seen from 2-7 days. The accumulation of metals (cadmium and zinc) in the root, stem and leaves of *A. halleri* ssp. *gemmifera* was evaluated within 7 days exposure period.

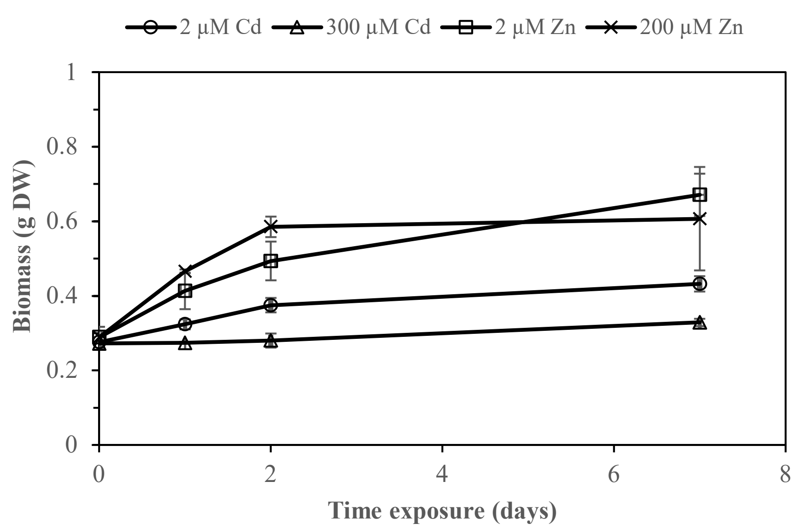

Figure 1. The effect of different concentrations in cadmium and zinc supplies on the growth of biomass plants (n = 3).

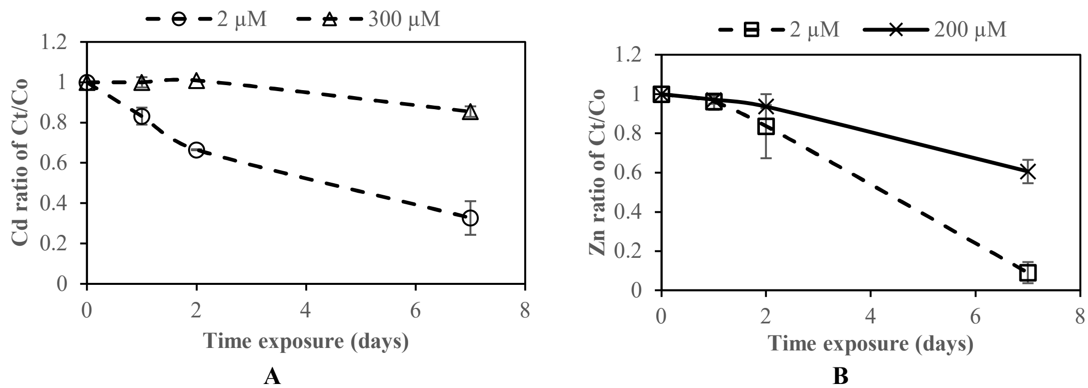

Figure 2. The ratio of cadmium and zinc decrement in the medium at different time exposure in *A. halleri* ssp. *gemmifera* hydroponic system after treated with 2 and 300 µM of cadmium (A) and 2 and 200 µM of zinc (B) (n = 3).

As presented in Figure 3, it is clearly seen that increasing metal concentration supplies in the medium resulted in increasing concentration of those metals in the root, stem and leaves of *A. halleri* ssp. *gemmifera*. Cadmium content was found to be about 90, 44 and 13-fold higher in the root, stem and leaves of *A. halleri* ssp. *gemmifera*, respectively, with the addition of 300 µM of cadmium supply. Moreover, this metal increased 25, 30 and 7 times higher in high-level zinc treatment compared to low-level treatment for 7 days. Furthermore, this plant preferred to accumulate cadmium in the stem (100 µg g^{-1} DW), followed by the root (75 µg g^{-1} DW) and leaves (20 µg g^{-1} DW). The same pattern was also found in *A. halleri* ssp. *gemmifera*'s organs after being treated with 300 µM of cadmium. In contrast, different accumulation pattern was recorded at zinc treatments. At low-level zinc treatment, the highest zinc accumulation was located in the leaves, while zinc content in the root and stem was nearly equal. Contrary to a low-level treatment, at high-level zinc treatment, the highest zinc accumulation was stored equally in the root and stem, and the leaves contained zinc three times lower than zinc content in the aerial parts.

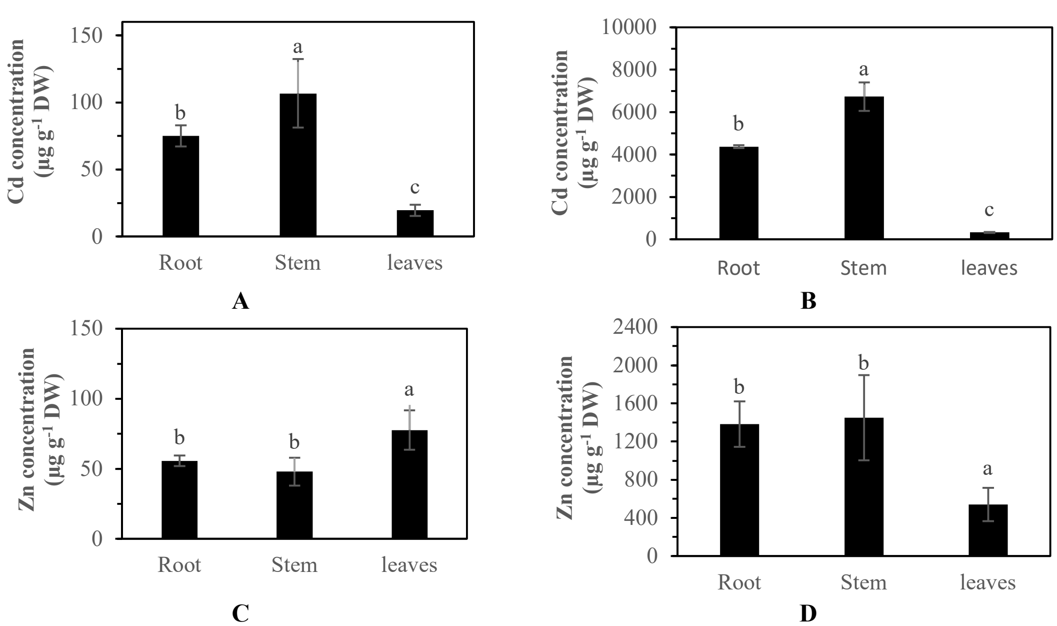

Figure 3. Accumulation of cadmium (A, B) and zinc (C, D) in the root, stem and leaves of *A. halleri* ssp. *gemmifera* upon the addition of 2 µM cadmium (A), 300 µM cadmium (B), 2 µM zinc (C) and 200 µM zinc for 7 days in the hydroponic method (n = 3). The different letter indicates a significant difference between organs in all cadmium/zinc treatments ($p<0.05$).

The metal speciation and their concentration in the medium might affect the metal accumulation behaviour in *A. halleri* ssp. *gemmifera* within 7 days exposure period.

Finally, to evaluate the ability of *A. halleri* ssp. *gemmifera* to uptake and translocate metals (cadmium and zinc), BCF and TF were calculated and presented in Table 1. At the same concentration treatments (2 μM), BCF in *A. halleri* ssp. *gemmifera* was 5-fold higher in zinc treatments compared to cadmium treatment, indicating uptake of zinc was faster than cadmium. In the case of high-level treatments (300 μM for cadmium and 200 μM for zinc), the BCF in both treatments showed an almost similar ratio. The TF of cadmium and zinc in high-level treatment exhibited 40-50% decreasing ratio compared to low-level treatment, suggesting that increasing metal supply concentrations might inhibit the translocation of metals to the aerial parts.

Table 1. BCF and TF of cadmium and zinc in *A. halleri* ssp. *gemmifera* after being treated with different concentrations of cadmium and zinc for 7 days.

Evaluation Index	Cd supply (μM)		Zn supply (μM)	
	2	**300**	**2**	**200**
BCF	0.7	0.2	3.6	0.1
TF	0.8	0.4	1.3	0.8

Discussion

As a non-essential element, cadmium causes Cd-toxic symptoms in plants, such as stunting, necrosis, chlorosis and necrosis (He et al., 2017). However, hyperaccumulator plants can concentrate metals in their tissues without exhibiting toxicity signs (Miransari, 2011). Specifically, in this study, the total biomass of *A. halleri* ssp. *gemmifera* increased significantly compared to the control treatment (0 days) for all treatments after 7 days (Table 1). The metal tolerance ability of hyperaccumulator plants shows a positive response of growth biomass while living under the presence of metals (Kacálková et al., 2009). However, this finding indicated that the presence of higher cadmium content seemed to induce greater inhibition of the growth effect. For example, the total biomass of *Youngia japonica* (L.) DC showed an increasing trend after exposure to 80 mg kg^{-1} Cd, while this plant experienced a significant reduction of biomass under >160 mg kg^{-1} Cd treatment for 60 days (Yu et al., 2021). In this study, the increase of total biomass in *A. halleri* ssp. *gemmifera* was also observed significantly under low-level Cd treatment, particularly for 2 days exposure period and had a minor biomass growth at high-level Cd treatment. Moreover, higher biomass growing found in all Zn treatments (Table 1). In *Noccaea caerulescens* (J. Persl and C. Persl) F.K. Mey, the dry mass of root and shoot also increased significantly during 7 weeks exposure period after being treated with <200 μM of Zn hydroponically and induced inhibition at >300 μM of Zn treatment (Dinh et al., 2015). Therefore, it can be concluded that the effect of metals (cadmium and zinc) on the biomass of hyperaccumulator plants depended on the metal concentrations in their tissues. Meanwhile, the accumulation of metals in the tissues of hyperaccumulator plants, including *A. halleri* ssp. *gemmifera* corresponded to the availability of metals in their environment.

The uptake ability and metal accumulation in root, stem, and leaves of *A. halleri* ssp. *gemmifera* were further evaluated in the present study. In all treatments, cadmium and zinc showed depleted concentration at different rates in the medium, indicating the plant had undergone a metal uptake mechanism. Generally, zinc decrement in the medium occurred faster than in cadmium treatment. The previous study also reported that in the same concentrations of cadmium and zinc for 20 minutes uptake experiment, *A. halleri* showed higher Zn uptake capacity than Cd uptake (Zhao et al., 2006). In addition, the low-level metal treatments demonstrated more rapid depletion than high-level treatments. After 7 days of treatments, only 35% of cadmium remained in the medium at low-level cadmium treatment, while at high-level cadmium treatment, the plants only absorbed around 10% of the total cadmium available in the medium. In the zinc case, almost 90% of zinc was absorbed by low-level zinc-treated plants, and 40% of zinc present in the medium was uptake by high-level zinc-treated plants.

Accumulation of cadmium and zinc in the root, stem and leaves clearly demonstrated an increase according to the enhancement of metals supplies in the medium. Cadmium was accumulated 13-90 times higher in a high-level cadmium treatment than in a low-level cadmium treatment. Similarly, zinc content in the organs after high-level zinc treatment increased 7-30-fold compared to thus in a low-level zinc treatment. This is a common behaviour that is regularly found in hyperaccumulator plants. For instance, the accumulation of cadmium and zinc in the root and shoot of *Thlaspi caerulescens* J. Presl and C. Presl associated with metals concentration in the soil in a log-liner analysis at the field site and pot experimental methods (Zhao et al., 2003). In pot treatment of *Impatiens glandulifera* and *Siegesbeckia orientalis* L., cadmium experienced a constantly increasing with the addition of cadmium concentration treatments (Xu et al., 2018; Coakley et al., 2019). The same pattern of cadmium accumulation also occurred in the organs of *Amaranthus mangostanus* L. using a hydroponic approach (Chi et al., 2019). In addition, the concentration of zinc found in roots, petioles and leaves of *Potentilla griffithii* Hook.f. increased gradually from approximately <5,000 to >12,000 mg kg^{-1} DW after exposure to 20, 40, 80 and 160 mg L^{-1} of zinc supply in the medium for 60 days under hydroponics (Hu et al., 2009). The metal uptake by *A. halleri* ssp. *gemmifera* was distributed to its root, stem and leaves after 7 days exposure period. In cadmium treatments, there were significant differences in cadmium content in root, stem and leaves (Figures 3A and 3B). The stem was confirmed as the highest cadmium accumulation site, followed by the root and leaves. This finding indicated that within 7 days, cadmium had been absorbed from medium to root and then translocated rapidly from root to stem. Unfortunately, the movement of cadmium from stem to leaves seemed to be slow compared to cadmium transferred from root to stem.

Like other hyperaccumulator plants, the hyperaccumulating mechanisms involve high metal absorption, increasing xylem loading, and finally, cadmium might store mostly in the leaves (Verbruggen et al., 2009). This process relied on membrane protein transporter-regulated cadmium, which facilitated cadmium ion transport and maintained its concentration cellularly (Jain et al., 2018). In the xylem, cadmium complexed with metal chelators was transported to the leaves via a water transpiration system (Song et al., 2017). Since transporting cadmium to the leaves is considered a long-distance translocation system, it was predicted that under 7 days, cadmium had not been unloaded to the leaves yet. Consequently, cadmium was localized primarily in the stem, whereby cadmium was found in a low concentration in the leaves. Interestingly, this pattern was not only found in a low-level cadmium treatment but also in high-level cadmium. It can be speculated that increasing cadmium supply at short time exposure (7 days) might not induce the decrease of transport protein and metal chelators synthesis. However, BCF and TF decreased in high-level cadmium treatment (Table 1). In *Hylotelephium spectabile* (Boreau) H. Ohba case, a high soil cadmium content might reduce BCF (Yang et al., 2018).

In the present study, zinc was mainly accumulated in leaves, while there was no significant difference between zinc in the root and stem at low-level supply (Figure 3C). This result indicated that high translocation of zinc from root to stem and stem to leaves undergone under low zinc treatment. Meanwhile, the transportation of zinc to the leaves might be suppressed as the impact of increasing 100 times higher of zinc supply level (Figure 3D). Contrary to *A. halleri* ssp. *gemmifera*, the higher accumulated zinc was in the shoot of *P. griffithii* upon the addition of 0-320 mg L^{-1} zinc supply for 21 days. BCF and TF of zinc in this treatment were lower than in the low-level zinc treatment (Table 1). In this case, zinc accumulated evenly in the root and stem, approximately 1400 µg g^{-1} DW, while in leaves only reached 540 µg g^{-1} DW. An effective zinc translocation system to the leaves, especially under low zinc treatment, is predicted due to the role of zinc as essential nutrition for the plants. A small amount of zinc is involved in plant metabolisms, while most of the remained zinc might transfer to the aerial parts under similar mechanisms with cadmium (Gupta et al., 2016).

At low-level zinc treatment, accumulation of zinc in the organs showed a contrary trend apart from the cadmium case. Similar to *P. griffithii* accumulation behaviour, at the same concentration supply in the medium (20 mg L^{-1}), the highest cadmium was spotted in the petioles and leaves part, while zinc was mainly accumulated in the root (Hu et al., 2009). Tang et al. (2009) reported in *Arabis paniculata* Franch Zn content was localized mainly in the shoot under the addition of 153 µM of cadmium, while cadmium preferred to store in the root in 9-267 µM of cadmium (Tang et al., 2009). Finally, the present study concludes that although uptake and translocation mechanisms of cadmium and zinc are common, the preference accumulation sites in organs of *A. halleri* ssp. *gemmifera* might be varied, reflecting an indirect approach to coping with the toxicity effects.

Conclusion

In this study, physiological response (biomass growth) and accumulation sites in roots, stems and leaves of *A. halleri* ssp. *gemmifera* under different concentrations (low and high levels) of cadmium and zinc was examined under a short time exposure period (7 days). The cadmium was uptake slower than zinc in the medium, but the biomass growth was more affected by cadmium treatments. As predicted, since zinc is an essential element, its availability might positively support biomass growth. However, plants under a high level of zinc supply showed less uptake and translocation to the leaves, suggesting that high zinc might inhibit unloading metal mechanisms into leaf tissues. In contrast, no difference of cadmium accumulation patterns between low and high cadmium supply (stem > root > leaves) due to the short-exposure period. Therefore, the study related to the cellular and metabolic approach to elucidate more deeply about cadmium and zinc uptake and translocation system is recommended to understand the cadmium/zinc hyperaccumulator *A. halleri* ssp. *gemmifera.*

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