Cadmium accumulation and tolerance of Macleaya cordata: *a newly potential plant for sustainable phytoremediation in Cd-contaminated soil*

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RESEARCH ARTICLE

Cadmium accumulation and tolerance of *Macleaya cordata*: a newly potential plant for sustainable phytoremediation in Cd-contaminated soil

Jian Nie[1,2] · **Yunguo Liu**[1,2] · **Guangming Zeng**[1,2] · **Bohong Zheng**[3] · **Xiaofei Tan**[1,2] · **Huan Liu**[1,2] · **Jieli Xie**[1,2] · **Chao Gan**[1,2] · **Wei Liu**[1,2]

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Abstract Heavy metal pollution is a major concern of the public due to their threats to the safety of food chains. A 60-day pot experiment was conducted using *Macleaya cordata* as plant material to investigate the phytoremediation potential and anti-oxidative responses of *M. cordata* under different Cd stress. Significant growth inhibition phenomenon and toxic symptoms were not detected in the experiment. The high biomass of the plant provided high accumulation capacity for Cd with an average dry weight of 3.6 g. The maximum extraction amount of Cd was 393 μg·plant^{-1}, suggesting that this species had potential for phytoremediation of Cd-contaminated soil. A slight increase of chlorophyll (CHL) content was observed in Cd10 treatment. The plant was confirmed to have relatively high tolerance to the Cd stress on the basis of tolerance indexes (TI), relative water content, and CHL_a/CHL_b ratio. *M. cordata* could maintain high level of superoxide dismutase (SOD) activity under Cd stress, indicating strong tolerance capacity for reactive oxygen species (ROS) in plant cells. Catalase (CAT) activity show a certain range of decline in the experiment compare to the control. And peroxidase (POD) activity in leaves changed irregularly when compared to the control. The malondialdehyde (MDA) content increased as Cd concentration elevated compared to the control. In addition, as an inedible crop with relatively high economic value, *M. cordata* have shown the advantage of high biomass and high tolerance under Cd stress, which can provide a new plant resource for sustainable phytoremediation.

Keywords Cadmium · Soil contamination · Phytoremediation · *Macleaya cordata*

Responsible editor: Elena Maestri

✉ Yunguo Liu
liuyunguo2012@yeah.net

[1] College of Environmental Science and Engineering, Hunan University, Changsha 410082, People's Republic of China

[2] Key Laboratory of Environmental Biology and Pollution Control (Hunan University), Ministry of Education, Changsha 410082, People's Republic of China

[3] School of Architecture and Art Central South University, Central South University, Changsha 410082, People's Republic of China

Introduction

Heavy metal pollution is an ever-increasing worldwide issue. The heavy metal pollutants released by industrial discharges have brought adverse effects on both ecological environment and human health. Compared with the unexpected pollution accidents, the issue of food security in agriculture areas is what we should pay more attention to. However, long period application of phosphate fertilizers have turned Cd into a major concern of the public among the most of the heavy metal pollutants, for which it poses a potential threat to human health due to its sequential bioaccumulation through the food chain (Ali et al. 2013; Xu et al. 2014; Yang and Jiang 2014). Exposure to exceeded level of Cd stress by long-term intake of Cd-contaminated food could probably result in harmful effects on human health such as itai-itai disease (Ji et al. 2011; Liu et al. 2007). The thought-provoking public nuisance events which caused by heavy metals have aroused particularly public attention on the control of heavy metals (Xu et al. 2012). Conventional soil remediation technologies entail large costs for the remediation of heavy metals-polluted sites. Therefore, it is a matter of great urgency to develop green

and efficient technologies which can remove heavy metals from contaminated soil (Zeng et al. 2015).

Compared with physical and chemical techniques of remediation, the developing phytoremediation technologies which aim to extract or inactivate metals from contaminated soil have attracted much public attention for their cost-effective and environmental friendly performances (Ji et al. 2011; Pilon-Smits 2005). Some plant species which have an extremely high capacity to take up metals by roots and translocate and store them in the shoot have been developed to resist the toxicity of heavy metals (Baker et al. 2000; Tang et al. 2009). *Thlaspi caerulescens* have the ability to co-hyperaccumalate Cd and Zn, and *Brassica napus* are also good candidates for Cd phytoremediation (Grispen et al. 2006; Zhao et al. 2003). Good Cd phytoremediation performances were show by *Solanum nigrum L.* in the lab studies (Ji et al. 2011). However, most of the hyperaccumulators tested are not commercially viable for phytoremediation.

Macleaya cordata (Willd.) R. Br. is a deciduous perennial plant in the family Papaveraceae which contains various bioactive alkaloids (Kosina et al. 2010; Liu et al. 2013). In China, *M. cordata* is a widely distributed wild plant which shows good adaptation to prevailing environmental in Shanxi, Guangxi, and Yunnan provinces. *Macleaya* herb has long been used for its analgesic and anti-inflammatory properties in humans, and *M. cordata* has been suggested to apply in the phytoextraction of uranium-contaminated soil (Li et al. 2015). Several possible advantages for selecting *M. cordata* as a candidate phytoremediation plant are listed as follows: (1) huge taproot, fast growth, and large biomass; (2) its relatively high economic values make a foundation for sustainable phytoremediation; (3) non-edible repulsion to herbivores to avoid food chain contamination (Ali et al. 2013); (4) perennial herb plant and do not have any reported environmental hazards (Khandare et al. 2011). Its other qualities, such as being used as a kind of pioneer plant in the control of Karst rocky desertification in Guizhou Province, have informed the need for exploring this plant for phytoremediation applications (Agunbiade et al. 2009; Zou and Lu 2009).

The tolerance to the Cd toxicity should ideally be taken into consideration when phytoremediation plants are subjected to Cd-contaminated conditions. As oxidative damages occurred, anti-oxidative enzymes including superoxide dismutases (SODs), peroxidases (PODs), and catalases (CATs) are of increasing importance to plants for they can scavenge the activated oxygen species produced by Cd stress. SODs is a major scavenger of $O_2^{\cdot-}$, and its enzymatic action results in the formation of H_2O_2 and O_2. The determination of these enzymes could be useful in identifying the anti-oxidant capacity of *M. cordata* to cope with Cd stress.

According to the descriptions above, the objectives of this study are therefore (1) to evaluate the potential of *M. cordata* as a phytoremediation plant in remediation of Cd-contaminated soil; (2) to investigate the anti-oxidative defense responses of *M. cordata* when it was subjected to different levels of Cd contamination.

Materials and methods

Plant preparation and soil materials

The soil material was collected from the horizon 0~20 cm depth from Yuelu Mountain in Hunan Province, China. Soil properties were characterized in Table 1. The collected soil was air-dried at room temperature, sieved through 2 mm sieves, and then the air-dried soil was completely mixed with four levels (10, 20, 40, 60 $mg \cdot kg^{-1}$ Cd/soil) of Cd solution. For each level of Cd solution, 30, 60, 120, and 180 mg pure Cd were artificially added in the quantitative distilled water using required amount of $CdCl_2 \cdot 2.5H_2O$. After adding heavy metals, the soils were equilibrated for 30 days, undergoing 5 cycles of saturation with de-ionized water and were thereafter air dried (Wang et al. 2009). Seedlings of *M. cordata* were obtained from Hunan Agricultural University, China. The seedlings were washed with redistilled water three times and then transplanted into plastic pot (20 cm in diameter and 25 cm in height) filled with 3 kg polluted soil for the pot experiments (Liu et al. 2011).

Experiment design

The pot culture experiment was conducted for a period of 60 days in the lab. All plants were grown under controlled environmental conditions with a 10-h photoperiod (from 8:00 am to 18:00 pm), a 26/19 °C day/night temperature regime, and 65 % relative humidity. Five treatments with three replicates were set up to access the efficiency of heavy metals extracted by *M. cordata*, which included a control (CK, without any Cd addition) and four Cd spiking treatment (10, 20, 40, 60 $mg \cdot kg^{-1}$) designated as Cd10, Cd20, Cd40, Cd60. All the pots were watered every day in order to keep soil moisture at 70 %.

Table 1 Chemical properties of the collected soil material from Yuelu Mountain

pH	Organic ($g \cdot kg^{-1}$)	CEC ($g \cdot kg^{-1}$)	Total Cd ($g \cdot kg^{-1}$)	Total N ($g \cdot kg^{-1}$)	Total P ($g \cdot kg^{-1}$)	Total K ($g \cdot kg^{-1}$)
4.75	17.3	15.8	ND	0.857	0.261	14.4

CEC cation exchange capacity, *ND* not detected

Assessment of phytoremediation efficiency

Heavy metal element analysis

After 60 days of cultivation, plants were carefully separated from soil and thoroughly washed with abundant distilled water. Roots were carefully removed from the substrate and dipped in a cold solution of HCl (0.01 M) during 5 min to eliminate heavy metals adsorbed at the root surface, and then washed three times with cold distilled water and blotted dry with filter paper (Aldrich et al. 2003). The fresh plants were dried at 105 °C in a vacuum oven for 30 min and then at 70 °C for 24 h until constant weight. And then the dry samples were ground into fine powder sieved through 1 mm nylon sieve.

Accurately weighted 0.2 g samples of dry plant materials were put into 100 ml beaker covered with a funnel and subsequently a 12 ml solution containing HNO_3 and $HClO_4$ (*v*/*v* 3:1) were added. The digested solution was diluted with 2 % (*v*/*v*) HNO_3 to a volume of 25.0 ml before filtration. The concentration of Cd in the digested plant sample was determined by using a flame Atomic Absorption Spectrometer (Analyst 700, Perkin Elmer, USA) at wavelength of 228.8 nm.

Cd accumulation and tolerance of M. cordata

Bioconcentration factor (BCF), translocation factor (TF), and metal extraction amount ($M_{extraction}$, μg·plant^{-1}) were used to evaluate plants phytoremediation efficiency and calculated by the following equations (Zhuang et al. 2005). BCF indicates the efficiency of a plant species in accumulating a metal into its tissues from the surrounding environment (Ladislas et al. 2012). TF indicates the efficiency of the plant in translocating the accumulated metal from its roots to shoots (Ghnaya et al. 2007). Metal extraction amount indicates the metal amount which the whole plant tissue extracted from the contaminated soil at the harvest time. The calculation equations are given as:

$$\mathrm{BCF} = \frac{C_{\mathrm{shoot}}}{C_{\mathrm{soil}}} \quad (1)$$

$$\mathrm{TF} = \frac{C_{\mathrm{shoot}}}{C_{\mathrm{root}}} \quad (2)$$

$$M_{\mathrm{extraction}} = C_{\mathrm{leaf}} \times M_{\mathrm{leaf}} + C_{\mathrm{stem}} \times M_{\mathrm{stem}} + C_{root} \times M_{\mathrm{root}} \quad (3)$$

Where C_{leaf} (μg·g^{-1}), C_{stem} (μg·g^{-1}), and C_{root} (μg·g^{-1}) represent the metal concentration in plant leaf, stem, and root, respectively. $M_{extraction}$ is the metal extraction amount (μg·plant^{-1}). M_{leaf}, M_{stem}, and M_{root} (g·plant^{-1}) are the dry biomass of each plant.

The tolerance index (TI) was expressed on the basis of plant growth parameters including root length, shoot length, root biomass, and shoot biomass (Wilkins 1978). Relative water content (RWC) was measured according to the method of Chen et al. (2009). Fresh weight was immediately measured and the samples were dried in an hot air oven for 48 h at 70 °C dry weight (DW). The calculation equations are given as:

$$\mathrm{TI} = 100 \times \frac{\mathrm{Growth\ Parameter_{Cd}}}{\mathrm{Growth\ Parameter_{CK}}} \quad (4)$$

$$\mathrm{RWC}(\%) = \frac{\mathrm{FW-DW}}{\mathrm{FW}} \times 100 \quad (5)$$

Where $\mathrm{Growth\ Parameter_{CK}}$ and $\mathrm{Growth\ Parameter_{Cd}}$ represent the shoot length, shoot biomass, root length, and root biomass of plant, respectively. RWC refers to the fresh weight of sample (g). FW (g) is fresh weight of plant sample and DW (g) is dry weight of plant sample.

Estimation of chlorophyll contents

Chlorophyll (*a* and *b*) and carotenoids were extracted from 0.2 g fresh leaf sample in the dark condition by grinding into homogenate with 95 % (*v*/*v*) ethanol and $CaCO_3$ powder according to the assay of Arvola (1981). The assay mixture was centrifuged at 4000 rpm for 10 min. The supernatants were then dilute with 95 % (*v*/*v*) ethanol to appropriate concentration for spectrophotometric analysis. The absorbance was evaluated against a blank of a pure 95 % (*v*/*v*) ethanol at wave lengths of 665, 649, and 470 nm using a UV–vis spectrophotometer (Lichtenthaler and Wellburn 1983; Sartory and Grobbelaar 1984). Chlorophyll (*a* and *b*) and total chlorophyll and carotenoids were calculated by the following equations:

$$\text{Chlorophyll } a\ \left(\mu g \cdot ml^{-1}\right) = 13.95 \times A_{665} - 6.88 \times A_{649} \quad (4)$$

$$\text{Chlorophyll } b\ \left(\mu g \cdot ml^{-1}\right) = 24.96 \times A_{649} - 7.32 \times A_{665} \quad (5)$$

$$\text{Total chlorophyll} = \text{chlorophyll } a + \text{chlorophyll } b \quad (6)$$

$$\text{Total carotenoids} = \frac{1000A_{470} - 2.05\mathrm{CHL}_a - 114.8\mathrm{CHL}_b}{245} \quad (7)$$

where A_{470}, A_{649}, and A_{665} represent the absorbance at wave lengths of 470, 649, and 665 nm, respectively.

Finally, the chlorophyll contents were calculated as milligram per gram fresh weight.

Determination of antioxidant enzymes

To control the level of activated oxygen species (AOS), plants have evolved enzymatic and non-enzymatic defense systems. Among these defense systems, anti-oxidative enzymes, especially SODs and CATs, play an important role in scavenging ROS through a series of complex reactions. Anti-oxidative defense responses of *M. cordata* were evaluated by

antioxidant enzymes including SOD, POD, and CAT of leaves and roots. The method of Giannopolitis and Ries (1977) was referenced to estimate the activities of SOD. One unit of SOD activity was defined as the amount of enzyme required to cause a 50 % inhibition rate of NBT as monitored spectrophotometrically at 560 nm. And POD activity was determined in terms of guaiacol oxidation rate in the presence of H_2O_2 (Upadhyaya et al. 1985). Absorbance was measured at 420 nm. Catalase activity was determined by the method of Aebi (1984). The assay mixture (3.0 ml) consisted of 100 μl enzyme extract, 100 μl H_2O_2 (300 mM), and 2.8 ml (50 mM) phosphate buffer with 2 mM CA (pH 7.0). One unit of CAT activity was defined as the required enzyme amount to decrease 1 μmol H_2O_2 min^{-1} mg^{-1} of total protein in absorbance at 240 nm.

Lipid peroxidation and soluble protein content

The content of malondialdehyde (MDA) was used to estimate the extent of lipid peroxidation in terms of the modified method of Heath and Packer (1968). A 1.0 g leaf sample was ground into homogenate with 10 ml 10 % trichloroacetic acid (TCA). Then the homogenate was centrifuged at 4000 r·min^{-1} for 10 min. Two milliliters of aliquot supernatant was added with 2 ml 0.5 % thiobarbituric acid (TBA), then the new mixture was heated at 100 °C for 15 min and cooled in the ice bath. After centrifuged at 4000 r·min^{-1} for 10 min, spectrophotometric analysis of supernatant was measured at the wave length of 450, 532, and 600 nm. The soluble protein content was determined by the method of Bradford (1976) using Coomassie brilliant blue G-250 as dye and bovine serum albumin (BSA) as standard.

Statistical analysis

All the experiment results were analyzed using the SPSS 18.0 package. Data in the texts and tables were expressed as means standard deviation, and error bars in the figures indicate standard deviations. The statistical significance of the differences between groups was evaluated by analysis of variance (ANOVA) and compared using least significant differences (LSD) at $p < 0.05$, $n = 3$. The Pearson correlation was calculated to examine the relationships with 95 % confidence intervals.

Results and discussion

Phytoremediation properties of plant

M. cordata has been researched for a long time, its medical properties including analgesic and anti-inflammatory have made a contribution to curing related disease, but little did we know about its phytoremediation properties (Zdarilova et al. 2008). The hyperaccumulators are characterized based on four basic features. Firstly, the concentration in the shoots of should be above 100 mg·kg^{-1} for Cd (Baker and Brooks 1989). Secondly, TF should be above 1. Thirdly, BCF should be greater than 1. Lastly, tolerance property, a hyperaccumulator should have high tolerance to toxic contaminants.

As Table 2 indicates, the maximum Cd concentration in the stem of *M. cordata* was 91.93 ± 4.02a mg·kg^{-1} at Cd60 treatment, which is close to achieve the criterion for a Cd hyperaccumulator (>100 mg·kg^{-1} Cd in shoot). And the maximum Cd concentration in the root was 163.39 ± 3.62b mg·kg^{-1} at Cd60 treatment. The accumulation amounts in shoot of each treatment were 44.72, 33.87, 24.80, and 25.67 % of the total Cd extraction amount. It indicated the fact that Cd ions are mainly retained in the roots (Cataldo et al. 1983). So far as the results demonstrated, Cd distribution in different tissues of *M. cordata* follow the order: (Cd concentration: root > stem > leaf). Additionally, both of the Cd accumulation concentration and extraction amount in *M. cordata* increased as the Cd concentration in soil increased (Table 2).

It has been highly recognized that high biomass of plant and high BCF are two key factors for successful phytoremediation (Zhao et al. 2003). Generally, almost all hyperaccumulators have high BCF but small biomasses, which lead to a low total metal extraction amounts from contaminated soil. For instance, the Cd hyperaccumulation plant *T. caerulescens* L. accumulated 600 mg·kg^{-1} Cd in shoots and the BCF reached 29.6, but this species only extracted 240 μg Cd per each plant (Perronnet et al. 2003). In the present studies, the Table 2 indicated that the maximum metal extraction amount had reached 393 μg·$plant^{-1}$ at Cd60 treatment, which was higher than that of *T. caerulescens* L. BCFs were totally above the reference value (1.0) at all levels of Cd treatment, which indicated that *M. cordata* have the potential to be used as a phytoremediation species (Yoon et al. 2006). However, what greatly improved the phytoremediation performance of *M. cordata* was the high Cd extraction amount. Its relatively high capacity which counted on the high biomass of plant made it possible to serve as a phytoremediation species.

According to the characteristics above, the present studies showed that *M. cordata* had potential to be used as a phytoremediation species. The maximum Cd concentration of shoot (91.93 mg·kg^{-1} DW) was very close to achieve the criterion for a Cd hyperaccumulator (>100 mg·kg^{-1} Cd in shoot). And BCFs were greater than 1.0, which showed a basic feature of hyperaccumulator. Meanwhile, high biomass provided high capacity of Cd in plant, confirming that biomass played a crucial role in effective phytoremediation (Klang-Westin and Eriksson 2003). Besides, the species could accumulate more amount of

Table 2 Cd accumulation in *M. cordata* and its phytoremediation properties after 60 days exposure

Treatment	Cd concentration (mg·kg^{-1})			BCF	TF	Extraction amount (μg·plant^{-1})
	Leaf	Stem	Root			
CK	ND	ND	ND	–	–	–
Cd10	16.11 ± 1.19d	22.81 ± 1.90d	31.11 ± 2.39d	2.03	0.65	94 ± 2.45d
Cd20	21.81 ± 1.76c	39.81 ± 3.76c	66.24 ± 3.21c	1.66	0.50	184 ± 3.96c
Cd40	30.23 ± 3.23b	52.28 ± 3.25b	127.52 ± 3.83a	1.12	0.35	304 ± 6.56a
Cd60	53.35 ± 4.02a	91.93 ± 2.42b	163.39 ± 3.62b	1.25	0.46	392 ± 5.61b

Values represent mean ± S.D. ($n = 3$). Different letters above the error bars refer to the significant differences ($p < 0.05$, LSD test)

ND not detected

Cd at high level of Cd according to data in Table 2 (Pandey et al. 2015). The facts that translocation factors were below 1.0 in the present studies might imply an exclusion mechanism of *M. cordata* to Cd (Baker 1987). The primary research of *M. cordata* on its phytoremediation potential was undertaken, the application of exogenous substance including EDDS and EDTA can be considered suitable for improving the phytoremediation efficiency in further studies (Wang et al. 2009; Zaier et al. 2010).

Cd tolerance of *M. cordata*

It has been well recognized that high tolerant Cd tolerance is of vital importance to the phytoremediation species (Salt et al. 2003). In our studies, there were no significant growth inhibition phenomenon and toxic symptoms existed in Cd treatment when compared to the control. As is shown in Fig. 1, the average plant height of the control was 37.3 cm while the height of Cd treatment ranged from 30.2 to 34.9 cm with an average of 32.4 cm. Analysis of the data reflected that there was a significantly negative correlation between Cd concentration and plant height ($r = -0.962$; $p < 0.05$). The shoot length and root length decreased as the Cd concentration in soil increased, showing 16 % reduction and 25 % reduction respectively at Cd60 treatment compared to the control.

And in Fig. 2, the total plant biomass (dry weight) varied from 3.2 g to 3.9 g·plant^{-1} DW with an average of 3.6 g·plant^{-1} DW. Similarly, a significantly negative correlation was also found between Cd concentration and plant dry biomass ($r = -0.963$; $p < 0.05$). The shoot biomass and root biomass (dry weight) decreased as the Cd concentration in soil increased, showing 24 % reduction and 12 % reduction respectively at Cd60 treatment compared to the control.

The tolerance of plants to Cd was measured on the basis of four plant growth parameters including root

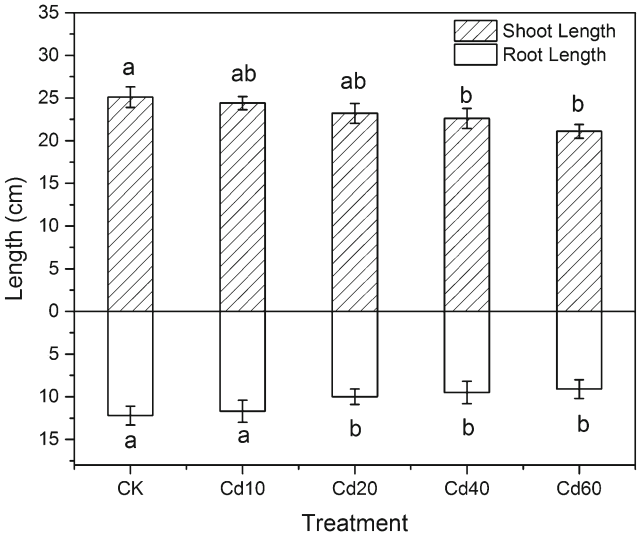

Fig. 1 The length of the shoots and roots of *M. cordata* under different level of Cd after 60 days exposure. Values represent mean ± S.D. ($n = 3$). *Different letters above the error bars* refer to the significant differences ($p < 0.05$, LSD test)

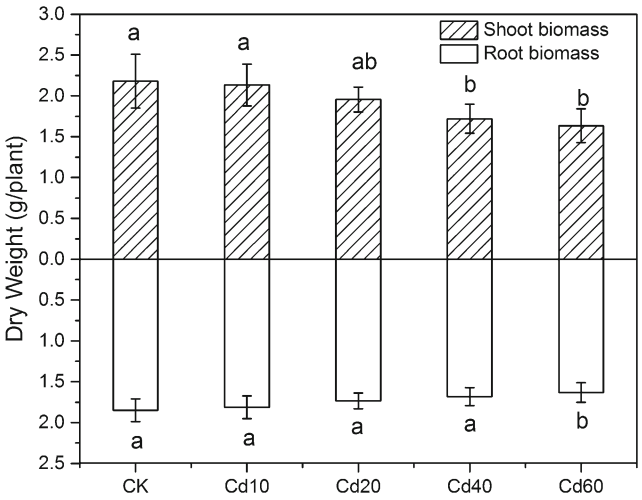

Fig. 2 Dry biomass (DW) of the shoots and roots of *M. cordata* under different level of Cd after 60 days exposure. Values represent mean ± S.D. ($n = 3$). *Different letters above the error bars* refer to the significant differences ($p < 0.05$, LSD test)

length, shoot length, root biomass, and shoot biomass. As Fig. 3 indicated, the TI decreased when the Cd concentrations in soil elevated. The root biomass showed the highest TI among the four growth parameters while the TI reflected by shoot biomass exhibiting a huge decline under Cd stress. It can be inferred that the shoot of *M. cordata* was relatively more suppressed by Cd treatment compared to the root. RWC has been recognized as an indicator to measure plant tolerance under heavy metal stress. In Fig. 4, there was no significant reduction in the RWC, showing 8 % reduction at Cd60 treatment compared to the control. The decreased RWC is commonly attributed to the stomatal closure caused by Cd stress.

In conclusion, all the plants survived in the Cd treatment, and based on the measurement of plant growth parameters and TI, the present data suggested that *M. cordata* were moderately tolerant to Cd stress.

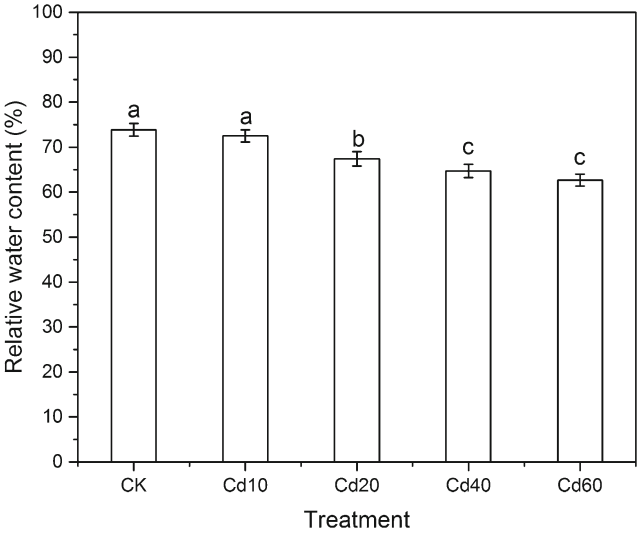

Fig. 4 Effects of Cd concentration on relative water content (RWC) of *M. cordata* after 60 days exposure. Values represent mean ± S.D. ($n = 3$). *Different letters above the error bars* refer to the significant differences ($p < 0.05$, LSD test)

Effects of heavy metals on photosynthesis

Cd damages the photosynthetic apparatus. Metal-induced changes of chlorophyll contents in leaves of plants were similar to the result of *B. napus*, Cd-induced toxic effects decreased total chlorophyll (CHL) content (Larsson et al. 1998). There was no significant difference between the Cd10 treatment and the control. A slight increase of total CHL content was depicted in the Cd 10 treatment as compared to the control (Fig. 5). Then CHL content decreased when exposed to higher Cd concentration. Additionally, the sign of a biomass decline began to emerge at Cd20 which indicated that Cd-induced damages had become a threat to the growth of the plant (Heged et al. 2001; Toppi and Gabbrielli 1999). Analysis of the data reflected that there was a significantly negative correlation between Cd concentration and total CHL content ($r = -0.979$; $p < 0.05$) while a significantly positive correlation was found between total CHL content and shoot biomass (DW) ($r = 0.945$; $p < 0.05$). It demonstrated the fact that adverse effects on the photosynthetic system by Cd ions have significant correlation with the growth of *M. cordata*. The CHL_a/CHL_b ratio was used to measure the senescence of leaves (Woolhouse 1974). As Fig. 5 indicated, the CHL_a/CHL_b ratio decreased gradually with the elevation of Cd stress, suggesting that

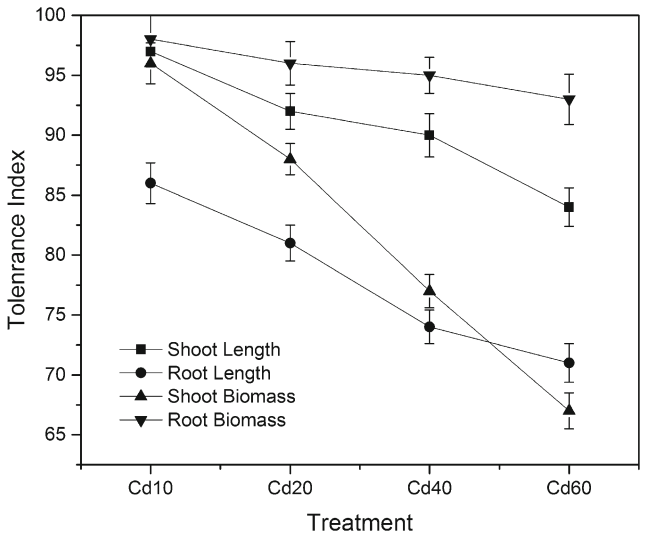

Fig. 3 Tolerance indexes of *M. cordata* under different level of Cd after 60 days exposure. Values represent mean ± S.D. ($n = 3$). *Different letters above the error bars* refer to the significant differences ($p < 0.05$, LSD test)

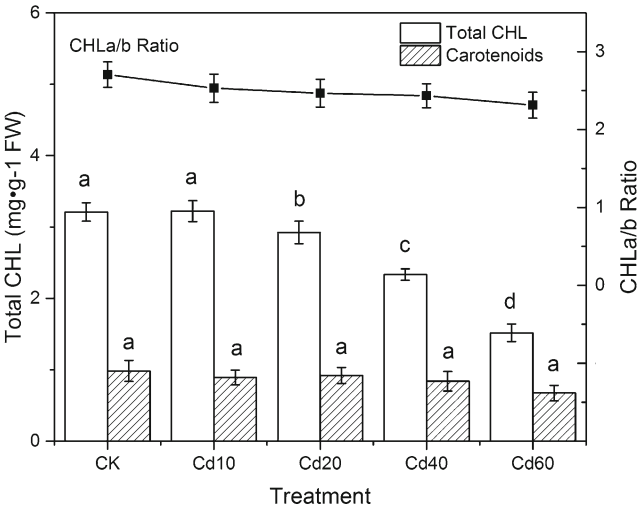

Fig. 5 Effects of Cd concentration on chlorophyll content, CHL_a/CHL_b ration, and carotenoids content in the leaves of *M. cordata* after 60 days exposure. Values represent mean ± S.D. ($n = 3$). *Different letters above the error bars* refer to the significant differences ($p < 0.05$, LSD test)

CHL_a was relatively more suppressed by Cd compared to the CHL_b. The maximum carotenoids content in leaves was 0.98 $mg \cdot g^{-1}$ FW and the carotenoids content was not significantly affected by the increasing Cd stress.

Anti-oxidative response to Cd stress

Cd is an important heavy metal pollutant in soil and does not have any beneficial physiological role in plants (Wahid et al. 2009). Cd was found to produce oxidative stress (Das 1997). When the plants were under Cd stresses, the generation of H_2O_2 and O_2^- were further intensified in physiological reactions. Plant display a range of mechanisms to cope with the adverse effects of Cd, and anti-oxidant system is of vital importance in detoxifying Cd toxicity. Anti-oxidant enzymes were exhausted to alleviate oxidative damages when the oxidative damages occurred. SOD, as a major scavenger of $O_2^{\cdot-}$, converts $O_2^{\cdot-}$ radicals to H_2O_2 (Noctor GFoyer 1998). Then, the crucial roles of disintegrating H_2O_2 in plant cells are dawned on CAT and POD, the injury effects of H_2O_2 can be eliminated when H_2O_2 were converted to H_2O and molecular oxygen.

Anti-oxidant enzymes activity in *M. cordata* cells responded variably to different level of Cd concentration. The SOD activity under Cd10 treatments was stimulated by the Cd stress, which exhibited a slightly growth (4.9 %) compared to the control (Fig. 6). SOD is considered as the first defense line against oxidative stresses, the Cd-induced SOD activity stimulation might be attributed to the hormesis effect (Stebbing 1982). Analysis of the data reflected that there was a statistically negative correlation between Cd concentration and SOD activity ($r = -0.664$; $p < 0.05$). The SOD activity merely exhibited a 7.2 % decline when the plant was under the highest Cd stress compared with the control, suggesting that maintaining high level of SOD activity might be one of the major anti-oxidant mechanisms to cope with oxidative damage induced by Cd toxicity in *M. cordata* cells.

CAT is one of the major regulators of H_2O_2 level in plant cells. CAT activity show a certain range of decline from 8.15 to 2.45 Units·mg^{-1} protein with the increase of Cd concentration (Fig. 7). The descending tendency may be ascribed to the fact that CAT enzymes were exhausted to mitigate the toxic effects of H_2O_2. Another possible explanation for the reduction of CAT activity was that proteolytic activity increased 20 % due to the Cd treatment and oxidized CAT proteins were more efficiently degraded (Romero-Puertas et al. 2002). Analysis of the data reflected that there was a significantly negative correlation between Cd concentration and CAT activity ($r = -0.990$; $p < 0.05$). POD enzyme possesses high affinity for H_2O_2. The detoxification of H_2O_2 is conducive to maintaining the integrity of cellular membranes. The POD activity increased significantly by 125 % in leaves at the level of 10 $mg \cdot kg^{-1}$ Cd compared with the control, and then fluctuated obviously as Fig. 8 shown. The maximum concentration of POD was at Cd40 treatment with an average of 9.35 Unit·mg^{-1} protein. Analysis of the data reflected that there was not a statistically positive correlation between Cd concentration and POD activity ($r = 0.413$; $p < 0.05$).

Concluded from the above, the anti-oxidant chain consist by SOD, CAT, and POD enzymes in the leaves have functioned well against the oxidative effects induced by Cd, indicating that the plant have efficient anti-oxidant response to scavenge excess of ROS. Maintaining high level of SOD activity might be one of the major anti-oxidant responses to cope with oxidative damage induced by Cd toxicity in *M. cordata* cells.

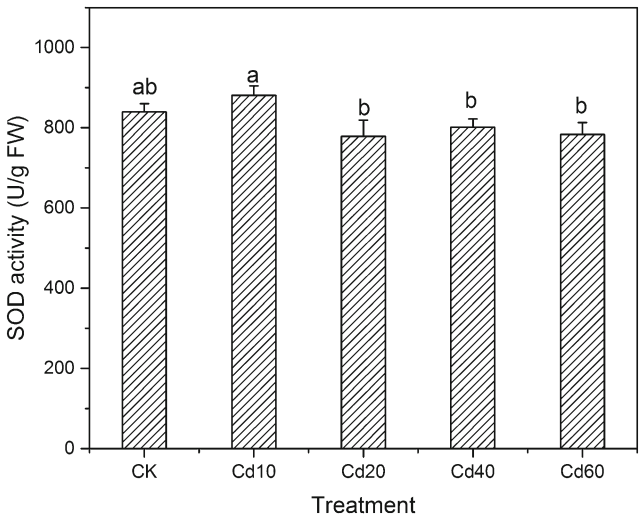

Fig. 6 Effects of Cd concentration on SOD activity in the leaves of *M. cordata* after 60 days exposure. Values represent mean ± S.D. ($n = 3$). *Different letters above the error bars* refer to the significant differences ($p < 0.05$, LSD test)

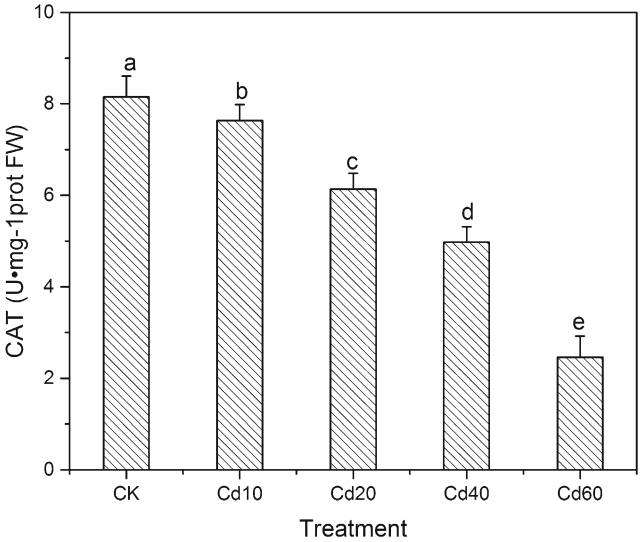

Fig. 7 Effects of Cd concentration on CAT activity in the leaves of *M. cordata* after 60 days exposure. Values represent mean ± S.D. ($n = 3$). *Different letters above the error bars* refer to the significant differences ($p < 0.05$, LSD test)

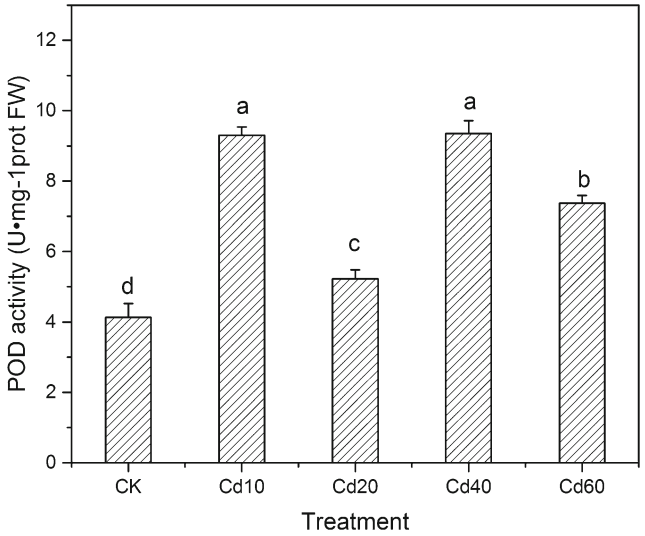

Fig. 8 Effects of Cd concentration on POD activity in the leaves of *M. cordata* after 60 days exposure. Values represent mean ± S.D. ($n = 3$). *Different letters above the error bars* refer to the significant differences ($p < 0.05$, LSD test)

Effects of Cd on lipid peroxidation and soluble protein content

The enhanced lipid peroxidation which was induced by free radicals was generally recognized to cause membrane destabilization (Tappel 1973). MDA, a product of lipid peroxidation, is widely used to assess the oxidative damage of membrane lipids. And increase of MDA content is generally regarded as signal of heavy metal stress. The dynamic changes of MDA contents in leaves were corresponded with the increasing trend of Cd concentrations in the leaves (Fig. 9). As Fig. 9 indicated, the maximum value was 11.724 μmol·g^{-1} at Cd60 treatment with an increase of 171 % compared to the control. The further intensified cellular membrane damage which is induced by Cd ions was evidenced by the increasing accumulation of MDA (Chaoui et al. 1997). Analysis of the data reflected that there was a statistically positive correlation between Cd concentration and MDA content ($r = 0.950$; $p < 0.05$).

The changes of soluble protein content were reflected in Fig. 10. Concentration of soluble protein in leaves increased at low level of Cd treatment and then decreased as the Cd stress elevated, showing 28 % reduction at Cd60 compared to the control. Soluble protein contents were stimulated at low level of Cd, this may be explained by the reason that some soluble proteins functioned with anti-oxidation and detoxification were synthesized (Yang et al. 2007) to cope with Cd stress. When plants were exposed to higher level of Cd, the Cd-induced damages in photosynthetic apparatus may be responsible for the reduction of protein content (Grill et al. 1989).

Environmental and engineering implications

The remediation of Cd contamination to arable land is of particularly importance for the public due to the Cd accumulation in food chains. The recent data indicated that agricultural activities accounted for 63 % of the total annual inventory of Cd in agricultural soils (Niu et al. 2013). Cd was believed to cause damage at low concentrations and can be taken up by crops easily (Cobb et al. 2000; Yang et al. 2008). The safety of food chain, as well as human health, is intimately linked to the environment of agricultural soil. As Fig. 11 indicated, edible crops including rice are facing the threat from Cd-contaminated soil, Cd contamination in food chain will exist for a long time and human health is challenged by these contaminated foods.

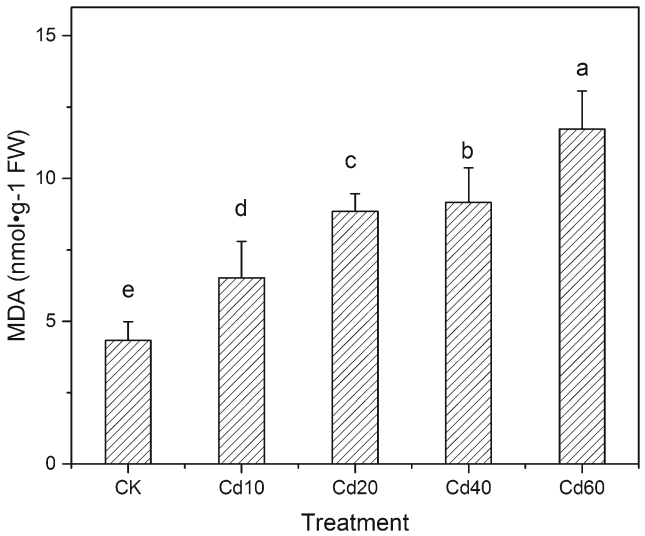

Fig. 9 Effects of Cd concentration on MDA content in the leaves of *M. cordata* after 60 days exposure. Values represent mean ± S.D. ($n = 3$). *Different letters above the error bars* refer to the significant differences ($p < 0.05$, LSD test)

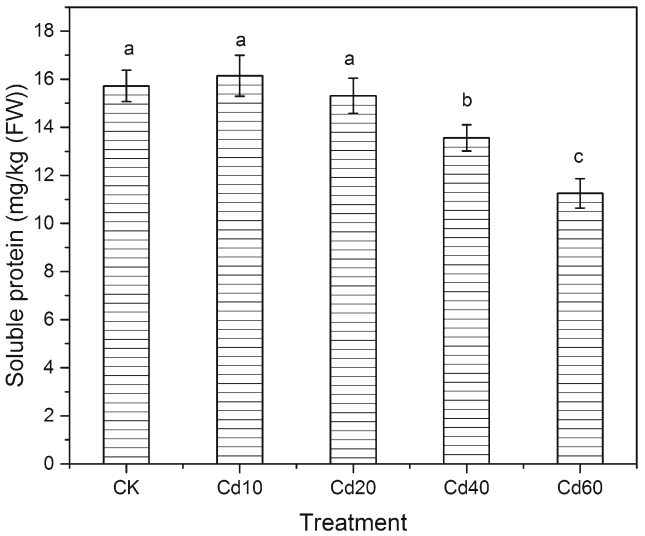

Fig. 10 Effects of Cd concentration on soluble protein content in the leaves of *M. cordata* after 60 days exposure. Values represent mean ± S.D. ($n = 3$). *Different letters above the error bars* refer to the significant differences ($p < 0.05$, LSD test)

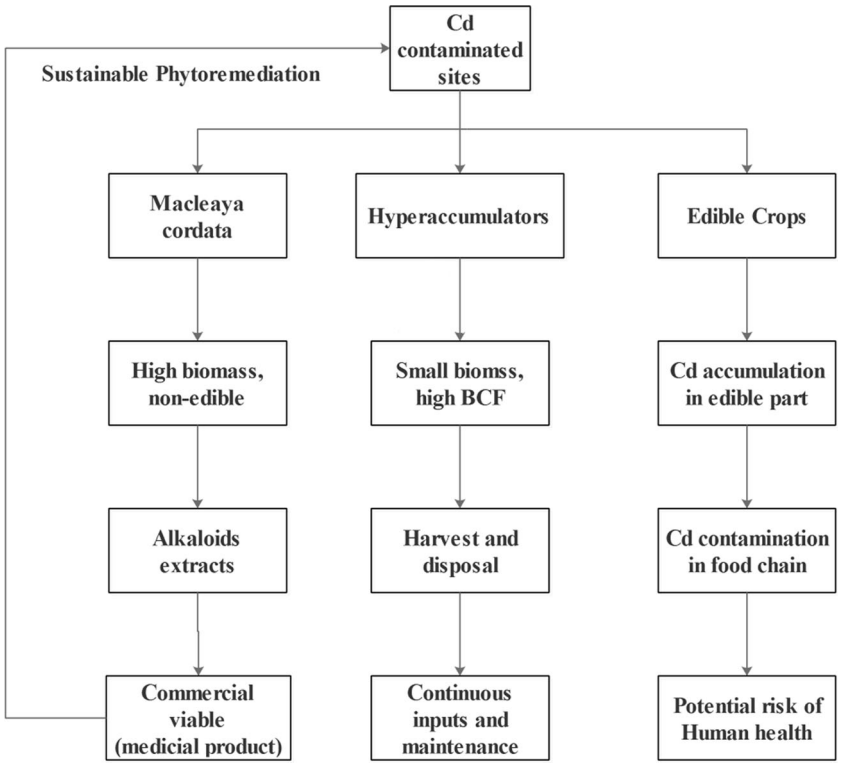

Fig. 11 A diagram which indicates the sustainable phytoremediation mode of *M. cordata*

Hyperaccumulators applied in the contaminated site were proved to be feasible in the removal of heavy metals. Li et al. (2003) have suggested that hyperaccumulator plant species could be used as a commercial phytoextraction technology in nickel (Ni) contaminated or Ni-rich soils. Generally, it takes a long time for hyperaccumulators to remediate the polluted sites. Therefore, continuous economic inputs will be greatly demanded during the long period of phytoremediation time and methods for the disposal of the harvested plant have not yet been well developed after phytoremediation. Fast pyrolysis and burning were thought to be the major methods for the disposal of harvest biomass (Bridgwater et al. 1999). Besides, deposition of the wastes at a hazardous waste site is expensive and can reach above 1000 €/t (Sas-Nowosielsk et al. 2004). Therefore, the vital issue of how can phytoremediation be sustainable must be paid special attention. Compared with the hyperaccumulators, the advantage of commercially viable is what makes *M. cordata* take a further step in sustainable phytoremediation. Possible risk of Cd residue in alkaloids extracts needs further investigation.

Conclusion

The data obtained from the present experiments have shown the possibility of applying *M. cordata* in phytoremediation for its accumulation potential of Cd. The results also suggested that *M. cordata* was relatively tolerant to high level of Cd stress. And there was no significant growth inhibition and toxic symptoms observed in the experiment. Anti-oxidants including SOD, CAT, and POD displayed positive responses to cope with the Cd-induced stress. To mitigate the increasing oxidative stress, anti-oxidative enzymes are in such great demand. Maintaining high level of SOD activity is one of the important anti-oxidative responses for *M. cordata* when subjected to Cd stress. As an inedible wild plant species, its application in Cd-contaminated agricultural land will be a good choice for reducing the threat which Cd posed to the food chain. On the other hand, *M. cordata* has been used in traditional Chinese medicine for its analgesic, anti-edemic, carminative, depurative, and diuretic properties. The relatively high economic value based on the medical properties of *M. cordata* has provided the possibility of sustainable phytoremediation compared with some well-known hyper-accumulating plant species. However, whether the plant species is suitable for field experiments needs to be further investigated. If further confirmed, *M. cordata* could have impact on practical phytoremediation application.

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