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PHOTOSYNTHETIC PERFORMANCE AND ANTI-OXIDATIVE RESPONSE OF *CORNUS CONTROVERSA* SEEDLINGS UNDER CADMIUM AND LEAD STRESS

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Abstract

The photosynthetic efficiency of *Cornus controversa* leaves was decreased significantly under Cd treatment while it was not affected by Pb exposure. Cd decreased while Pb treatment increased the chlorophyll contents of *Cornus controversa* leaves. Furthermore, the peroxidase (GPX) activities were decreased after Cd treatment while elevated by Pb exposure in *Cornus controvera* seedlings. In addition, both Cd and Pb exposures increased the malondialdehyde (MDA) and proline contents and elevated the superoxide dismutase (SOD) activities of *Cornus controvera* seedlings. Collectively, these results indicated that *Cornus controversa* may be more tolerant to Pb than Cd toxicity. This finding will contribute to the evaluation of planting *Cornus controversa* in heavy metal polluted soil conditions.

Introduction

Environmental heavy metal contamination is becoming a worldwide problem that has attracted a great deal of attention. The release of heavy metals into the environment is mainly caused by various anthropogenic activities associated with agricultural practices, mineral exploration, industrial processes and solid waste management (Alumaa *et al*. 2002). Heavy metal pollution causes serious physiological damage to the plants. Exposure to high levels of heavy metals induces inhibition of nutrient uptake, hindering the activity of photosynthetic apparatus, inhibiting the activation of antioxidant enzymes (Sandalio *et al*. 2001), and displacing the essential elements in plants. Heavy metals also damage the plant cellular components, such as membranes, nucleic acids, chloroplast pigments, and peroxidizing lipid.

Among the heavy metal pollutants, cadmium (Cd) and lead (Pb) are considered as the most toxic element. The plants subjected to heavy metal contaminations display various morphological, physiological, and biochemical changes, and these changes are usually considered to be the indicators of the degree of heavy metal injury. Photosynthesis is one the most fundamental and intricate physiological processes in higher plants. The mechanism of photosynthesis involves various components, including photosynthetic pigments and photosystems, the electron transport system, and CO_2 reduction pathways, any damage caused by a stress may reduce the overall photosynthetic capacity of a green plant (Ashraf and Harris 2013). Heavy metal pollutions such as Cd, copper (Cu), and chromium (Cr) can inhibit the photosynthetic performance of plants by affecting the fluorescence emission kinetic characteristics and photosynthetic pigments of plants (Huseynova *et al*. 2009, Nagajyoti *et al*. 2010). Furthermore, the responses of plant natural

antioxidant system is a another efficient indicator for heavy metal stresses, which involves a number of enzymes including superoxide dismutase (SOD), peroxidase (GPX), catalase (CAT), and ascorbate peroxidase (APX) (Clijsters *et al*. 1999). These antioxidant enzymes can protect plant cells from damage caused by superoxide radical reaction products and build a physical barrier against toxic heavy metals (Krantev *et al*. 2008). Collectively, the photosynthetic performance and anti-oxidative response are two sensitive indicators of plants in response to heavy metal stresses, which can reflect the ability of plants surviving on polluted areas. The photosynthesis performance and anti-oxidant activity of *Cornus controversa* plants under different concentrations of Cd and Pb treatment were studied.

Materials and Methods

Two-year-old healthy *Cornus controversa* seedlings of a uniform height (15 - 20 cm) were grown in the Southwest Forestry University with a climate condition similar to that of the original site of the *Cornus controversa.* The seedlings were transplanted to 5 litre plastic pots filled with red soil, perlite and humus soil in the proportion of 3 : 3 : 2. The experimental treatments started from 10 September to 10 October, 2012 after the seedlings were transplanted. The seedlings were randomly divided into five groups, each group including10 pots. One was the control and the others were treated with different levels of Cd (10 mmol/l and 20 mmol/l) and Pb (5 mmol/l and 10 mmol/L). The Cd was applied as $CdCl_2$, and the Pb was applied as $Pb(NO_3)_2$. For each Pb treatment, the pots were watered with 10 ml of $Pb(NO_3)_2$ solution every other day. It is the same as for Cd treatment. The control was watered 10 ml of water instead of heavy metals solution. The seedlings were collected at days 30, respectively. Various chlorophyll fluorescence, physiological, and biochemical parameters were measured at the end of the experiment. The third or fifth pair of leaves completely unfolded were selected as materials to measure the fluorescence parameters and physiological and biochemical indexes, and at least five seedlings of each repetition were chosen in each treatment.

Chlorophyll fluorescence parameters were performed using the Imaging-PAM M-series (Walz, Effeltrich, Germany) as described by Brugnoli and Björkman (1992). After full dark adaption, leaves were used to determine the *Fo* (the minimal fluorescence after the dark adaptation), the maximum efficiency of *PSII* and *Fv/Fm*. Non-photochemical quenching coefficients *qN* and photochemical quenching *qP* coefficients were calculated as described by van Kooten and Snel (1990). The concentrations of chlorophyll (*a*, *b*) were calculated using adjusted extinction coefficients (Inskeep and Bloom 1985). The malondialdehyde (MDA) content were measured as described by Hodges *et al*. (1999). The absorbance of the free proline concentration was measured at 520 nm. Free proline was measured as described by Bates *et al*. (1973). The proline content was expressed as µg per gram of fresh weight. The total superoxide dismutase (EC 1.15.1.1, SOD) activity was measured spectrophotometrically based on inhibition in the photochemical reduction of nitrobluetetrazolium (NBT) (Beauchamp *et al*. 1971). The guaiacol peroxidase (EC 1.11.1.7, GPX) activity of leaves was measured as described by Chance and Maehly (1955). Statistical analyses were performed with the statistical software package for social science (SPSS), version 17.0. One-way analyze of variance (ANOVA) were conducted to evaluate the significance of the heavy metal effects. Among all treatments, the means were compared by Duncan's tests at the significance level ($p < 0.05$).

Results and Discussion

The *Fv/Fm* and *Y(II)* values of *Cornus controversa* leaves were obviously decreased upon 10 and 20 mmol/l Cd exposure, suggesting that Cd treatment may depress the photosynthesis efficiency of the plants (Table 1). In contrast, the *Fv/Fm* and *Y(II)* values were not obvious altered

after Pb treatment (Table 1). *Fv/Fm* indicates the maximum photochemical efficiency of the photosystem II, and *Y(II)* illustrates the amount of energy used in photochemistry by photosystem II. *Fv/Fm* and *Y(II)* values are two important indictors reflecting the photosynthesis efficiency of plants. Chlorophyll content is considered as an indictor of damages to photosynthetic system induced by adverse environment (Maxwell and Johnson 2000). Cd treatments decreased both chlorophyll *a* and *b* contents of *Cornus controversa.* In contrast, chlorophyll *a* and *b* contents were increased upon Pb treatment with the increasing concentrations of Pb (Table 2). Collectively, Cd might be more toxic to the photosynthetic systems of *Cornus controversa* than Pb pollution. On the other hand, one of the most important causes of Cd toxicity is that it induces strong iron (Fe) deficiency. Fe is required for the biosynthesis of chlorophyll, and iron-deficient plants often showed decreased levels of chlorophyll. A number of studies have showed that acute Cd stress decreased the chlorophyll content and perturbed the composition of thylakoid complexes (Kapoor *et al.* 2014). These findings suggested that the decreased level of chlorophyll in *Cornus controversa* seedlings upon Cd may be caused by Cd induced Fe deficiency. Furthermore, Fe has an intermediate redox potential relative to other electron donors used in photosynthesis, and Fe is also necessary for the biosynthesis of iron-sulfur proteins in plants. Cd induced Fe deficiency may interfere with the electron transport of the photosystem and thus decrease the photosynthesis efficiency of *Cornus controversa* seedlings. High level of Pb toxicity could inhibit chlorophyll

Table 1. Effects of lead and cadmium on chlorophyll fluorescence parameters.

Treatment	Fo	Fv/Fm	Y(II)	qN	qP
CK	$0.1077 \pm 0.0141a$	$0.7295 \pm 0.0094b$	$0.5238 \pm 0.0115b$	$0.5437 \pm 0.0881ab$	$0.8509 \pm 0.0257ab$
$Pb^{2+}A$	$0.1001 \pm 0.0022a$	$0.7158 \pm 0.0065b$	$0.5533 \pm 0.0314b$	$0.5363 \pm 0.0345ab$	$0.9127 \pm 0.0419b$
$Pb^{2+}B$	$0.0942 \pm 0.0128a$	$0.7525 \pm 0.0191b$	$0.5788 \pm 0.0425b$	$0.4717 \pm 0.0736a$	$0.8713 \pm 0.0203ab$
$Cd^{2+}A$	$0.1441 \pm 0.0120b$	$0.6672 \pm 0.0252a$	$0.4114 \pm 0.0653a$	$0.6490 \pm 0.0723b$	$0.8004 \pm 0.1202ab$
$Cd^{2+}B$	$0.1477 \pm 0.0096b$	$0.6582 \pm 0.0300a$	$0.3995 \pm 0.0194a$	$0.6374 \pm 0.0408b$	$0.7795 \pm 0.0512a$
p	0.000^{***}	0.001^{**}	0.000^{***}	0.036^{*}	$0.145ns$

CK, control treatment; $Pb^{2+}A$, treatment with 5 mmol/l $Pb(NO_3)_2$ solution; $Pb^{2+}B$, treatment with 10 mmol/l $Pb(NO_3)_2$ solution; $Cd^{2+}A$, treatment with 10 mmol/l $CdCl_2$ solution; $Cd^{2+}B$, treatment with 10 mmol/l $CdCl_2$ solution. Values followed by the same letter within a column indicate nonsignificant differences at p < 0.05 (DMRT). Each value represents the mean $\pm$ SE of five replicates; the same as follow.

Table 2. Effects of lead and cadmium on chlorophyll content.

Treatment	Chl *a* (mg/g FW)	Chl *b* (mg/g FW)	Chl *b/a*
CK	1.026 ± 0.048 ab	$0.521 \pm 0.079ab$	$0.5063 \pm 0.0201a$
$Pb^{2+}A$	1.291 ± 0.024 b	$0.651 \pm 0.035b$	$0.5041 \pm 0.0177a$
$Pb^{2+}B$	$1.706 \pm 0.299c$	$0.842 \pm 0.122c$	$0.4956 \pm 0.0180a$
$Cd^{2+}A$	$0.860 \pm 0.031a$	$0.444 \pm 0.028a$	$0.5155 \pm 0.0226a$
$Cd^{2+}B$	$0.778 \pm 0.050a$	$0.411 \pm 0.017a$	$0.5286 \pm 0.0120a$
p	0.000^{***}	0.000^{***}	$0.257ns$

synthesis causing impaired uptake of essential elements and even accelerate the decomposition of chlorophyll (Cenkci *et al.* 2010), such as, 50 mmol/l Pb could decrease chlorophyll content of *Pisum sativum* (Sengar and Pandey 1996). However, present work showed that 5 and 10 mmol/l $Pb(NO_3)_2$ treatments could increase the chlorophyll a/b content of *Cornus controversa* seedlings. Similar work also showed that low concentration of Pb exposure elevated the chlorophyll content of *Hydrilla verticillata* and *Jatropha curcas* L. (Shu *et al.* 2012, Singh *et al.* 2013). These findings suggested that low level of Pb might have a simulating effect on chlorophyll contents of plants.

In addition, MDA is a reliable marker that determines oxidative stress under heavy metal pollutions in plants. Therefore, the MDA content was measured in *Cornus controversa* after exposed to either Cd or Pb treatments. A significant increment of MDA content was detected after either Cd or Pb treatment (Fig. 1), suggesting that both Cd and Pb may promote seriously oxidative damage in the plant cells of *Cornus controversa*. The result agrees with many previous studies (Posmyk *et al.* 2005, Foyer and Noctor 2005). To protect from oxidative damage, antioxidant enzymes, such as SOD and GPX are activated to scavenge the radicals. SOD is an essential enzyme, which catalyzes the conversion of superoxide anion to O_2 and H_2O_2, and its activity is often explained as a way of O_2 radical-eliminating ability (Wang *et al.* 2005). In present study, the SOD activity in *Cornus controversa* leaves was increased significantly under either Cd or Pb treatment (Table 3). Enhanced SOD activities in *Cornus controversa* seedlings under Cd and Pb exposure suggested that the plants may start the natural anti-oxidative system to scavenge free O_2 radicals. In contrast, a number of studies also revealed the heavy metal toxicities may interfere with the antioxidant defense systems by affecting the enzyme activities (Cheng 2003, Nagajyoti *et al.* 2010). GPX is reported to catalyze the reduction of H_2O_2, organic hydroperoxides, and lipid hydroperoxides to H_2O and alcohol using GSH and/or other reducing equivalents (Foyer and Noctor 2011). In present work, Cd exposure decreased the GPX activities of *Cornus controversa* seedlings (Table 3), which probably caused by either the directly inactivation or indirect repression of the production of GPX proteins by Cd. The inhibition of GPX upon Cd may impair the ability of *Cornus controversa* seedlings in response to oxidative stresses. Additionally, previous studies demonstrated proline is a potent scavenger of ROS in response to oxidative stress (Chen and Dickman 2005). In present work, the proline content was increased 60.5 and 128.2% under 5 and 10 mmol/l Pb treatments respectively, while 77.9 and 221% under 10 and 20 mmol/l Cd treatments respectively (Fig. 2). The results were consistent with the report of Schat *et al.* (1997) on the *Silene vulgaris*. Hence, the increased accumulation of proline under the Cd and Pb treatments, this will help the *Cornus controversa* seedlings to maintain an osmotic balance within plant cells.

Table 3. Effects of lead and cadmium on antioxidative enzyme activities.

Treatment	GPX (mmol guaiacol/min/g/ FW)	SOD (Unit/g FW)
CK	1.783 ± 0.284ab	245.98 ± 41.04a
Pb^{2+}A	2.801 ± 0.883c	471.58 ± 104.70b
Pb^{2+}B	1.908 ± 0.226b	437.02 ± 96.87b
Cd^{2+}A	0.912 ± 0.059a	493.41 ± 65.79b
Cd^{2+}B	1.191 ± 0.597ab	431.26 ± 51.06b
p	0.004**	0.018*

It is suggested that 2-year-old *Cornus controversa* seedlings may be more tolerant to Pb than Cd. These findings will contribute to the evaluation of planting *Cornus controversa* in heavy metal polluted areas.
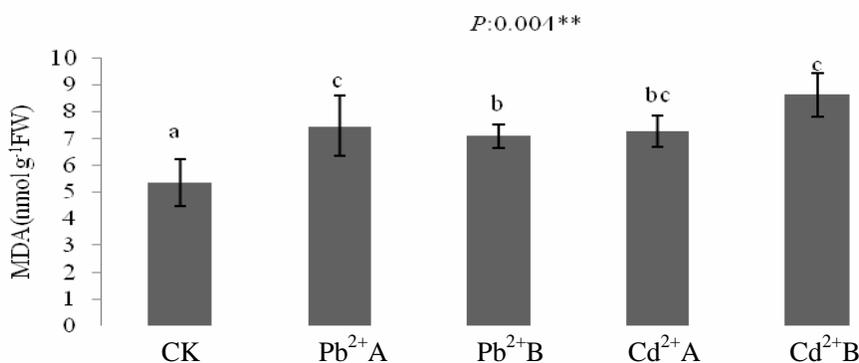
Fig.1 Effects of Pb and Cd on MDA content. CK, control treatment; $Pb^{2+}A$, treatment with 5 mmol/l $Pb(NO_3)_2$ solution; $Pb^{2+}B$, treatment with 10 mmol/l $Pb(NO_3)_2$ solution; $Cd^{2+}A$, treatment with 10 mmol/l $CdCl_2$ solution; $Cd^{2+}B$, treatment with 10 mmol/l $CdCl_2$ solution. Values followed by the same letter indicate nonsignificant differences at $p < 0.05$ (DMRT). Each value represents the mean ± SE of five replicates.
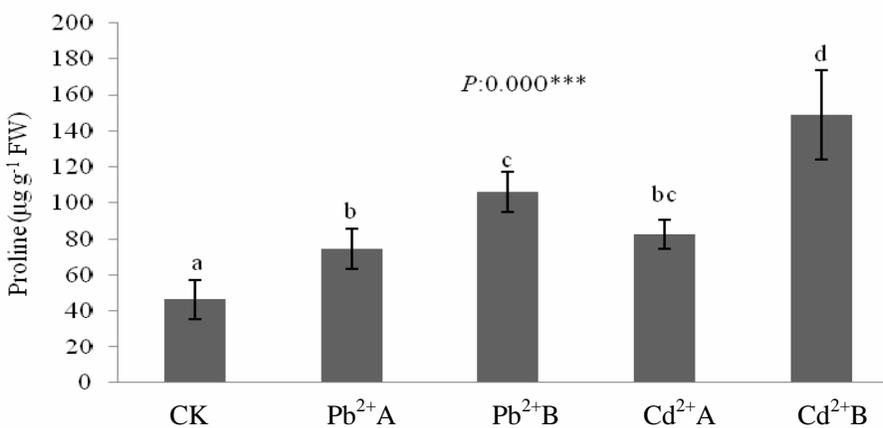

Fig. 2. Effects of Pb and Cd on proline content. CK, control treatment; $Pb^{2+}A$, treatment with 5 mmol/l $Pb(NO_3)_2$ solution; $Pb^{2+}B$, treatment with 10 mmol/l $Pb(NO_3)_2$ solution; $Cd^{2+}A$, treatment with 10 mmol/l $CdCl_2$ solution; $Cd^{2+}B$, treatment with 10 mmol/l $CdCl_2$ solution. Values followed by the same letter indicate nonsignificant differences at $p < 0.05$ (DMRT). Each value represents the mean ± SE of five replicates.

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