Ecotoxicology and Environmental Safety 162 (2018) 35–41

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Screening ornamental plants to identify potential Cd hyperaccumulators for bioremediation

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ARTICLE INFO

Keywords:
Cadmium stress
Bioaccumulation coefficient
Ornamental plant
Phytoremediation
Translocation factor

ABSTRACT

To identify possible cadmium (Cd) accumulators or hyperaccumulators among ornamental plants, a pot experiment involving increasing Cd concentration (0, 5, 15, 30, 60, and 100 mg kg^{-1}) was conducted among seven species. The principal objective was to screen for ornamental plants with an exceptional ability to accumulate and translocate Cd ions as well as sufficient biomass for harvesting. Regarding shoot biomass, root biomass, plant height and tolerance index (TI), *Malva rotundifolia* showed high tolerance to Cd and *Malva crispa*, *Sida rhombifolia*, *Celosia argentea* and *Celosia cristata* medium tolerance; *Althaea rosea* and *Abutilon theophrasti* were more sensitive to Cd than the other plants. A hormetic response was induced by Cd in *M. crispa*, *C. argentea*, *C. cristata* and *M. rotundifolia*. Based on its capacity for Cd accumulation, bioaccumulation coefficients (BCFs) and translocation factors (TFs), *M. rotundifolia* was selected from candidate plants after 60 days of exposure to Cd-contaminated soil and found to have accumulated more than 200 mg kg^{-1} Cd in its roots and 900 mg kg^{-1} in its shoots. Moreover, *M. rotundifolia* BCFs and TFs were higher than 1.0, with the former ranging from 1.41 to 3.31 and the latter from 1.03 to 7.37. Taken together, these results indicate that *M. rotundifolia* can be classified as a model hyperaccumulator.

1. Introduction

Heavy metal soil contamination is a severe environmental problem, especially in China (Fan et al., 2016; Liu et al., 2018). A comparison of metal concentrations in different Chinese environments showed that urban soils were more seriously contaminated than were agricultural soils (Wei and Yang, 2010). Among these elements, cadmium (Cd) exceeds the standard limit for China by up to 7.0% in soil samples and throughout the food chain, constituting a serious threat to both plants and animals (Fan et al., 2016; Mahar et al., 2016). For example, itai-itai disease is a well-known health risk caused by consumption of Cd-contaminated rice (Koji et al., 1983). The main sources of Cd pollution in soil are untreated industrial waste from smelting, mining and electroplating and excessive use of chemical fertilizers and pesticides (Zhang et al., 2010).

Over the last 20 years, many different technologies, including biological, physical and chemical approaches, have been developed to remediate metal-contaminated soils (Järup and Åkesson, 2009). Compared with other methods, phytoremediation is the most promising approach for removing or stabilizing soil pollutants because it generates no secondary pollution, is low in cost and does not alter soil aggregations (Zhang et al., 2013). Based on different uptake mechanisms, modern phytoremediation technology is divided into phytoextraction, phytostabilization, rhizofiltration, rhizodegradation and phytoevaporation (Sarwar et al., 2017); among these, phytoextraction is an attractive option for rehabilitating contaminated soils because it uses accumulator or hyperaccumulator plants to remove metals from soil or water through root-shoot translocation and aboveground accumulation (Mahar et al., 2016; Zhang et al., 2013).

To date, 721 hyperaccumulator species (7 Cd hyperaccumulators) from 52 families are known, most of which belong to either Phyllanthaceae (59 species) or Brassicaceae (83 species) (Reeves et al., 2017). Although Cd-hyperaccumulative plants can absorb 50–500 times more Cd than can normal plants and can accumulate more than 100 mg kg^{-1} dry weight Cd (Krämer, 2010; Sun et al., 2008), most of these plants produce a small amount of biomass, possess weak adaptive ability or cannot be practically applied. Thus, hyperaccumulator plants with higher biomasses and more effective Cd-transfer capacities must be identified for urban use (Krämer, 2010; Verbruggen et al., 2008). In addition, herbaceous ornamental plants generally have features such as wide distributions, high adaptability, large biomasses and short growth cycles. If herbaceous ornamental plants with hyperaccumulative properties can be screened for these features, they might be more economical and have greater practical application value. Some herbaceous

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https://doi.org/10.1016/j.ecoenv.2018.06.049
Received 2 March 2018; Received in revised form 18 May 2018; Accepted 14 June 2018

ornamental plants belonging to the families *Celosia* L., *Malva* L., and *Althaea* L. have high tolerance to heavy metal stress and effectively accumulate these metals (Liu et al., 2014, 2008; Wu et al., 2017; Zhang et al., 2010). Therefore, the present study aims to identify additional Cd accumulators or hyperaccumulators from these plant families. Studying the growth responses of herbaceous ornamental plants under different Cd gradients will contribute to future research.

2. Materials and methods

2.1. Soil preparation and plant material

Six Cd treatments (T0–T5) were designed with five replicates each: 0, 5, 15, 30, 60, and 100 mg kg^{-1}. In April 2016, soil samples were obtained from the field near Sichuan Agriculture University, Chengdu (30°41ˈ38˝N, 103°57ˈ86˝E) and passed through a 1-cm sieve. The selected soil had the following basic properties: soil texture of 49.41% sand (0.02–2 mm), 38.45% silt (0.002–0.02 mm) and 12.14% clay (< 0.002 mm); pH of 6.5; organic matter content of 23 mg kg^{-1}; available nitrogen (N) concentration of 19.2 mg kg^{-1}; Olsen phosphorus (P) concentration of 62.4 mg kg^{-1}; available potassium (K) concentration of 59.2 mg kg^{-1}; and total Cd concentration of 0.07 mg kg^{-1}. The prepared soils were mixed with a solution of analytical grade $CdCl_2 \cdot 2.5H_2O$ and completely equilibrated for 30 days. Before transferring these seedlings into pots (¢ = 46 cm, H = 31 cm), the processed soils were randomly sampled, and the Cd concentration was measured. The actual total Cd concentrations were 5.10, 15.18, 30.13, 60.08 and 100.20 mg kg^{-1}, and the available Cd concentrations were 2.00, 7.63, 11.06, 24.38 and 48.44 mg kg^{-1}.

Seeds of *Malva crispa*, *Althaea rosea*, *Malva rotundifolia, Sida rhombifolia*, *Abutilon theophrasti*, *Celosia argentea*, and *Celosia cristata* were collected from the Lanxiang seed company in Chengdu, China and germinated in a light growth chamber (8 h dark and 16 h light). After germination, the 30-day-old, uniformly sized seedlings were transplanted into pots at the rate of two plants per pot. All pots were watered with tap water to maintain a 70–80% soil water-holding capacity; no fertilizer was added during the entire experiment.

2.2. Determination of plant growth and Cd content

All plants were sampled after 60 days of growth; their roots were gently removed from the soils and rinsed several times with running tap water followed by deionized water. Then, the shoot length and leaf area of both control and treated plants were measured. The Cd ions adhering to the surface of the roots were removed by soaking the roots in 15 mM Na_2EDTA for 20 min. Finally, the samples were divided into leaves, stems and roots and dried at 80 °C to a constant weight, after which the weights of the parts were measured and recorded.

Dried plant samples were ground until they passed through a 0.15-mm mesh sieve and digested with concentrated HNO_3:$HClO_4$ (v: v, 4:1). Dried soil samples from each pot were ground until they passed through a 2-mm mesh sieve and were then digested with an HNO_3:HCl: $HClO_4$ mixture (v: v:v, 1:2:2) to obtain the total Cd concentration (Wu et al., 2017). The plant Cd concentrations and the total soil Cd concentration were determined using an atomic absorption spectrophotometer (AAS, Shimadzu AA-700, Shimadzu Corp., Kyoto, Japan) at the wavelength of 228.8 nm. The bioavailable Cd concentrations of the soil samples were determined using the diethylenetriaminepentaacetic acid (DTPA) extract method (Lindsay and Norvell, 1978). The GBW 10010 plant and GWA 7405 soil certified reference materials provided by the Chinese National Research Center for Certified Reference Materials (Beijing, China) were used to verify the accuracy of the Cd analysis (Xie et al., 2017). Each batch for verification included the certified reference materials and a reagent blank, and the recovery rates of the two certified reference materials were between 90.21% and 100.69%, respectively.

2.3. Calculation of relevant parameters

The effect of heavy metal accumulation and transportation inside the plants in both contaminated soils and control soils was calculated using the bioaccumulation coefficient (BCF) and the translocation coefficient (TF), respectively. The Cd tolerance of each species was evaluated using the tolerance index (TI) (Feng et al., 2018).

$$\text{Bioaccumulation coefficient(BCF)} = \frac{\text{Cd content in shoots}}{\text{Cd concentration in soil}}$$

$$\text{Translocation factor(TF)} = \frac{\text{Cd content in shoots}}{\text{Cd concentration in roots}}$$

$$\text{Tolerance index(TI)} = \frac{\text{RH} + \text{RL} + \text{RR} + \text{RS}}{4}$$

Where RH, RL,RR, and RS are the ratios for height, leaf biomass, root biomass and shoot biomass of the control and treatment groups, respectively.

2.4. Statistical analyses

To analyze the data, a variance analysis, *t*-test, Pearson's correlation and regression analysis were performed using SPSS version 17.0. A significance level of $p < 0.05$ was used to

assess differences among treatments. The Cd dose × plant species interaction was evaluated by the F values from two-way analysis of variance (ANOVA), and cluster analysis was applied using the Ward algorithmic method based on the parameters of root concentration, shoot concentration, BCF and TF.

3. Results and discussion

3.1. Plant growth response under Cd stress

As a potentially hazardous metal in the environment, Cd inhibits plant growth and interferes with physiological and biochemical processes (Jia et al., 2016; Tian et al., 2015). In our study, the morphologies of seven herbaceous ornamental plants showed significant differences when treated with different Cd concentrations for 60 days (Figs. 1–3). Cadmium stress seriously affected the root biomass, shoot biomass and plant height of *A. rosea* and *A. theophrasti*, which decreased, respectively, by 6.6 − 15.6% and 14.8 − 32.9%, 20.5 − 44.7% and 40.2 − 64.8%, and 18.9 − 55.7% and 22.6 − 52.3% under treatments T1–T5 compared with those of plants without Cd treatment (Figs. 1–3). The tolerance index (TI) was lower than 1 in T1-T5 (Fig. 4), indicating that growth was inhibited in *A. rosea* and *A. theophrasti*. Roots are the first part of a plant to be exposed to metals, and they represent the accumulative capacity and tolerance to metal stress of the plant (Singh et al., 2009). Root growth inhibition under Cd stress can result from cell wall lignification, which limits cell expansion and nutrient uptake (Finger-Teixeira et al., 2010), and the response of the above-ground parts can result from photosynthetic reaction inhibition, which prevents organic accumulation (Küpper et al., 2007). In this research, several plants, such as *S. rhombifolia*, *C. argentea*, *M. crispa*, *C. cristata*, and *M. rotundifolia*, showed slightly stimulated root biomass production at low Cd concentrations, and their root biomasses significantly increased, reaching peak values of 6.23% and 84.62% for *S. rhombifolia* and *C. cristata*, respectively, at 5 mg kg^{-1} Cd, 19.38% and 25.41% for *M. crispa* and *C. argentea*, respectively, at 15 mg kg^{-1} Cd and 12.31% for *M. rotundifolia* at 30 mg kg^{-1} Cd (Fig. 1). These results were consistent with those of previous studies (Feng et al., 2018; Liu et al., 2009). In particular, *M. rotundifolia* showed a higher TI under the high Cd concentration treatment (Fig. 4), suggesting higher tolerance to Cd stress, which is the crucial characteristic for an accumulator or hyperaccumulator (McGrath and Zhao, 2003; Rascio and Navari-Izzo, 2011).