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# EFFECTS OF CADMIUM ON THE ANTIOXIDATIVE DEFENSE SYSTEM AND BIOMASS ACCUMULATION OF *POPULUS* × *CANESCENS*

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**Abstract**

This study investigated the Cd-tolerance of *Populus* × *canescens* under five levels of $CdSO_4$ for 7, 14, 21 and 28 days by hydroponic experiment. The results showed that Cd significantly increased the activities of GR, DHAR and MDHAR in roots, wood, bark and leaves with the increase in Cd concentrations. While the increases in SOD activities are observed at 10 μM Cd and then decreased from 30 to 70 μM Cd. High levels of Cd decreased the GPX activity. Pretreatment with Cd had little effect on the enzymatic components of seedlings under normal conditions. Among all tissues, the pretreatment with 10 μM Cd showed better results, compared to 70 μM Cd. These results indicated that low concentrations of exogenous Cd applications increased the tolerance of *Populus* × *canescens* by enhancing antioxidant defense systems.

## Introduction

Cadmium (Cd) is a globally distributed toxic element. It enters the aquatic environment from natural and anthropogenic sources, and then is transferred to the food chain. Cd can inhibit enzyme activities, cause water imbalance, alter the membrane permeability and disturb mineral nutrition. Cd is a widespread heavy metal pollutant with great potential toxicity and high mobility in the environment. Cd contamination has been a worldwide public health concern for its bioaccumulation in plants (Dai *et al*. 2013a). Its level has been increased in the environment due to the anthropogenic activities, including the expansion of the industry, waste water irrigation, application of sewage sludges and the excessive use of fertilizers and pesticides (Tran and Popova 2013). Cd has negative effects on growth of most plant species, even at low concentrations. For its high affinity to thiol groups, Cd affects the cellular sulfhydryl homeostasis by inhibiting SH-containing redox regulated enzymes (Dai *et al*. 2012, Dai *et al*. 2013b), which leads to oxidative stress (Dai *et al*. 2015). Dai *et al*. (2012) have discovered that the accumulation of reactive oxygen species (ROS) could give rise to oxidative stress, which could lead to cell damage, mutation, and/or even death. Changes in ROS levels were observed in many plants treated by Cd (Duman *et al*. 2011, Bharwana *et al*. 2014). To alleviate those damages, plants can enhance scavenging systems including non-enzymatic antioxidants and antioxidant enzymes, such as SOD, CAT and enzymes in ascorbate-glutathione cycle (Bharwana *et al*. 2014). Understanding the detoxification strategies of plants under heavy metal stress is very important for the manipulation of heavy metal tolerance.

In the present study, we investigated the capacity of *Populus* × *canescens* to uptake Cd and the detoxification mechanism of Cd in all tissues of *Populus* × *canescens*, a hybrid of *Populus tremula* × *Populus alba*. The aim of the study to evaluate the role of antioxidative system in the phytoremediation capability of the *Populus* × *canescens* under Cd stress and determine the concentration which *Populus* × *canescens* is suitable for the phyto-remediation of Cd-polluted soils.

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## Materials and Methods

The experiments were performed in the orchard of Northwest Agriculture & Forestry University, Yangling (34°20′N, 108°24′E), P. R. China. Plantlets of *Populus* × *canescens* (*P. tremula* × *P. alba*) were produced by micropropagation and cultivated in a climate chamber. Culture conditions: a day/night temperature of 25/18°C, 50 - 60% relative air humidity, 150 μmol/$m^2$/$s^1$ photosynthetic photon flux and a 16 hrs photoperiod. After 4 weeks, the rooted plantlets were transferred to an aerated Hoagland nutrient solution in a growth room with the same environmental condition as in the climate chamber. The nutrient solution was changed every 3 days. After 12-week cultivation, the plants were treated by 0 (control), 10, 30, 50 and 70 μM $CdSO_4$ by adding its into the nutrient solution, respectively.

The seedlings were harvested after 7, 14, 21 and 28 days of treatment. The roots were immersed in 5 mM $CaCl_2$ for 15 min, and then the whole plants were rinsed with deionized water. The leaf and shoot (bark and wood) were separated and dried in an air oven at 80°C until a constant weight to obtain dry weight. The effects of Cd on the fresh weight of root, shoot (bark and wood) and leaf were assessed by using two-way ANOVA.

Each sample of leaves, bark, wood and root (0.5 g) was grinded in porcelain mortars by using liquid $N_2$. The extraction buffer was a 50 mM phosphate buffer (pH 7.0) containing 1 mM phenyl-methanesulfonyl fluoride, 0.1 mM ethylenediamine tetra acetic acid. Homogenized leaf, bark, wood and root material were centrifuged for 20 min at 15000 g and the supernatant was used for the determination of the enzymatic activities. The activities of superoxide dismutase (SOD, EC 1.15.1.11), glutathione reductase (GR, EC 1.6.4.2), glutathione peroxidase (GPX, EC 1.11.1.9), monodehydroascorbate reductase (MDHAR, EC 1.6.5.4), dehydroascorbate reductase (DHAR, EC 1.8.5.1) were determined according to Zhao and Dai (2015) and *Dai et al*. (2012).

The experiment had a completely randomized design with six replicates per treatment for each time point. Data were subjected to analysis of variance (ANOVA) to examine the effects of time, treatment and organ. Statistical analysis was conducted by using the software CoStat 6.2 (CoHort Software, CA, USA). Separation of means was performed by using LSD test at $P<0.05$ significance level.

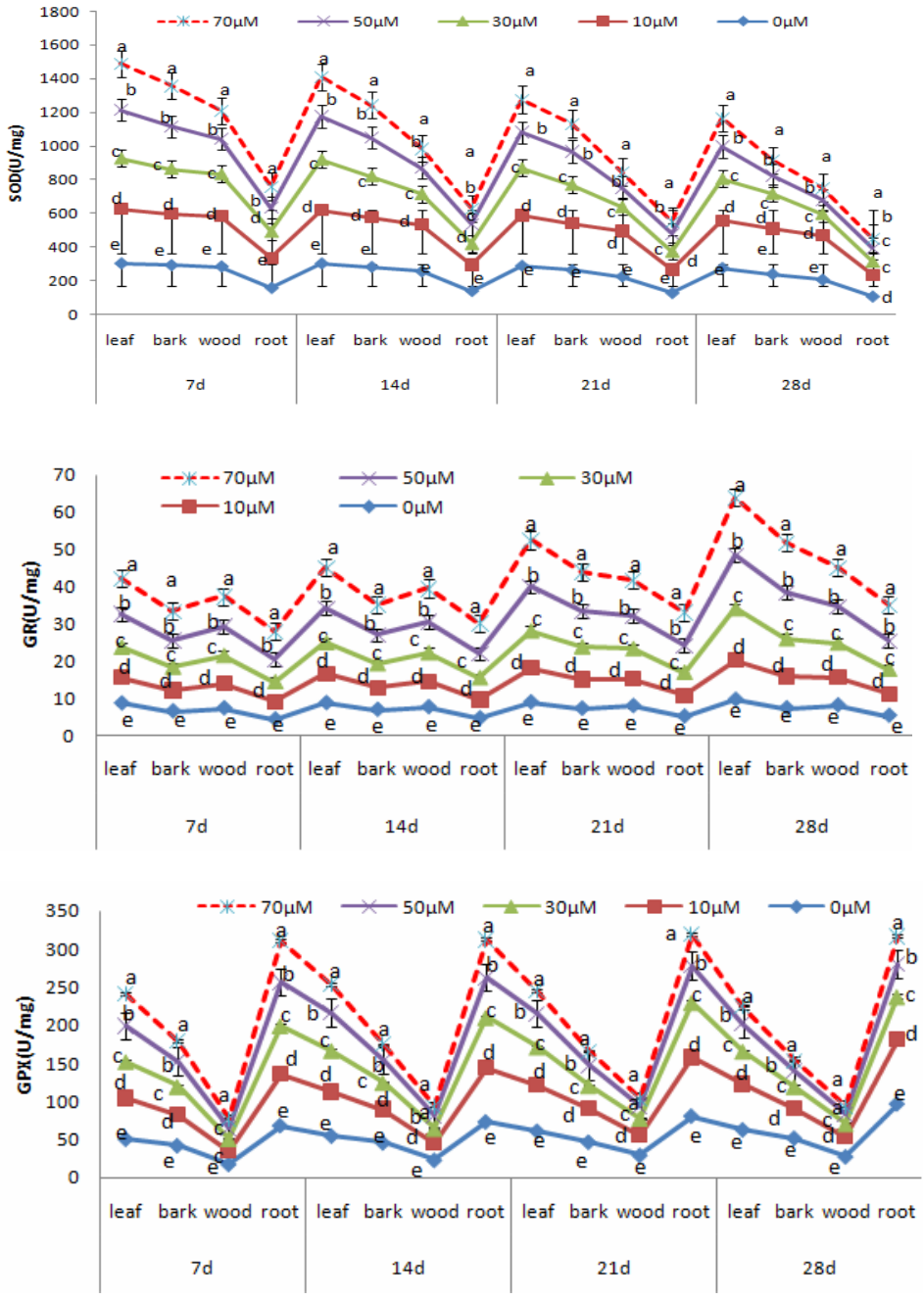
## Results and Discussion

Components of ROS scavenging system (i.e. enzymatic antioxidants; SOD and GR, GPX) were studied in *P.*×*canescens* plants treated with increasing Cd concentration (Fig.1). The activities of SOD were significantly increased under 10 μM Cd and decreased under 50 and 70 μM Cd ($p < 0.05$). Among different tissues, the decline in SOD activity was more rapidly in roots than in wood or leaves. The activities of SOD were decreased at different concentrations of Cd on 28 d. Significant decreases in GR activities were observed after 14 d of exposure to 10 μM Cd. GR activity was declined more strongly in leaf than in barks or woods. On the contrary, 50 and 70 μM Cd increased the activities of GR in different tissues. However, after 28 days of 70 μM Cd exposure, the activities of GPX in woods, barks, leaves and roots were decreased by 35.8, 25.2, 33.0 and 37.8%, respectively.

After 7 to 28 days of treatment, the activities of MDHAR and DHAR in different tissues were increased by the addition of $Cd^{2+}$, compared to the control (Fig. 1). There were significant increases in the activities of MDHAR and DHAR in roots under 10 and 30 μM Cd. However, there were significant decreases in the activities of MDHAR and DHAR in roots under 50 and 70 μM Cd ($p < 0.05$).

To analyze the toxic effects of Cd on the plant growth, the biomass of root, bark, wood and leaf was recorded (Table 1). After Cd exposure for 7 days, the biomass of all tissues in *Populus* ×

*canescens* was not affected by Cd exposure. However, Cd exposure for 28 days, compared to the control, 70 µM Cd reduced the biomass of leaf, wood, bark, and root by 36.0, 35.8, 33.0 and 25.1%, respectively.

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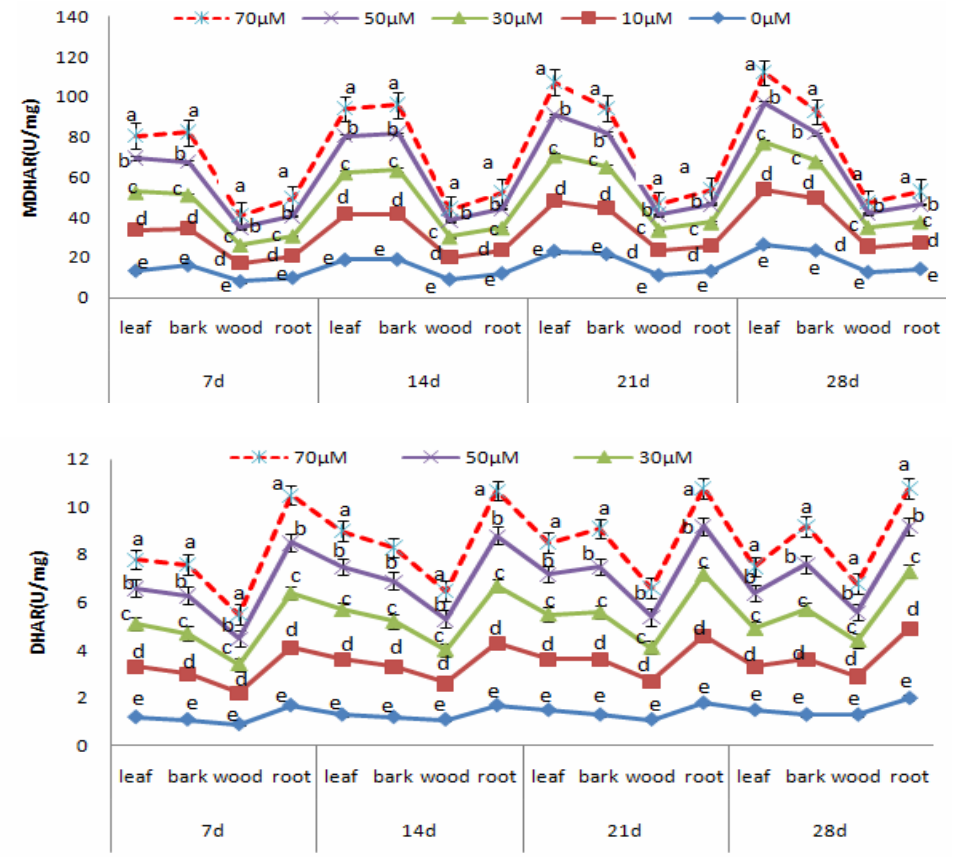


Fig. 1. Changes in SOD, GR, GPX, MDHAR and DHAR activities (U/mg FW) of *Populus* × *canescens* grown with 0 (control), 10, 30, 50, and 70 μM $CdSO_4$ for 7($T_1$), 14($T_2$), 21($T_3$) and 28($T_4$) days. Values are means of six replicates ± Sd. Different small alphabets representing significant difference at $p \leq 0.05$.

It has been reported that Cd inhibits the growth of several plant species with different sensitivities to Cd (Dai *et al*. 2013a). Recent studies have revealed that Cd can modulate the capability of antioxidative defense system in many plants (Qiu *et al*. 2008). Hasanuzzaman *et al*. (2012) found that the extent of oxidative stress is associated with endogenous Cd levels in plants. Increasing evidences showed that plants could resist oxidative damage by enhancing the activities of antioxidative enzymes (Dai *et al*. 2013b). In this study, Cd decreased the activities of SOD and GPX in different tissues. Similar effects of Cd were reported in several aquatic biosystems (Kim *et al*. 2013). Cd did not affect the activity of DHAR and stimulated MDHAR activity in grey poplar plants, which indicated that the regeneration of ascorbate was accomplished through DHAR and MDHAR under Cd stress. While the increases in GR activities were observed at 28 days of lower concentration (10 and 30 μM) Cd treatment. Many studies have shown that the increase in GR activity is important mechanism of the defence against Cd stress. Thus, our results suggest that *Populus* × *canescens* does not suffer from oxidative stress at low levels of Cd and would be expected as a species that can successfully adapt to low polluted environments. Therefore, our results can be used to illustrate how *Populus* × *canescens* responds to Cd stress. The ability of *Populus* × *canescens* to tolerate Cd could be partly derived from ROS detoxification through an efficient antioxidant system.

**Table 1. The leaf dry weight (LDW), bark dry weight (BDW), wood dry weight (WDW) and root dry weight (RDW) of *Populus* × *canescens* grown in 0 (control), 10, 30, 50, and 70 μM $CdSO_4$ for 7($T_1$), 14($T_2$), 21($T_3$) and 28($T_4$) days.**

| Time | Cd (μM) | LDW (mg/g) | WDW (mg/g) | BDW (mg/g) | RDW (mg/g) | Total W (mg/g) |
|---|---|---|---|---|---|---|
| $T_1$ | 0 | 1.1 ± 0.14** | 0.22 ± 0.05** | 0.5 ± 0.06** | 0.59 ± 0.05 | 2.42 ± 0.30** |
| | 10 | 1.02 ± 0.23** | 0.18 ± 0.08** | 0.44 ± 0.14** | 0.51 ± 0.17** | 2.27 ± 0.64** |
| | 30 | 0.85 ± 0.39** | 0.17 ± 0.11** | 0.39 ± 0.25** | 0.44 ± 0.26** | 1.87 ± 1.02** |
| | 50 | 0.71 ± 0.57** | 0.16 ± 0.17** | 0.37 ± 0.31** | 0.42 ± 0.34** | 1.67 ± 1.41** |
| | 70 | 0.63 ± 0.14** | 0.15 ± 0.05** | 0.32 ± 0.06** | 0.39 ± 0.05 | 1.51 ± 0.30** |
| $T_2$ | 0 | 1.32 ± 0.09** | 0.44 ± 0.03* | 0.76 ± 0.08** | 0.82 ± 0.12** | 3.36 ± 0.33** |
| | 10 | 1.14 ± 0.25** | 0.42 ± 0.06** | 0.68 ± 0.18** | 0.75 ± 0.21** | 3.01 ± 0.72** |
| | 30 | 1.07 ± 0.43** | 0.38 ± 0.12** | 0.62 ± 0.25** | 0.63 ± 0.29** | 2.75 ± 1.11** |
| | 50 | 0.89 ± 0.23** | 0.36 ± 0.08** | 0.58 ± 0.17** | 0.57 ± 0.17** | 2.42 ± 0.64** |
| | 70 | 0.76 ± 0.09** | 0.26 ± 0.03* | 0.5 ± 0.10** | 0.48 ± 0.12** | 2.02 ± 0.33** |
| $T_3$ | 0 | 1.68 ± 0.16** | 0.67 ± 0.02* | 1.12 ± 0.10** | 1.04 ± 0.09** | 4.53 ± 0.38** |
| | 10 | 1.53 ± 0.34** | 0.59 ± 0.09** | 1.06 ± 0.17** | 1.05 ± 0.17** | 4.24 ± 0.77** |
| | 30 | 1.51 ± 0.39** | 0.51 ± 0.11** | 0.94 ± 0.25** | 0.89 ± 0.26** | 3.9 ± 1.02** |
| | 50 | 1.42 ± 0.25** | 0.55 ± 0.06** | 0.82 ± 0.18** | 0.78 ± 0.21** | 3.55 ± 0.72** |
| | 70 | 1.24 ± 0.16** | 0.45 ± 0.02* | 0.76 ± 0.10** | 0.62 ± 0.09** | 3.09 ± 0.38** |
| $T_4$ | 0 | 2.33 ± 0.18** | 0.67 ± 0.06** | 1.30 ± 0.06** | 1.55 ± 0.08** | 5.95 ± 0.38** |
| | 10 | 2.17 ± 0.58** | 0.60 ± 0.17** | 1.23 ± 0.31** | 1.47 ± 0.34** | 5.5 ± 1.41** |
| | 30 | 2.05 ± 0.43** | 0.55 ± 0.12** | 1.19 ± 0.25** | 1.37 ± 0.29** | 5.16 ± 1.11** |
| | 50 | 1.82 ± 0.34** | 0.51 ± 0.09** | 0.94 ± 0.17** | 1.21 ± 0.17** | 4.49 ± 0.77** |
| | 70 | 1.49 ± 0.18** | 0.43 ± 0.06** | 0.87 ± 0.06** | 1.16 ± 0.08** | 3.96 ± 0.38** |

| Biomass | Primary effects | | Interactions |
|---|---|---|---|
| | Metal | Time | M×T |
| ANOVA p values | | | |
| Leaf | 0.000 | 0.000 | 0.009 |
| Bark | 0.000 | 0.000 | 0.002 |
| Stem | 0.000 | 0.000 | 0.000 |
| Root | 0.000 | 0.000 | 0.003 |
| Total | 0.000 | 0.000 | 0.000 |

Means ± SE from at least six independent measurements. * and ** significantly differ from the control at $p < 0.05$ and $p < 0.01$, respectively. ANOVA *a* value for the primary effects and interactions of metal × time (M × T) are provided.

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