Acclimatization of photosynthetic apparatus and antioxidant metabolism to excess soil cadmium in **Buddleja** spp.

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Heavy metal (HM) pollutants can cause serious phytotoxicity or oxidative stress in plants. *Buddleja* L., commonly known as “butterfly bushes”, are frequently found growing on HM-contaminated land. However, to date, few studies have focused on the physiological and biochemical responses of *Buddleja* species to HM stress. In this study, potted seedlings of *B. asiatica* Lour. and *B. macrostachya* Wall. ex Benth. were subjected to various cadmium (Cd) concentrations (0, 25, 50, 100, and 200 mg kg⁻¹) for 90 days. Both studied *Buddleja* species showed restricted Cd translocation capacity. Exposure to Cd, non-significant differences (p > 0.05) were observed, including quantum yield of photosystem II (PSII), effective quantum yield of PSII, photochemical quenching and non-photochemical quenching in both species between all studied Cd concentrations. Moreover, levels of cellular reactive oxygen species (ROS) significantly declined (p < 0.05) with low malondialdehyde concentrations. In *B. asiatica*, high superoxide dismutase and significantly enhanced (p < 0.05) peroxidase (POD) activity contributed greatly to the detoxification of excess ROS, while markedly enhanced POD activity was observed in *B. macrostachya*. Additionally, *B. macrostachya* showed higher membership function values than did *B. asiatica*. These results suggested that both *Buddleja* species exhibited high Cd resistance and acclimatization.

**Heavy metal (HM) pollution is a serious environmental problem, and the area of land contaminated with HM is growing rapidly and endangering animals and plants. Cadmium (Cd) is an extremely dangerous and widespread pollutant. Furthermore, not only is Cd, in contrast to several other HMs, a biologically non-essential nutrient, but the use of Cd is also increasing due to the presence of Cd in rocks mined for widely applied phosphate fertilizers and because of other human agricultural behavior. In plants, Cd-induced phytotoxicity or plant oxidative stress is a complex phenomenon, involving plant morphological, physiological, and biochemical responses. Physical symptoms of Cd-induced phytotoxicity include leaf chlorosis (such as reduced chloroplast organization, or impaired photosynthetic pigment or enzyme function) or withering, growth retardation (e.g., altered root–shoot ratios), reduction of photosynthetic activity, and lipid peroxidation. Under HM stress, plants generally overproduce reactive oxygen species (ROS), which disrupts the intracellular balance and raises the degree of lipid peroxidation, incurring severe redox reactions. Cd is known to both induce the generation of excess ROS, and to inhibit enzyme activity by masking catalytically active groups through interaction with their ligands. It therefore not only causes damage to the enzymatic systems of cells, but simultaneously causes damage to the leaf photosynthetic apparatus (e.g., photosynthetic Calvin-Benson cycle), and impairs the metabolism of phosphorus (P) and nitrogen (N), leading to declines in photosynthesis. To overcome Cd-induced toxicity, plants have developed diverse defensive mechanisms to lessen or detoxify oxidative stress, such as enzymatic and non-enzymatic antioxidants, and osmoprotectants. In certain tolerant plants, or hyperaccumulators, enzymatic antioxidants, including superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) can efficiently break down ROS and can thereby maintain intracellular ROS at a moderate level. Production of antioxidative enzymes is an important part of the plant defense system in the protection against various environmental stresses. Because of the high efficiency of these mechanisms in counteracting oxidative stress, they are considered to be a key mechanism in evaluating the tolerance and fitness of plant species.**

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translocation factor (TF) were calculated (Table 1). At soil treatments of 25–200 mg Cd kg⁻¹, both species showed low BCFs, ranging from 0.12 ± 0.00–5.86 ± 0.01 (Table 1). Moreover, the roots of *B. macrostachya* had accumulated 184.31 ± 0.27, while, the shoots had relatively low BCFs, ranging from 0.07 ± 0.00–0.74 ± 0.08 (Table 1).

### Results

**Cd uptake, transfer, and bioaccumulation.** The Cd concentrations, the calculated translocation and bioconcentration factors in *B. asiatica* and *B. macrostachya* are listed in Table 1. After being cultivated for 90 days, the amount of Cd that had accumulated in both *Buddleja* species was slightly higher when the plants had been grown on increased soil Cd concentrations. In this study, large amounts of Cd were taken up by the large amounts of heavy metals. *Buddleja* species, commonly known as “butterfly bushes,” are widely distributed throughout Asia, Africa, and America. *Buddleja* plants are commonly found along roadsides or abandoned land, but also grow in HM-contaminated land, and several species are known to have the capacity to accumulate large amounts of heavy metals. *B. asiatica* Lour., *B. paniculata* Wall., and *B. macrostachya* Wall. ex Benth. to increase soil Cd concentrations. The objective of this study was (1) to determine the capacity of two *Buddleja* species to bioaccumulate Cd; (2) to investigate physiological and biochemical mechanisms to prevent Cd toxicity in these two species; and (3) to evaluate the potential of these two species to acclimatize to Cd-contaminated land.

| Treatment ([mg Cd kg⁻¹ dry weight soil]) | Content (mg Cd kg⁻¹ DW; mean ± SE) | BCF¹ | TF  |
|----------------------------------------|-----------------------------------|------|-----|
|                                        | Root | Stem | Leaf | Root | Shoot | TF   |
| *Buddleja asiatica*                    |      |      |      |      |       |      |
| 0                                      | 18.17 ± 0.89a | 16.46 ± 3.37a | 17.81 ± 2.97a | -    | -     | 1.91 ± 0.25a |
| 25                                     | 34.52 ± 5.31a | 16.92 ± 0.00a | 22.54 ± 1.01a | 1.38 ± 0.21a | 1.58 ± 0.04a | 1.20 ± 0.18ab |
| 50                                     | 37.29 ± 2.63a | 22.65 ± 0.36a | 29.25 ± 2.34b | 0.75 ± 0.10b | 1.04 ± 0.05b | 1.41 ± 0.12ab |
| 100                                    | 75.00 ± 9.90b | 22.38 ± 2.81a | 27.38 ± 0.65b | 0.73 ± 0.17b | 0.50 ± 0.03cd | 0.70 ± 0.14bc |
| 200                                    | 75.94 ± 9.71b | 26.15 ± 1.52a | 28.23 ± 1.18b | 0.38 ± 0.05b | 0.27 ± 0.01b | 0.74 ± 0.08bc |
| *Buddleja macrostachya*                 |      |      |      |      |       |      |
| 0                                      | 8.54 ± 0.03a | 3.63 ± 0.05a | 3.19 ± 0.06a | -    | -     | 0.80 ± 0.01a |
| 25                                     | 146.48 ± 0.29b | 5.70 ± 0.22a | 4.16 ± 0.23b | 5.86 ± 0.01a | 0.39 ± 0.02a | 0.07 ± 0.00b |
| 50                                     | 140.35 ± 1.33b | 4.43 ± 0.20a | 6.54 ± 0.13e | 2.81 ± 0.03b | 0.22 ± 0.01b | 0.08 ± 0.00b |
| 100                                    | 173.99 ± 0.36c | 6.80 ± 0.38a | 4.99 ± 0.20bc | 1.74 ± 0.04c | 0.12 ± 0.06d | 0.07 ± 0.00bc |
| 200                                    | 184.31 ± 0.27d | 32.11 ± 1.89b | 5.43 ± 0.03d | 0.92 ± 0.06d | 0.19 ± 0.01bc | 0.20 ± 0.01c |

Table 1. Characteristics of cadmium uptake, transfer, and accumulation (mean ± S.E.; n = 3) of *B. asiatica* and *B. macrostachya* under different Cd treatments. Different letters indicate different significant differences (One-way ANOVA; p < 0.05).

Photosystem II (PSII) is known to be more sensitive to HM stress than photosystem I (PSI). Chlorophyll a fluorescence of Cd has often been used as a tool to determine the effects of abiotic stress on the photosynthetic apparatus. Moreover, the evaluation of chlorophyll a fluorescence can also provide indirect information about plant physiological responses and growth performance under any growth conditions or abiotic stresses. Chlorophyll fluorescence represents the energy that is re-emitted from chlorophyll molecules that return from an excited state to a non-excited state. Chlorophyll molecules in their excited state are able to dissipate absorbed light either through photochemical processes, especially photosynthesis, or by emitting fluorescence. Cd-induced stresses may not only cause seriously damage to the photosynthetic apparatus, reducing the photosynthetic capacity, but may also reduce plant fitness. }

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1. Qi et al. 2019. 
2. Qi et al. 2020. 
3. Qi et al. 2021. 
4. Qi et al. 2022. 
5. Qi et al. 2023. 
6. Qi et al. 2024. 
7. Qi et al. 2025. 
8. Qi et al. 2026.
Compared with *B. macrostachya*, *B. asiatica* plants exhibited high TF at the same Cd concentrations (Table 1). At soil Cd concentrations of 50 mg Cd kg\(^{-1}\) and higher, neither of our study plant species were able to uptake, accumulate, or translocate Cd to the same extent as hyperaccumulators with low TFs (<1.0). However, at soil Cd concentrations of 50 mg kg\(^{-1}\) and less, the TFs values in *B. asiatica* plants were greater than one, ranging from 1.91 ± 0.25–1.20 ± 0.18 (Table 1). These, however, dramatically declined (*p* > 0.05) with increasing soil Cd concentrations.

**Photosynthetic activity.** To assess the effect of soil Cd on *Buddleja* photosynthesis, leaf chlorophyll a fluorescence was determined in our two *Buddleja* study species, *B. asiatica* and *B. macrostachya* (Fig. 1). Both species showed similar response patterns to different Cd concentrations within a species, while there were divergent patterns between species (Fig. 1). Both *B. asiatica* and *B. macrostachya* showed great quantum yield of PSII (Fv/Fm), 0.86 ± 0.02–0.87 ± 0.02 and 0.77 ± 0.01–0.78 ± 0.01, respectively (Fig. 1a). Following exposure to soil Cd, though *B. asiatica* demonstrated a larger effective quantum yield of PSII (ΦPSII) and greater photochemical quenching (Qp) than did *B. macrostachya* with significant differences (*p* < 0.05), the two species showed similar response patterns to increasing Cd concentrations (Fig. 1b,c). Furthermore, there were no significant differences (*p* > 0.05) between treatments with different soil Cd concentrations at a species level.

Following exposure to Cd, *B. asiatica* plants showed lower non-photochemical quenching (NPQ), quantum of regulated energy dissipation [*Y*(NPQ)], and quantum yield of non-regulated energy dissipation [*Y*(NO)] than that in *B. macrostachya* (Fig. 1d–f). Furthermore, there were significant differences (*p* < 0.05) in NPQ, *Y*(NPQ) and *Y*(NO) between the two studied *Buddleja* species at all investigated soil Cd concentrations. However, Cd exposure did not increase their NPQ, *Y*(NPQ) and *Y*(NO) levels, with only nonsignificant differences (*p* > 0.05).

**Membrane lipid peroxidation.** After exposure to Cd for 90 days, seedlings of both *Buddleja* species survived and appeared to be growing normally. To evaluate the membrane lipid peroxidation, we measured the malondialdehyde (MDA) content in the leaves (Fig. 2a). There were significant differences (*p* < 0.05) in the leaf MDA concentrations between *B. asiatica* and *B. macrostachya* at all concentrations of Cd studied (Fig. 2a). In our study, *B. macrostachya* leaves showed consistently lower MDA concentrations (always lower than 0.002 μmol g\(^{-1}\)) than that did *B. asiatica* leaves, which had MDA concentrations ranging from 0.36 ± 0.02 to 0.49 ± 0.01 μmol g\(^{-1}\) (Fig. 2a).

The two *Buddleja* species showed different patterns of MDA response to increasing soil Cd concentrations (Fig. 2a). At Cd concentrations up to 100 mg kg\(^{-1}\), the concentration of MDA in *B. asiatica* leaves significantly decreased (*p* < 0.05) with increasing soil Cd concentrations (Fig. 2a). However, leaf MDA concentrations in *B. macrostachya* leaves remained at a low level with no significant differences following increasing soil Cd concentrations (*p* > 0.05; Fig. 2a).

**Antioxidant enzyme activities.** To evaluate the antioxidant enzyme defense system in our two *Buddleja* study species in response to different soil Cd concentrations, we measured the activities of several enzymatic antioxidants in the leaves (Fig. 2b–d). Following exposure to Cd, *B. asiatica* and *B. macrostachya* showed different response patterns in the sod and POD activities (Fig. 2b,c). Compared with *B. macrostachya* (62.39 ± 5.05 to 96.17 ± 4.71 U g\(^{-1}\)), *B. asiatica* presented high SOD activity (307.65 ± 3.94–391.69 ± 17.92 U g\(^{-1}\)) at all investigated soil Cd concentrations (Fig. 2b). Moreover, SOD activity significantly declined (*p* < 0.05) with increasing soil Cd concentrations in *B. asiatica* species, besides the markedly increase at 100 mg Cd kg\(^{-1}\) (Fig. 2b). The highest SOD activity occurred following the 100 mg Cd kg\(^{-1}\) and control treatments, 391.69 ± 17.92 and 388.83 ± 33.78 U g\(^{-1}\), respectively (Fig. 2b). In contrast, *B. macrostachya* showed much lower SOD activity (*p* < 0.05) compared to *B. asiatica* at all Cd concentrations. Furthermore, there were no significant differences (*p* > 0.05) between any of the investigated Cd treatments with increasing soil Cd concentrations (Fig. 2b). SOD activity in *B. macrostachya* peaked following the 25 mg Cd kg\(^{-1}\) treatment, with a value of 96.17 ± 4.71 U g\(^{-1}\) (Fig. 2b).

In contrast, POD activity responded very differently to increasing Cd concentrations than did SOD activity in both *B. asiatica* and *B. macrostachya* (Fig. 2b,c). After exposure to soil Cd, *B. macrostachya* showed greater POD activity than *B. asiatica* at all Cd concentrations, with values ranging from 943.75 ± 168.14–2,002.92 ± 253.57 U g\(^{-1}\) min\(^{-1}\) and 57.87 ± 1.63–137.24 ± 1.37 U g\(^{-1}\) min\(^{-1}\), respectively (Fig. 2c). Up to 200 mg Cd kg\(^{-1}\), POD activity in *B. macrostachya* showed a gradual increase with increasing soil Cd concentrations, but the differences between all Cd treatments were nonsignificant (*p* > 0.05). In *B. asiatica*, the POD activity increased at low Cd concentrations (25 mg Cd kg\(^{-1}\)) with significantly (*p* < 0.05) differences compared to the control (Fig. 2c). However, POD activity declined significantly (*p* < 0.05) following exposure to Cd concentrations above 25 mg kg\(^{-1}\) (Fig. 2c).

After exposure to low concentrations of Cd, both *B. asiatica* and *B. macrostachya* presented low catalase (CAT) activities with a similar response pattern (Fig. 2d), and at Cd concentrations up to 50 mg kg\(^{-1}\), CAT activity was similar in both between and within species with nonsignificant differences (*p* > 0.05; Fig. 2d). However, following exposure to high soil Cd concentrations (100 and 200 mg Cd kg\(^{-1}\)), *B. asiatica* plants showed high CAT activities with nonsignificant differences (*p* > 0.05), while those in *B. macrostachya* markedly decreased (Fig. 2d).

**Membership function.** To evaluate the Cd resistance and accumulation characteristics in *B. asiatica* and *B. macrostachya*, a membership function based on all the investigated physiological and biochemical parameters was calculated (Table 2). Following exposure to increasing soil concentrations of Cd, *B. macrostachya* presented greater comprehensive membership function values (D, 6.82–10.04) than did *B. asiatica* (5.00–7.17) at all studied Cd treatments. Compared to the control, exposure to increasing concentrations of Cd caused the D values
in *B. asiatica* to decline, while the D values in *B. macrostachya* were increased markedly (Table 2). Moreover, *B. macrostachya* plants showed higher D value (8.88) than that in *B. asiatica* (5.94) at a species level (Table 2).

**Figure 1.** The effects of Cd treatments on (a) Fv/Fm, (b) ΦPSII, (c) Qp, (d) NPQ, (e) Y(NPQ), and (f) Y(NO) in the leaves of *B. asiatica* and *B. macrostachya*. Values are represented as the mean ± S.E. (n = 5). Asterisks indicate significant differences between different species at the same Cd level according to LSD-tests (**, p < 0.01). (a–f) were separately created by using ORIGIN (Version 2019b, OriginLab Corporation, USA) and then adjusted and assembled using Adobe Illustrator CS4 software (Adobe Systems, San Jose, CA). (Fv/Fm): the quantum yield of PSII, ΦPSII: the effective quantum yield of PSII, Qp: photochemical quenching, NPQ: non-photochemical quenching, Y(NPQ): the quantum of regulated energy dissipation, Y(NO): the quantum yield of non-regulated energy dissipation.
Phytoremediation is considered to be an excellent choice for environmental HM management. Plant species have evolved different mechanisms to cope with HM exposure, either acting as contaminant accumulators or excluders. The HM accumulation capacity of hyperaccumulator and hypertolerant plant species has been widely discussed. In our present study, both Buddleja species extracted and accumulated large amounts of Cd in the roots, but not in the stems or leaves (Table 1). Moreover, the Cd concentrations in all studied plant tissues markedly increased with increasing soil Cd concentrations. According to the standard of Sun et al.17, the Cd concentrations in neither roots nor shoots in either Buddleja species reached the criterion to qualify as hyperaccumulators.
Buddleja asiatica were efficient. Furthermore, our results suggested that both the extent of photodamage in either of the species, and that their protective regulatory mechanisms Buddleja exhibited high Y(NO) values (as NPQ and Y(NPQ)) (Fig. 1f). This implied that exposure to Cd did not increase experimental conditions17,19,23,36,37. Enzymatic antioxidants are predominantly produced in the sensitive foliage ing the cell membrane13,36. Both stressed and unstressed plant cells might produce ROS, but HM-tolerant plants Buddleja generally have a well-developed antioxidant system for the removal of ROS11,23,36. Malondialdehyde, a product of lipid peroxidation has been used as a key indicator for the determination of oxidative damage in plants19,13,23,36. In this study, leaves from both Buddleja species under any of the Cd treatments. Compared with B. asiatica, B. macrostachya plants exhibited high Y(\(N\)PQ) values (as NPQ and Y(\(N\)PQ)) (Fig. 1f). This implied that exposure to Cd did not increase the extent of photodamage in either of the Buddleja species, and that their protective regulatory mechanisms were efficient. Furthermore, our results suggested that both B. asiatica and B. macrostachya showed high tolerance and acclimatization to artificial soil Cd stress.

Environmental stress often causes oxidative damage and can lead to the excessive production of ROS harming the cell membrane13,36. Both stressed and unstressed plant cells might produce ROS, but HM-tolerant plants generally have a well-developed antioxidant system for the removal of ROS11,23,36. Malondialdehyde, a product of lipid peroxidation has been used as a key indicator for the determination of oxidative damage in plants19,13,23,36. In this study, leaves from both Buddleja study species produced low amounts of MDA following treatment with elevated Cd concentrations (Fig. 2a). This implies that both studied Buddleja species can efficiently activate their antioxidant defense systems and quench free radicals. Furthermore, these efficient ROS scavenging mechanisms protect Buddleja species from destructive reactions under soil Cd stress.

Plants have evolved diverse mechanisms and various protective antioxidant defense systems to eradicate oxidative stress16,18,20,21. Antioxidants' response against Cd toxicity varies among different plant species and experimental conditions17,19,23,36,37. Enzymatic antioxidants are predominately produced in the sensitive foliage and protect plants from damage by quenching free radicals. Compared with Cd-sensitive plants, Cd-tolerant plants respond positively in antioxidant enzyme activities to Cd-induced stress38. Superoxide dismutase, which is a first line of defense against ROS, can successfully catalyze the dismutation of \(O_2^-\) to \(H_2O_2\) and \(O_2\). POD and CAT enzymes can effectively decompose \(H_2O_2\) at the intracellular level, and convert it into \(H_2O\). In this study, both two studied Buddleja species can efficiently regulate the steady-state level of cellular ROS through different antioxidant mechanisms. After exposure to soil Cd stress, B. asiatica maintained high SOD levels at all soil Cd concentrations, while high POD levels were maintained in B. macrostachya (Fig. 2b,c). Buddleja asiatica...
markedly reduced the activities of SOD and POD following exposure to Cd, however, POD activity significantly improved \((p<0.01)\) following the 25 and 50 mg Cd kg\(^{-1}\) treatments (Fig. 2b,c). In contrast, POD activity in B. macrostachya gradually increased following exposure to soil Cd, and a stable low level of SOD activity was maintained throughout (Fig. 2b,c). All our results suggest that both our Buddleja study species were equipped with efficient mechanism to manage cellular redox homeostasis at its optimum after exposure to soil Cd stress. These enzymatic antioxidants improved plant tolerance to soil Cd stress, and preserved normal plant growth and metabolism in these species following increasing soil Cd concentration.

In addition, to evaluate overall plant resistance and acclimatization to environmental stresses, the comprehensive membership function value (D) was also calculated\(^{39,40}\). The D value is used to identify Cd-resistant plants, where a large D value is positively correlated with high resistance and acclimatization\(^{39,40}\). After exposure to soil Cd stress, both B. asiatica and B. macrostachya exhibited not only different response mechanisms and growth performance, but also excellent acclimatization with high D values (Table 2). Combined with their capacities to accumulate Cd in their tissues, our results suggest that B. asiatica and B. macrostachya show species-specific responses in their resistance and acclimatization to Cd biotoxicity. Moreover, they have efficient ROS detoxification mechanisms in response to soil Cd stress, and are potentially Cd-hypertolerant plants.

### Conclusion

Buddleja asiatica and B. macrostachya plants growing in Cd-contaminated soil presented high tolerance to Cd stress with limited Cd accumulation capacity. In neither Buddleja species did Cd exposure affect their photosynthetic activity or photoprotection capacity. On the contrary, levels of ROS significantly declined, the plants preserving cellular redox homeostasis through different enzymatic antioxidant mechanisms. In B. asiatica, SOD and POD activities contributed greatly to the detoxification mechanism in response to oxidative stress, while B. macrostachya relied only on POD activity. Moreover, both species showed great Cd tolerance and resistance, and both are therefore potential Cd-hypertolerant plants for use in phytoremediation.

### Materials and methods

#### Cultivation of plants and soil treatments.  
This study was carried out between April 2017 and February 2019 in the greenhouses of Honghe University, Yunnan, China. Cuttings were taken from a mature B. asiatica specimen and biennial seedlings of B. macrostachya were used. Before the experiments, all individuals (about 40) were collected and cultivated in a greenhouse at Honghe University for 1 week. Twenty-five individuals of each species, having similar heights and growth vigor were then selected for use in the experiments.

Soil was collected from the base of the mature B. asiatica specimen and was used in each of the experiments. The organic carbon content of the soil sample was 32.85 ± 8.18 mg g\(^{-1}\), the total nitrogen was 0.34 ± 0.10 mg g\(^{-1}\), and the total phosphorus was 0.20 ± 0.02 mg g\(^{-1}\). The soil sample was slightly Cd-contaminated, and Cd content was ~ 4.60 mg kg\(^{-1}\). Soil samples were air-dried and sieved through a 2 mm mesh, then subpackaged into separate bags with 10 kg in each bag. The levels of Cd in the soil were then adjusted to 0, 25, 50, 100, and 200 mg kg\(^{-1}\) DW using Cd supplied as Cd(NO\(_3\))\(_2\)·4H\(_2\)O. All soil samples were then watered with distilled water and incubated in bags for 20 days with ~ 60% humidity\(^{41}\). Plants were then transplanted into the soil and were cultivated under normal greenhouse conditions for 90 days.

#### Determination of Cd concentration.  
Following exposure to Cd for 90 days, the Cd concentrations in different plant tissues were investigated. Plant samples were washed clean of soil and were rinsed with deionized water. Subsequently, plant roots, stems, and leaves were separately oven-dried at 70 °C for a week. The dried tissues were weighed, ground, and sieved through a 2 mm stainless steel mesh.

Plant tissues (roots, stems, and leaves) were separately microwave digested using the Speedwave ENTRY system (Berghof, Germany) with a mixed solution of HNO\(_3\), HClO\(_4\), and HF = 3:1. The Cd content was determined using Flame Atomic Absorbance Spectrometry (FAAS; TAS-990, Beijing Purkinje General Instrument Co., Ltd, China) and a standard Cd solution (GSB04-1721-2004) provided by the National Center for Reference Materials.

#### Evaluation of Cd translocation potential.  
To evaluate the potential of the test plants to bioaccumulate Cd, the translocation factor (TF) and bioconcentration factor (BCF) indexes were further calculated following Liu et al.\(^{29}\):

\[
\text{TF} = \frac{[\text{Cd}]_{\text{shoot}}}{[\text{Cd}]_{\text{root}}}
\]

\[
\text{BCF} = \frac{[\text{Cd}]_{\text{shoot or root}}}{[\text{Cd}]_{\text{soil}}}
\]

#### Evaluation of photosynthetic activity.  
Chlorophyll a fluorescence traits were recorded using a Li-6400XT with a fluorescent leaf chamber (LiCOR Inc., USA). Ten mature leaves from each treatment were selected for the measurement of chlorophyll a fluorescence and were marked. After being dark-adapted for 30 min, Fo and Fm were recorded. The ratio of variable to maximal fluorescence \([Fv/Fm = (Fm − Fo)/Fm]\), which characterizes the quantum yield of photosystem II, was determined following Figueroa et al.\(^{30}\).

The following day (9:00–11:00), chlorophyll a fluorescence parameters were measured using the same leaf. Before the test, all marked leaves were light-adapted with natural light for half an hour. A saturating light pulse (1200 µmol quanta m\(^{-2}\) s\(^{-1}\), 1 s) was applied for closing all reaction centers. Then Fo', Fm', and Fs were recorded\(^{4}\).

On top of this, the effective quantum yield of PSII \([\Phi PSII = (Fm' − Fs)/Fm']\) was calculated following Redondo-Gómez et al.\(^{43}\). The non-photochemical quenching \([NPQ; NPQ = (Fm − Fm')/Fm']\), photochemical
quenching \([Q_p; Q_p = 1 - (F_s - F_o)/(F_m' - F_o')]\), and photochemical efficiency of PSII in light \((F_v/F_m')\) were calculated following Lima et al.\(^3\) and Ware et al.\(^5\). The quantum yield of non-regulated energy dissipation \([Y(\text{NO})]; Y(\text{NO}) = F_s/F_m']\) and quantum of regulated energy dissipation \([Y(\text{NPQ})]; Y(\text{NPQ}) = 1 - Y(\text{II}) - Y(\text{NO})]\) were further calculated according to Huang et al.\(^3\).

**Determination of lipid peroxidation.** To evaluate the extent of lipid peroxidation of plants, the concentrations of malondialdehyde (MDA) in the leaves were measured following Sun et al.\(^7\). The seventh or eighth healthy mature leaves from the apex of the branches were used for analysis. About 0.2 g of fresh leaf tissue was ground and dissolved in 10 ml cold trichloroacetic acid (TCA; 10%). The homogenate was then centrifuged for 20 min at 4000 rpm and 4 °C for 10 min. The liquid supernatant was taken and used for analysis. Three replicates for each Cd treatment were performed.

**Assay of antioxidant enzymes.** To evaluate the antioxidant enzyme defense system in the study plants, the antioxidant metabolism was investigated. The seventh or eighth healthy mature leaves from the apex of the branches were used for analysis. About 0.2 g of fresh leaf tissues was ground and dissolved in 10.0 ml of cold phosphoric buffer solution (PBS) at PH 7.0. The homogenate was then centrifuged at 4000 rpm for 15 min. The supernatant liquid was taken and used for analysis. Superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) activities were subsequently determined following Sun et al.\(^7\) and Sidhu et al.\(^11\). Three replicates for each Cd treatment were performed. The SOD activity was determined by measuring the inhibition in the photoreduction of nitroblue tetrazolium (NBT). The POD activity was evaluated using the guaiacol oxidation assay. The CAT activity was determined by monitoring the disappearance of \(H_2O_2\).

**Statistical analyses.** To evaluate the performance and acclimatization of plants under different Cd stresses, the membership function value of Cd resistance in each *Buddleja* species was also calculated using the modified equations following Liu et al.\(^39\) as follows:

\[
u(X_j) = \frac{(X_j - X_{\text{min}})}{(X_{\text{max}} - X_{\text{min}})}, \quad j = 1, 2, \ldots, n
\]  

\[
W_j = \frac{|P_j|}{\sum_{j=1}^{n} |P_j|}, \quad j = 1, 2, \ldots, n
\]

\[
D = \sum_{j=1}^{n} [(\nu(X_j) \times W_j)], \quad j = 1, 2, \ldots, n
\]

where \(\nu(X)\) is the membership function value for adaptability of the trait \((j); X_j, X_{\text{min}}\) and \(X_{\text{max}}\) are the means, minimum and maximum values of the trait \((j)\), respectively; \(P_j\) is the contribution rate of the trait \((j)\) based on principal component analysis; \(W_j\) is rate of the contribution of the trait \((j)\) in all studied traits, and \(D\) is the comprehensive membership function value for the adaptability of the species.

One-way analysis of variance (ANOVA) was performed using PAST version 2.0 to reveal any differences between species or different Cd treatments. Fisher’s least significant difference (LSD) tests were performed using PAST version 2.0\(^44\) to analyze statistically significant differences among different samples at the level of 0.05 and 0.01. A significant difference was considered at two different levels \((p < 0.05\) or \(p < 0.01)\). Unless indicated, data presented represent the mean value plus or minus the standard error (± SE). To reduce the heterogeneity of variances, the data were log\(_{10}\)-transformed, if necessary. All bar charts were created by using ORIGIN (Version 2019b, OriginLab Corporation, USA) and then adjusted and assembled using Adobe Illustrator CS4 software (Adobe Systems, San Jose, CA) as shown in Figs. 1 and 2.

Received: 20 December 2019; Accepted: 26 November 2020
Published online: 08 December 2020

**References**

1. Benavides, M. P., Gallego, S. M. & Tomaro, M. L. Cadmium toxicity in plants. *Braz. J. Plant Physiol.* **17**, 21–34 (2005).

2. Sharma, S. S. & Dietz, K. J. The relationship between metal toxicity and cellular redox imbalance. *Trends Plant Sci.* **14**, 43–50 (2009).

3. Gill, S. S., Khan, N. A. & Tuteja, N. Cadmium at high dose perturbs growth, photosynthesis and nitrogen metabolism while at low dose it up regulates sulfur assimilation and antioxidant machinery in garden cress (*Lepidium sativum* L.). *Plant Sci.* **182**, 112–120 (2012).

4. Moustaka, J., Tanou, G., Adamakis, I. D., Eleftheriou, E. P. & Moustakas, M. Leaf age dependent photoprotective and antioxidative mechanisms to paraquat-induced oxidative stress in *Arabidopsis thaliana*. *Int. J. Mol. Sci.* **16**, 13989–14006 (2015).

5. Ruley, A. T., Sharma, N. C., Sahi, S. V., Singh, S. R. & Sajwan, K. S. Effects of lead and chelators on growth, photosynthetic activity and Pb uptake in *Sesbania drummondii* grown in soil. *Environ. Pollut.* **144**, 11–18 (2006).

6. Mobin, M. & Khan, N. A. Photosynthetic activity, pigment composition and antioxidative response of two mustard (*Brassica juncea*) cultivars differing in photosynthetic capacity subjected to cadmium stress. *J. Plant Physiol.* **164**, 601–610 (2007).

7. Najeeb, U. et al. Insights into cadmium induced physiological and ultra-structural disorders in *Juncus effusus* L. and its remediation through exogenous citric acid. *J. Hazard Mater.* **186**, 565–574 (2011).

8. Li, X., Zhao, M., Guo, L. & Huang, L. Effect of cadmium on photosynthetic pigments, lipid peroxidation, antioxidants, and artemisinin in hydropponically grown *Artemisia annua*. *J. Environ. Sci.* **24**, 1511–1518 (2012).
9. Anjum, S. A. et al. Morphophysiological growth and yield responses of two contrasting maize cultivars to cadmium exposure. *Clean Soil Air Water* **44**, 29–35 (2015).

10. Khan, M. I. R., Nazir, F., Asgher, M., Per, T. S. & Khan, N. A. Selenium and sulfur influence ethylene formation and alleviate cadmium induced oxidative stress by improving proline and glutathione production in wheat. *J. Plant Physiol.* **173**, 9–18 (2015).

11. Siddiqui, A. S. Singh, H. P., Rabbani, D. R. & Kohli, R. K. Tolerance and hyperaccumulation of cadmium by a wild, unpalatable herb *Coronopus didymus* (L.) Sm. (Brassicaceae). *Ecotaxon. Environ. Saf.* **135**, 209–215 (2017).

12. Sharma, P. & Dubey, B. S. Cadmium uptake and its toxicity in higher plants. *Cadmium Toxicity and Tolerance in Plants* (eds Khan, N. A. & Samiullah, S.) 63–86 (Narosa Publishing House, New Delhi, 2006).

13. Anjum, N. A., Khan, N. A., Sofo, A., Baier, M. & Kizek, R. Editorial: Redox homeostasis managers in plants under environmental stresses. *Front. Environ. Sci.* **4**, 00035. https://doi.org/10.3389/fenvs.2016.00035 (2016).

14. Semane, B. et al. Leaf proteome responses of *Arabidopsis thaliana* exposed to mild cadmium stress. *J. Plant Physiol.* **167**, 247–254 (2010).

15. Asgher, M., Khan, M. I. R., Anjum, N. A. & Khan, N. A. Minimizing toxicity of cadmium in plants—Role of plant growth regulators. *Protoplasma* **252**, 399–413 (2015).

16. Hegedüüs, A., Erdély, S. & Horváth, G. Comparative studies of *H₂O₂* detoxifying enzymes in green and greening barley seedlings under cadmium stress. *Plant Sci.* **160**, 1085–1093 (2001).

17. Sun, Y., Zhou, Q., Wang, L. & Liu, W. Cadmium tolerance and accumulation characteristics of *Bidens pilosa* L. as a potential Cd-hyperaccumulator. *J. Hazard Mater.* **161**, 808–814 (2009).

18. Anjum, N. A. et al. Catalase and ascorbate peroxidase—Representative *H₂O₂*-detoxifying heme enzymes in plants. *Environ. Sci. Pollut. Res.* **23**, 19002–19029 (2016).

19. Murtaza, B. et al. A multivariate analysis of physiological and antioxidant responses and health hazards of wheat under cadmium and other lead stress. *Environ. Sci. Pollut. Res.* **26**, 362–370 (2019).

20. Slama, I., Abdellah, C., Bouchereau, A., Flowers, T. & Savouré, A. Diversity, distribution and roles of osmoprotective compounds accumulated in halophytes under abiotic stress. *Ann. Bot.* **115**, 433–447 (2015).

21. Smokvarska, M., Francis, C., Platre, M. & Martinière, A. A Plasma membrane nanodomain ensures signal specificity during osmotic signaling in plants. *Curr. Biol.* **30**, 1–11 (2020).

22. Kalaji, H. M. et al. Chlorophyll a fluorescence as a tool to monitor physiological status of plants under abiotic stress conditions. *Acta Physiol. Plant* **38**, 102 (2016).

23. Wu, M. et al. Physiological and biochemical mechanisms preventing Cd toxicity in the new hyperaccumulator *Abelmoschus manihot*. *J. Plant Growth Regul.* **37**, 799–718 (2017).

24. Guidi, L., Landi, M., Penella, C. & Cultatayud, A. Application of modulated chlorophyll fluorescence and modulated chlorophyll fluorescence imaging in studying environmental stress effects. *Annali di Botanica* **6**, 5–22 (2016).

25. Norman, E. M. Buddlejaeae. Flora neotropica monograph, vol. 81, 1–190 (The New York Botanical Garden, Bronx, 2000).

26. Waranusantigul, P., Kruatrachue, M., Pokethitiyook, P. & Auesukaree, C. Evaluation of Pb phytoremediation potential in *Euphorbia poiretii* L. and *Bidens pilosa* L. *Ann. Bot.* **108**, 193–201 (2006).

27. Qi, C. et al. New genes for cadmium stress improve cadmium tolerance in *Oryza sativa L.* *Plant Sci.* **182**, 28–35 (2011).

28. Tian, S. K. et al. Uptake, sequestration and tolerance of cadmium at cellular levels in the hyperaccumulator plant species *Sedum alfredii*. *J. Exp. Bot.* **68**, 2387–2398 (2017).

29. Liu, Z. et al. Accumulation and tolerance characteristics of cadmium in a potential hyperaccumulator—*Loniceria japonica* Thunb. *J. Hazard Mater.* **169**, 170–175 (2009).

30. Figueroa, M. E., Fernández-Baco, L., Luque, T. & Davy, A. J. Chlorophyll fluorescence, stress and survival in populations of *Medicago truncatula* grassland species. *J. Veg. Sci.* **8**, 881–888 (1997).

31. Björkman, O. & Demming, B. Photon yield of *O₂* evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. *Planta* **170**, 489–504 (1987).

32. Sheng, M. et al. Influence of arbuscular mycorrhizae on photosynthesis and water status of maize plants under salt stress. *Myco- rrhiza* **18**, 287–296 (2008).

33. Huang, W. Zhang, S. B. & Cao, K. F. Stimulation of cyclic electron flow during recovery after chilling-induced photoinhibition of PSII. *Plant Cell Physiol.* **51**, 1922–1928 (2010).

34. Zaiyou, J., Xiu-ren, Z. & Jing, T. Photosynthetic and chlorophyll fluorescence characteristics of *Isodon rubescens* (Hemsley) H. Har. *Sci. Rep.* **10**, 10043 (2020).

35. Lima, C. S. et al. Antioxidant protection and PSII regulation mitigate photo-oxidative stress induced by drought followed by high light in cashew plants. *Environ. Exp. Bot.* **149**, 59–69 (2018).

36. Tsikas, D. Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges. *Anal. Biochem.* **524**, 13–30 (2017).

37. Pandhair, V. & Sekhon, B. S. Reactive oxygen species and antioxidants in plants: An overview. *J. Plant Biochem. Biotechnol.* **15**, 71–78 (2006).

38. Guo, J. et al. Cadmium stress increases antioxidant enzyme activities and decreases endogenous hormone concentrations more in Cd-tolerant than Cd-sensitive wheat varieties. *Ecotoxicol. Environ. Saf.* **172**, 380–387 (2019).

39. Liu, N. et al. Evaluation of mercury resistance and accumulation characteristics in wheat using a modified membership function. *Ecol. Indic.* **78**, 292–300 (2017).

40. Yan, C. et al. Screening diverse soybean genotypes for drought tolerance by membership function value based on multiple traits and drought-tolerant coefficient of yield. *BMC Plant Biol.* **20**, 321 (2020).

41. Huang, H. et al. The phytoremediation potential of bioenergy crop *Ricinus communis* for DDTs and cadmium co-contaminated soil. *Bioresour. Technol.* **167**, 11034–11038 (2011).

42. Redondo-Gómez, S. et al. Growth and photosynthetic responses to salinity in an extreme halophyte, *Sarcocornia fruticosa*. *Physiol. Plantarum* **128**, 116–124 (2006).

43. Ware, M. A., Belgo, E. & Ruban, A. V. Photoprotective capacity of non-photochemical quenching in plants acclimated to different light intensities. *Photosynth. Res.* **126**, 261–274 (2015).

44. Hammer, O. & Harper, D. A. T. Palaeontological Data Analysis (Blackwell, Oxford, 2006).

**Acknowledgements**

We thank X. D. Qi, P. Ding, L. Li, and W. T. Hai for the help in plant cultivation and management, and G. Chen, L. Z. Meng and Y. H. Liu for editing the manuscript. This work was supported by grants-in-aid from the National Natural Science Foundation of China (grant no. 31660111) and Scientific Research Project (XJ15B17; HX20012) of Honghe University.
Author contributions
W.G. designed the experiment. Y.C. and Y.S. carried out the experiment and collected the data. W.G. and Y.C. analyzed data and wrote the paper. B.D. revised the manuscript, and improved the language.

Competing interests
The authors declare no competing interests.

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