Dose-Response, 13:1, 2015
Formerly Nonlinearity in Biology, Toxicology, and Medicine

ISSN: 1559-3258
DOI: 10.2203/dose-response.14-033.He

International Dose-Response Society
www.Dose-Response.org

HORMETIC RESPONSES OF *LONICERA JAPONICA THUNB.* TO CADMIUM STRESS

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□ The hormetic responses of *Lonicera japonica* Thunb. to cadmium (Cd) stress were investigated in a hydroponic experiment. The present results showed that root length and total biomass dry weight increased in comparison with the control at low concentrations Cd. The height of the plant exposed to 2.5 and 5 mg L^{-1} Cd increased significantly by 11.9% and 12.8% relative to the control, and with the increase of Cd concentrations in the medium, plant height began to decrease. The responses of photosynthetic pigments contents and relative water content to Cd stress had a similar trend, which all showed significantly an inverted U-shaped dose–response curve and confirmed that the stimulatory effect of low concentrations Cd occurred in the plant. Furthermore, *L. japonica,* as a new Cd-hyperaccumulator, could be considered as a new plant model to study the underlying mechanisms of the hormesis.

Key words: Hormetic responses, Lonicera japonica *Thunb., cadmium, hyperaccumulator.*

INTRODUCTION

In recent years, environmental pollutants such as heavy metal have significant adverse biological effects to humans and other living organisms (Sun *et al.* 2009; Bačkor *et al.* 2010; Birgül and Tasdemir 2011). However, some toxic chemical stressors may have beneficial protective effects at low doses (Calabrese and Baldwin 2001; Calabrese and Baldwin 2003a; Stebbing 2003). This biphasic dose–response phenomenon has been known as hormesis, which are always characterized by J- or inverted U- shaped dose response curve, depending on the different endpoints measured (de la Rosa *et al.* 2004). Hormesis is the stimulatory effect of low-dose toxic stressors and have been conducted in many organisms (Calabrese and Baldwin 2003b; Calabrese and Blain 2005; Cedergreen *et al.* 2007; Calabrese 2008; Calabrese and Blain 2009; Calabrese *et al.* 2010). Cadmium (Cd) has attracted the most attention due to its great toxicity and high water solubility, neurotoxic and carcinogenic effects (López-Millán *et al.* 2009; Qiu *et al.* 2011; Han *et al.* 2012). Excessive amount of

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Cd can reduce chlorophyll contents, inhibit growth, damage root tips and perturb water homeostasis (Dong *et al.* 2006; Chen *et al.* 2011). Several studies are more concerned about the toxic effects of Cd at high concentrations (Liu *et al.* 2003; Aina *et al.* 2007; Han *et al.* 2007). However, much less information is available about the effect of Cd at low concentrations.

Lonicera japonica Thunb., as a popular ornamental, has become established in temperate and tropical regions worldwide in the past 150 years (Larson *et al.* 2007). It possesses the characteristics of high biomass, easy cultivation, extensive competitive ability, wide geographic distribution, and strong resistance to environmental stresses (Tae *et al.* 2003; Park *et al.* 2005; Thanabhorn *et al.* 2006). In our previous study, it was shown that *L. japonica* had a strong tolerance to Cd and could be known as a new potential Cd-hyperaccumulator (Liu *et al.* 2011a). When exposed to low concentrations Cd, *L. japonica* did not show any visual symptoms, even the plant growth seemed to be improved (Liu *et al.* 2009). In the present study, we investigated the hormetic responses of growth characteristics and photosynthetic pigment composition in *L. japonica* to Cd stress. It will be helpful to have a better understanding of the hormesis mechanisms and may provid a new model for further studying the hormetic effects in plants.

MATERIALS AND METHODS

Plant culture and Cd exposure

Cuttings of *L. japonica* were collected from a non-contaminated field–Shenyang Arboretum of Chinese Academy of Sciences and propagated in perlite in the laboratory. After 2 months, plants were transformed to slightly modified Hoagland solution containing the following ingredients (mmol l^{-1}): $Ca(NO_3)_2 \cdot 4H_2O$ 5.00, $MgSO_4 \cdot 7H_2O$ 2.00, KNO_3 5.00, KH_2PO_4 1.00, H_3BO_3 0.05, $ZnSO_4 \cdot 7H_2O$ 0.80×10^{-3}, $MnCl_2 \cdot 4H_2O$ 9.00×10^{-3}, $CuSO_4 \cdot 5H_2O$ 0.30×10^{-3}, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ 0.02×10^{-3}, Fe-EDTA 0.10. Uniform plants were cultivated in black plastic boxes (15 plants per box) containing the culture medium with continual aeration; the volume of solution in each box was 1.5 L. The experiment was performed in a growth chamber under controlled conditions: 14 h illumination per day (5.00 a.m. to 7.00 p.m.), 800 μmolm^{-2} s^{-1} PPFD, with a 25 °C/18 °C day/night temperature and a relative humidity of 60 %. Solutions were renewed once every 3 days to prevent nutrient depletion, and the pH was daily adjusted to 5.8 ± 0.1 with 0.1 M HCl or 0.1 M NaOH. Plants, which had been cultivated hydroponically there for 2 weeks, were transformed into similar solutions but containing 0, 0.5, 2.5, 5, 10 and 25 mg L^{-1} Cd^{2+}(added in the form of $CdCl_2 \cdot 2.5H_2O$), respectively. The experiment was repeated three times. After 28 days Cd exposure, the plants were harvested for analysis.

Measurements of plant biomass and Cd content

The harvested plants were rinsed with distilled water, and the roots were immersed in 20 mM Na_2-EDTA for 15 min to remove Cd adhering to the root surface (Yang *et al.* 2004). Then the plants were separated into leaves, stems and roots. These portions were then separately rinsed with distilled water and finally with deionized water, wiped with tissues and weighed. They were then dried at 105 °C for 10 min, then at 70 °C until the weight was constant.

Dried plant materials were weighed and ground. The powders were digested with a concentrated acid mixture of HNO_3 / $HClO_4$ (3:1, v/v). The concentrations of Cd in plant tissues and soils were determined with an Optima3000 ICP-AES instrument (Perkin-Elmer, USA).

Measurement of photosynthetic pigments

The extraction procedure was similar to Booker and Fiscus (2005) with small modification. Tissue leaf samples (0.2 g) were soaked in 25 ml 95 % (v/v) ethanol at 4 °C in darkness until the tissues became white. Extract was used to measure the absorbance at 649, 665 and 470 nm. Chlorophyll and carotenoid contents were calculated according to Lichtenthaler and Wellburn (1983).

Measurements of relative water content

Fully expanded third function leaf was sampled, and fresh weight (FW) was determined right after excision, and the leaves were immersed into double distilled water to saturate them in the dark for at least 18 h to determine the weight of leaves with saturated water (TW). Dry weight (DW) was weighed after drying the leaves to a constant weight in an oven at 80 °C. Relative water content (RWC) was calculated using the following formula (Smart and Bingham 1974): RWC (%) = (FW - DW)/(TW - DW) 9 100 %.

Statistical analyses

Average values and standard deviations (SD) were calculated using Microsoft Office Excel 2003. One-way analysis of variance was carried out with SPSS13.0 for windows. LSD test was used to determine the difference (p <0.05) among samples.

RESULTS

Effect of Cd stress on plant growth

After 28 days Cd exposure, the effect of Cd in the medium on the growth of *L. japonica* in terms of root length, height and total biomass dry weight was shown in Table 1. When the concentration of Cd exposure in the medium was 0.5 and 2.5 mg L^{-1}, root length of the plant increased compared with the control, but showed significantly decreased exposed to 10 and 25 mg L^{-1} Cd. The height of the plant exposed to 2.5 and 5 mg L^{-1} Cd increased significantly by 11.9% and 12.8% relative to the control. With the increase of Cd concentrations in the medium, plant height began to decrease, and showed no significant differences compared with the control (p >0.05). The total biomass dry weight increased in comparison with the control at Cd concentration from 0.5 to 5 mg L^{-1}, and then turned to decrease from 10 to 25 mg L^{-1}. The responses of root length, height and total biomass dry weight to Cd stress all showed an inverted U-shaped dose–response curve, which confirmed that the stimulatory effect of low concentrations Cd occurred in *L. japonica*.

Effects of Cd stress on photosynthetic pigment composition

The contents of chlorophyll a (Chla), Chlorophyll b (Chlb), chlorophyll (Chl) and carotenoid (Car) in leaves of *L. japonica* after 28 days were presented in Figure 1. When exposure to the lower Cd concentrations from 0.5 to 5 mg L^{-1}, all of the photosynthetic pigments contents increased, and the maximum increase rate of Chla, Chlb, Chl and Car contents was 14.0, 10.8, 9.75 and 54.5 % compared with the control, respectively. When at the high concentration Cd (>10 mg L^{-1}), the contents of photosynthetic pigments tended to decline with the increase of Cd concentrations in the medium. The photosynthetic pigment composition generally showed an inverted U-shaped curve with the increase of Cd concentrations in the medium, which was similar to the variation pattern of plant growth. The result indicated that low concentrations Cd may be beneficial to plant.

Effects of Cd stress on the relative water content of leaves

As shown as Figure 2, when the concentration of Cd exposure in the medium was 0.5 and 2.5 mg L^{-1}, the relative water content of leaves had an increased trend after 28 days Cd exposure. With the increase of Cd concentrations in the medium, the relative water content of leaves began to decrease. When Cd concentrations in the medium got up to the highest (25 mg L^{-1}), the relative water content of leaves decreased by 19.4% compared with the control, which indicated that high concentration Cd had an influence on the water balance of the plant.

Cd accumulation in *L. japonica*

With the increase of Cd concentrations in the medium, Cd concentrations in roots and shoots of *L. japonica* all increased significantly (Figure 3). Cd mainly accumulated in roots and at the same level of Cd

TABLE 1. Root length, height and total biomass dry weight of *L. japonica* under Cd stress.

Cd concentration in the medium (mg L^{-1})	Root length (cm)	Height (cm)	Total biomass dry weight (g $plant^{-1}$)
0	7.84±0.73b	23.5±2.68b	0.83±0.03bc
0.5	8.91±0.65a	24.6±3.11ab	0.84±0.04bc
2.5	8.58±0.15a	26.3±3.09a	0.93±0.02a
5	7.43±0.42b	26.5±4.06a	0.88±0.03ab
10	6.13±0.47c	25.7±2.41ab	0.77±0.03c
25	6.10±0.16c	24.1±2.79b	0.70±0.05d

Data are means ± SD (n=3). Different letters within the columns indicate significant differences at the 5 % level according to the LSD test.

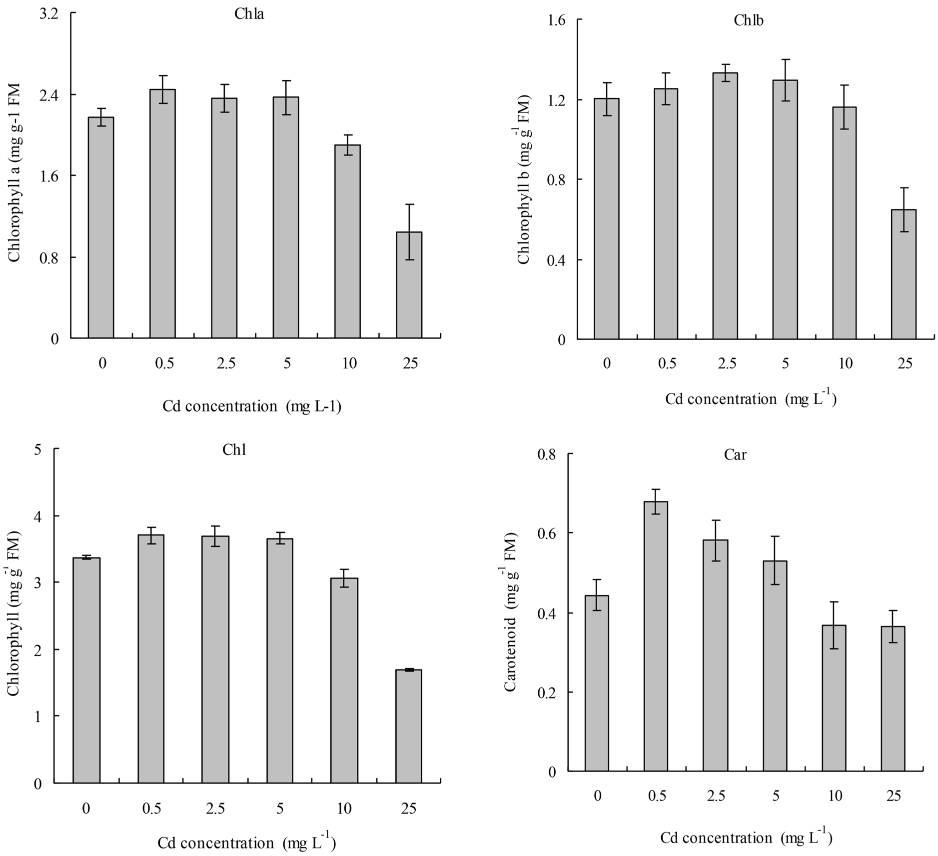

FIGURE 1. The chlorophyll a (Chla), Chlorophyll b (Chlb), chlorophyll (Chl) and carotenoid (Car) contents in leaves of *L. japonica* under Cd stress. Values represent mean ± SD.

concentration, there was a significant gradient of Cd concentrations from roots to shoots. Shoots and roots Cd concentrations exposed to 25 mg L^{-1} Cd were 622.93 ± 66.93and 1575.64 ± 82.15 mg kg^{-1} DW respectively, more than 100 mg kg^{-1} dry tissue, which is the threshold value of

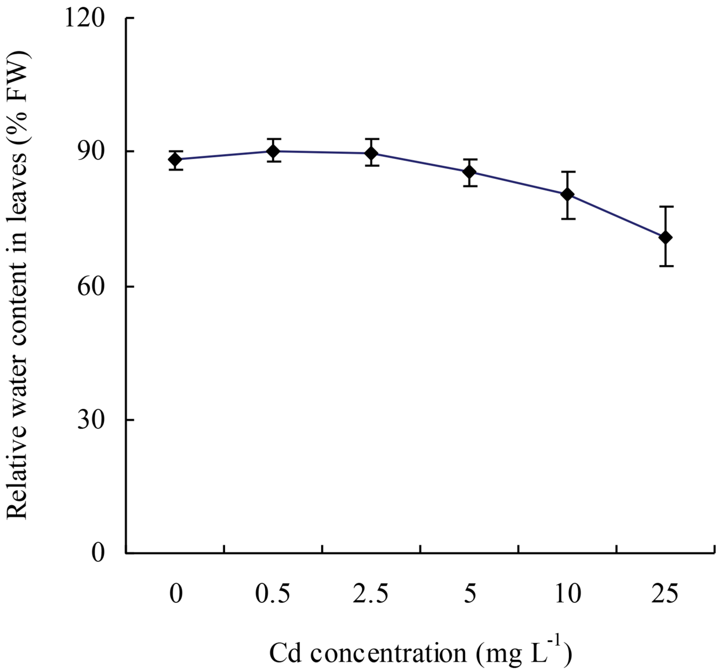

FIGURE 2. Relative water content in leaves of *L. japonica* under Cd stress. Values represent mean ± SD.

Cd-hyperaccumulator (Baker and Brooks 1989). It is suggested that *L. japonica* has a good ability for Cd hyperaccumulation.

DISCUSSION

Hormesis is described that a toxic substance or stressor has a stimulatory effect at low doses but a deleterious effects at high doses (Calabrese and Baldwin 2003a, b). Cd is non-essential element for plants and animals, and it can be easily transferred into the food chain and threatens human health (Han *et al.* 2012). Excessive Cd levels usually lead to the inhibition of plant growth as the heavy metal easily accumulates within plant tissues and negatively interferes with essential physiological processes (di Toppi and Gabbrielli 1999; He *et al.* 2008). However, in the present study, the grow characteristics of *L. japonica*, such as the root length, height and total biomass dry weight increased at low concentrations Cd, and then turned to decrease at high concentrations Cd. Similar phenomena has been reported in barley by Aery and Rana (2003). The phenomenon is agreement with the definition and description of hormesis in toxicology.

It is found that Cd stress often affects photosynthetic pigment composition and causes visual phytotoxic symptoms (Valentovičová *et al.* 2010). In the present study, after 28 days Cd exposure, the contents of chlorophyll

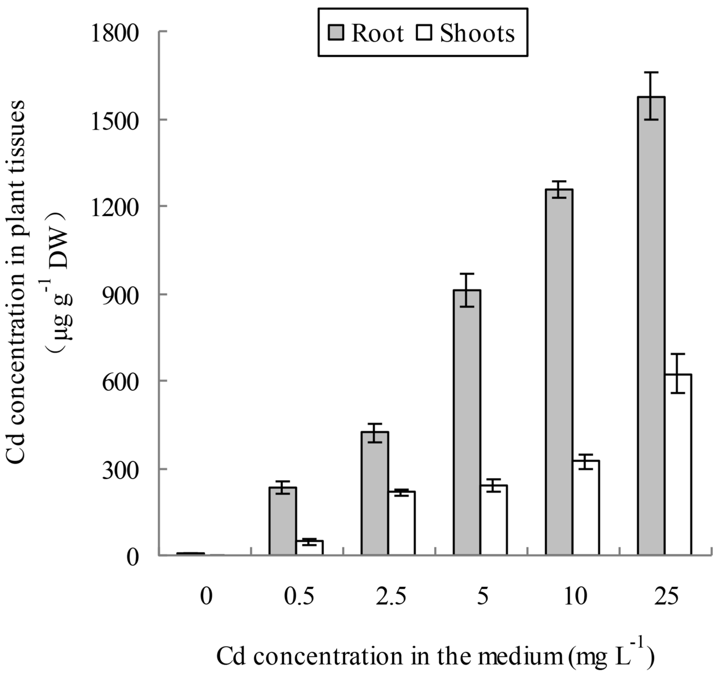

FIGURE 3. Effect of Cd stress on roots and shoots Cd concentrations in *L. japonica*. Values represent mean ± SD.

a (Chla), Chlorophyll b (Chlb), chlorophyll (Chl) and carotenoid (Car) in leaves generally showed an inverted U-shaped curve with the increase of Cd concentrations in the medium, which indicated that low concentrations Cd may be beneficial to plant growth. The reasons resulted from low concentration Cd had a stimulatory effect on photosynthetic pigment composition of the plant, promoted the accumulation of dry matter, and increased the seedling biomass. The similar results have been reports by Zhou and Qiu (2005), and Seth *et al.* (2008), which is mainly explained by Cd-induced Fe uptake increase. Our previous study also showed that there is a synergistic interaction in accumulation and translocation between Cd and Fe in *L. japonica*, and Zn or Mn uptake in leaves has been improved at low concentrations Cd (Liu *et al.* 2011b).

It is proposed that the critical standard of Cd hyperaccumulator is 100 mg kg^{-1}, and the key feature of hyperaccumulators is strong tolerance of the plant to heavy metals (Chaney *et al.* 1997). In the present study, after 28 days Cd exposure, Shoots and roots Cd concentrations exposed to 25 mg L^{-1} Cd were 622.93 ± 66.93and 1575.64 ± 82.15 mg kg^{-1} DW respectively, far more than the critical standard of Cd hyperaccumulators, and *L. japonica* seedlings did not show toxic phenomenon obviously. when Cd concentration in the medium got up to the highest, shoots and roots accumulated Cd contents reached the maximum, indicating that *L. japon-*

ica still keep a good accumulation ability is very strong. The plant can still maintain a normal growth, which showed that *L. japonica* has a strong tolerance and hyper accumulation potential to Cd. In the real life, it is more faced the low concentration Cd environment. *L. japonica* may keep better growth and accumulated more Cd, which will have important practical significance to remediate contaminated soil by Cd.

CONCLUSIONS

In the present study, the hormetic responses of Lonicera japonica Thunb. to Cd stress were shown. The evidence points were indicated by the fact that the plant growth, photosynthetic pigments contents and relative water content were increased at low concentrations Cd, and then turned to decrease at high concentrations Cd. These results confirmed the occurrence of hormetic phenomenon again. Furthermore, *L. japonica* had a good ability of hyperaccumulation in phytoremediation application for Cd-contaminated soil. The special characteristics will be useful to be considered as a new plant model to study the underlying mechanisms of the hormesis.

ACKNOWLEDGEMENTS

Authors wish to express their gratitude to the reviewers for the manuscript. This work was supported by the National Natural Science Foundation of China (41301340), the National Science & Technology Pillar Program (2012BAC05B05) and the major National Science & Technology project "water pollution control and management" (2012ZX07202008) of China.

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