

# Accumulation of cadmium and its effects on physiological characteristics in *Arundo donax* L.

PU G. Z.<sup>\*</sup>, Zhang D. N., Xu G.P. And HUANGA Y. Q.

Guangxi Key Laboratory of Plant Conservation and Restoration Ecology in Karst Terrain, Guangxi Institute of Botany, Guangxi Zhuang Autonomous Region and Chinese Academy of Sciences, Guilin, 541006, China

\*corresponding author:

e-mail: pukouchy@163.com

## Abstract

A wetland simulated experiment was employed to investigate the accumulation of Cadmium (Cd) in *Arundo donax* L., and the effects of Cd addition on its growth and photosynthesis characteristics. Results showed that the morphological characteristics, the leaves and roots biomass didn't show statistically significant differences. The order of Cd accumulation is stem < roots < leaves. There were no differences on relative chlorophyll content, quantum yield of PSII electron transport (Yield) and maximum quantum efficiency of photosystem II (Fv/Fm) (except 0.125 mg/L), photosynthetic rate (Pn), while significant differences on initial chlorophyll fluorescence (Fv/Fo). The activity of superoxide dismutase (SOD) was stimulated by Cd treatments. Although the activities of peroxidase (POD) and catalase (CAT) were decreased at 2.5 mg/L, and at 0.05 and 0.125 mg/L, respectively, the activity of CAT was increased at 2.5 mg/L and POD increased at 0.05 and 0.125 mg/L, indicating the decrease in CAT and POD was compensated by the induction of POD and CAT activity, respectively. The results suggested that the oxidative stress may involve in the mechanism of Cd toxicity and *A. donax* showed a strong tolerance to the increased Cd pollution, which may have a potential use for phytoremediation purposes in wetland environment.

**Keywords:** *Arundo donax*, antioxidant enzymes, chlorophyll fluorescence, gas exchange parameters.

## 1. Introduction

Cadmium (Cd) is regarded as one of the most toxic heavy metals in ground water, soil and sediments due to its wide industrial application, hence posing a serious environmental concern. It can undermine the ecosystem function and human health at trace level due to its high toxicity (Zhi *et al.*, 2016). Hence, how to effectively control the cadmium pollution and restore the ecological environment is one of the most urgent environmental problems. It is well known that Cd is a highly toxic metal pollutant in soil and is highly mobile between soil-plant systems and can be quickly absorbed by plants and hence transported to upper parts causing toxicity (Irfan *et al.*, 2014). Many studies indicated that excess Cd may cause nutrient deficiency, disruption of ATPase activity, decrease of photosynthesis, inhibition of various enzyme activities,

induction of oxidative stress and reduce genotoxicity (Johna *et al.*, 2009 ; Alshaal *et al.*, 2015 ; Moussa *et al.*, 2016 ; Shahid *et al.*, 2017). These studies also suggested that accumulation of Cd in plants varied greatly not only among plant species, cultivars and genotypes but also among different environmental chemical properties of the same species, and the plants' Cd detoxification and tolerance mechanisms (Shahid *et al.*, 2017). Obviously, plant species in different environments would show a wide range of plasticity in Cd tolerance. Therefore, it is important to understand the response of plants to Cd in different environments, and then carry out phytoremediation. Phytoremediation is a growing field of research in environmental studies owing to the advantages of its environmental friendliness, safe, and cost-effective (Malik, 2007; Mirza *et al.*, 2010; Elhawat *et al.*, 2014). Although hyperaccumulation as a tool for cleaning up metal contaminated environments has been widely suggested (Leitenmaier and Küpper, 2013), they are often linked with slow growth rate and low biomass production, so that net removal of metals via phytoextraction is quite limited (Elhawat *et al.*, 2014). There for, the use of low cost, fast growing indigenous plants with efficient biomass producing plant species such as giant reed (*Arundo donax* L.) are highly desirable for phytoremediation of metal contaminated sites and waters. *A. donax* is a perennial rhizomatous grass (Poaceae family), native to the freshwater regions of Eastern Asia. Because of its high biomass, stronger adaption and unique physiological features whereby it readily absorbs and concentrates toxic chemicals from contaminated soil (Elhawat *et al.* 2014). Furthermore, *A. donax* has gained spacious reputation as good candidate for energy production and paper industry (Elhawat *et al.* 2014). Many studies indicate that *A. donax* may have a potential use for phytoremediation purposes (Alshaal *et al.* 2013; Shabana *et al.* 2012; Miao *et al.* 2012; Mirza *et al.* 2010; Elhawat *et al.*, 2014 Papazoglou *et al.* 2005; Alshaal *et al.*, 2015 ; Liu *et al.*, 2017). As a C3-grass, *A. donax* shows high photosynthetic rates and unsaturated photosynthetic potential compared to C4 plants (Alshaal *et al.*, 2015). Previous studies show that, under higher level Cd, As, Pb, Cr and Ni stress, *A. donax* can healthy growth and the photosynthetic system is not harmed (Alshaal *et al.*, 2015), depending on its higher anti-oxidant capacity by which its anti-oxidative enzymes catalyze the dismutation of highly reactive O<sub>2</sub><sup>-</sup> into non-

**Table 1.** Basic physiochemical properties of tested soil and water

Soil	pH	TN(g/kg)	TP(g/kg)	TK(g/kg)	AN(mg/kg)	AP(mg/kg)	Cd(mg/kg)
	6.50	1.167	0.80	0.78	181.26	3.72	0.01
Water	PH	DO(mg/L)	SpC( $\mu$ s/cm)	Ca <sup>2+</sup> (mg/L)	Mg <sup>2+</sup> (mg/L)	HCO <sub>3</sub> <sup>-</sup> (mmol/L)	Cd( $\mu$ g/L)
	7.38	1.56	235.00	53.00	7.63	1.70	<0.001

toxic forms like O<sub>2</sub> and H<sub>2</sub>O (Miao *et al.* 2012). These results indicate that metals such Cd, Ni, As, Cr and Pb are most likely sequestered in a very effective approach within *A. donax* plant, thus providing a potent protection of the photosynthetic machine. However, there is limited data on the physiological responses of *A. donax* to Cd stress in wetland environment and the capacity of *A. donax* to recover Cd-contaminated wetland bodies. Therefore, the objective of this study was to monitor the physiological responses (including chlorophyll fluorescence, photosynthetic parameters and antioxidant enzymes activities) responses of *A. donax* to Cd stress in a wetland simulated experiment and also to highlight the uptake and translocation of Tl in the plant.

## 2. Material and Methods

### 2.1. Test soil and plant

The tested (0–20 cm) sample and water was collected from the garden of the Guangxi Institute of Botany and the basic physiochemical properties of soil and water were analyzed (Table 1). Air dried soils of 5 kg were put into container with diameter of 35 cm and height of 50 cm. In order to simulate wetland environment, each container was covered with 4L of water. Test soil in each pot was homogeneously sprayed with aqueous solutions containing 0.01, 0.025, 0.05, 0.125 and 2.5 mg Cd per L water, which were prepared by dissolving salts of CdCl<sub>2</sub>·2.5H<sub>2</sub>O. The soil-grown plants were obtained from growth of young meristematic buds grown in sterile aqueous medium. **2.2. Culture and harvest for plant.** After four months of cultivation, chlorophyll, chlorophyll fluorescence parameters, photosynthetic gas exchange parameters and antioxidant enzymes activities were determined. After that, the aboveground of *A. donax* was harvested and thoroughly washed with tap water, then rinsed with deionized water. Parts of fresh leaves were selected for the determination of enzyme activities. The remaining samples including leaves, stems and roots were then separated and cut with stainless steel scissor, and dried at 40 °C for 72 h for elemental analysis. Total Cd in the plant material was estimated after digestion of oven-dried plants (100 mg) following the protocol of Liu *et al.* (2017).

### 2.3. Photosynthetic parameters

Chlorophyll fluorescence parameters and photosynthetic gas exchange parameters were determined by the method described by Lichtenthaler (2005) using LI-6400XT (Li-Cor, Inc., USA) and portable fluorometer (Monitoring-PAM, Walz, Germany), separately. Photosynthetic rate (Pn), transpiration rate (Tr, intercellular CO<sub>2</sub> concentration (Ci), and stomatal conductivity (Gs) were measured from the middle region of the topmost fully

expanded leaf at 25°C under a light intensity of 1,200  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>, relative humidity of 40%, and CO<sub>2</sub> concentration of 370  $\mu$ mol mol<sup>-1</sup>. The topmost fully expanded leaves of treated and control plants were first light- and dark-adapted for 20 min to obtain F and Fo. The Fm' and Fm values (maximum fluorescence yield of light- and dark-adapted leaves, respectively) were calculated with a saturation pulse, and then the maximum photosystem II quantum yield was calculated by the formula [(Fm-Fo)/Fm=Fv/Fm]. The effective quantum yield of PSII, Y(II)=(Fm-F)/Fm, was determined according to Genty (1989). All measurements were taken from five plants of each replication during 8:00 to 11:00 a.m.

### 2.4. Antioxidant enzymes activities

The activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) were assayed by following the protocols of Shah *et al.* (2012) with slight modification. Leaves (0.3g) were homogenized in 5 cm<sup>3</sup> of ice-cold 50 mM phosphate buffer pH 6.5 (for POD, SOD) and pH 7.5 (CAT). The extracts were centrifuged at 10000 g for 20 min at 0 to 4 °C in a Beckmann refrigerated centrifuge, and the supernatants were used for the enzyme activity assays.

### 2.5. Data analysis

All data were statistically analyzed using the SPSS package (Version 18.0). Cd accumulation values are expressed as means  $\pm$  standard deviation (SD) of the four replicates. ANOVA using was applied to examine statistical significant differences among addition levels of soil amendments. A probability level of  $P < 0.05$  was considered significant, unless otherwise stated.

## 3. Results and discussion

### 3.1. Effects of Cd on biomass and growth of *A. donax*

During the vegetative stages of plant development, all plants of all treatments showed no detrimental or toxic symptoms (Table 2). The morphological characteristics, as well as the leaves and roots biomass of *A. donax*, did not show statistically significant differences compared with control (Table 2). However, the stems biomass showed statistically significant differences between control and heavy-metal-treated (Table 2). This indicated that the stems may be the Cd-sensitive organs in *A. donax* in agree with previous studies which showed that the reduction of shoot dry biomass caused by Cd application has been demonstrated in many plants (Çikili *et al.*, 2016). On the basis of the reduction rate in the shoot dry biomass of plants, *A. donax* was determined to be Cd-tolerant

**Table 2.** The biomass of *A. donax* after four months of growth in the Cd-contaminated soil-water medium

Concentration (mg/L)	Height (cm)	Number of nodes	Biomass of <i>A. donax</i> (g)		
			Leaves	Stems	Roots
0.00	56 <sup>a</sup>	11 <sup>a</sup>	10.0 <sup>a</sup>	18.4 <sup>a</sup>	14.8 <sup>a</sup>
0.01	62 <sup>a</sup>	9 <sup>a</sup>	8.9 <sup>a</sup>	13.5 <sup>b</sup>	8.8 <sup>a</sup>
0.025	52 <sup>a</sup>	13 <sup>a</sup>	12.8 <sup>a</sup>	13.8 <sup>b</sup>	10.3 <sup>a</sup>
0.05	65 <sup>a</sup>	10 <sup>a</sup>	10.2 <sup>a</sup>	14.7 <sup>b</sup>	9.2 <sup>a</sup>
0.125	66 <sup>a</sup>	9 <sup>a</sup>	10.2 <sup>a</sup>	14.0 <sup>b</sup>	11.3 <sup>a</sup>
2.50	60 <sup>a</sup>	10 <sup>a</sup>	9.1 <sup>a</sup>	13.1 <sup>b</sup>	13.8 <sup>a</sup>

Note: Data represent average mean from four repetitions and standard deviation, respectively. Data with different superscript letters indicate a significant difference at  $P < 0.05$ .

**Table 3.** Concentrations of Cd in Soil, Water and dry *A. donax* plants after 4-month cultivation (mg/kg)

Concentration (mg/L)	Soil (mg/kg)	Aboveground (mg/kg)			Belowground/ (Roots, mg/kg)
		Leaves	Stems	Total	
0.00	<0.1 <sup>f</sup>	0.1 <sup>e</sup>	0.1 <sup>e</sup>	0.1 <sup>f</sup>	0.2 <sup>e</sup>
0.01	11.7 <sup>e</sup>	46.0 <sup>d</sup>	6.2 <sup>d</sup>	52.2 <sup>e</sup>	36.8 <sup>d</sup>
0.025	19.2 <sup>d</sup>	53.2 <sup>cd</sup>	9.7 <sup>cd</sup>	62.9 <sup>d</sup>	42.7 <sup>c</sup>
0.05	34.1 <sup>c</sup>	60.8 <sup>bc</sup>	14.0 <sup>bc</sup>	74.8 <sup>c</sup>	46.7 <sup>c</sup>
0.125	45.7 <sup>b</sup>	67.3 <sup>b</sup>	17.9 <sup>b</sup>	85.2 <sup>b</sup>	52.2 <sup>b</sup>
2.50	58.7 <sup>a</sup>	83.6 <sup>a</sup>	23.1 <sup>a</sup>	106.7 <sup>a</sup>	56.9 <sup>a</sup>

Note: Data with different superscript letters indicate a significant difference at  $P < 0.05$ .

according to the scale suggested by Shahbaz *et al* (2011) as tolerant for the reduction rate of < 30%.

### 3.2. Accumulation of Cd in *A. donax*

The order of Cd accumulation was stem < roots < leaves, and the accumulation of Cd in the plants (including roots, leaves and stems) and soil all showed a positive correlation with Cd concentration ( $P < 0.05$ ; Table 3). The maximum accumulation of Cd was found in plants at 2.50 mg/L treatments, e. g. Cd concentrations in leaves, roots and stems were  $83.63 \pm 6.92$ ,  $56.88 \pm 0.84$  and  $23.05 \pm 1.73$ , respectively (Table 3). In addition, the accumulation of Cd in the belowground parts was significantly higher than that in the aboveground parts of *A. donax* ( $P < 0.05$ ; Table 3). In general, Cd is accumulated more in the roots than in the shoots of plants (Çikili *et al.*, 2016). However, in present study, the highest determined Cd concentrations were in the leaves of *A. donax*, and the content of Cd in aboveground parts (leaves and stems) was nearly 2 times than that in belowground part (roots), indicating *A. donax* has a higher translocation factor to Cd. Song *et al.* (2014) found that, at solutions containing 200 mM Cd, the concentration of Cd in shoots was 3 times more than that of the roots. This suggests that *A. donax* could have a higher capacity to uptake Cd and have different mechanisms of tolerance, physiology of transport, and

accumulation to Cd. The possible reason may be that the better work condition of Cd<sup>2+</sup> with soil and water as the medium in the experiment. In this condition, the state of Cd was mostly Cd<sup>2+</sup>, which was easily absorbed by plants.

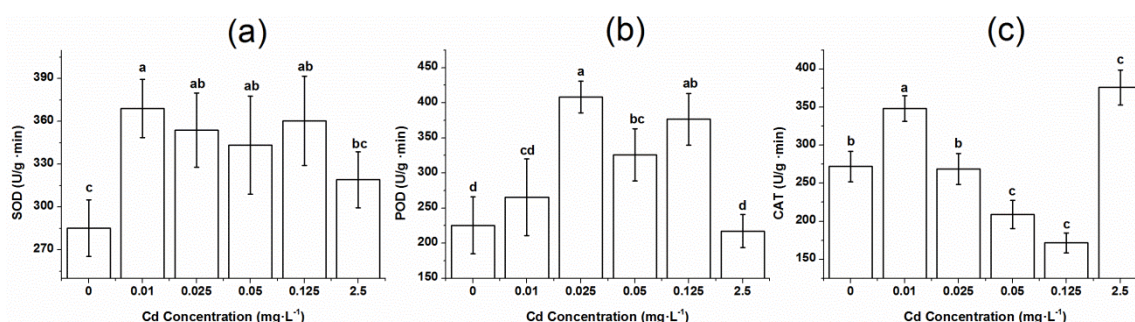
### 3.3. Chlorophyll fluorescence parameters

There were no differences on relative chlorophyll content (SPAD), Yield and Fv/Fm (except that at 0.125 mg/L) while significant differences on Fv/Fo when compared to control values (Table 4). Gas exchange measurements showed that there were no differences on Pn, while 0.01 mg/L Cd treatments decreased Tr and Gs, and 0.05 mg/L Cd treatments decreased Ci (Table 4). Obviously, within the present range of Cd concentration, it didn't affect the photosynthetic parameters including SPAD, Yield, Fv/Fm and Pn (except both of Yield and Fv/Fm at 0.125 mg/L), suggesting that PSII reaction centers were not damaged since SPAD being a measurement of photosynthesis, Fv/Fm providing the most frequently applied Chl fluorescence ratio and Yield giving a more realistic impression of the overall leaf photosynthetic condition (Wang *et al.*, 2016). However, Fv/Fo was inhibited by Cd application. The possible reason may be that the ratio Fv/Fo shows higher amplitude at stress conditions, since all changes of Fv and/or Fo are immediately reflected in it. Thus, in leaves with partial photoinhibition the values of

**Table 4.** Chlorophyll fluorescence parameters and gas exchange parameters of *A. donax* in different level Cd treatments.

Concentration (mg/L)	SPAD	Yield	Fv/Fo	Fv/Fm	Pn	Tr	Gs	Ci
0.00	34.57 <sup>a</sup>	0.80 <sup>a</sup>	4.34 <sup>a</sup>	0.81 <sup>a</sup>	8.33 <sup>a</sup>	0.25 <sup>ab</sup>	3.90 <sup>a</sup>	1.73 <sup>a</sup>
0.01	35.13 <sup>a</sup>	0.76 <sup>a</sup>	3.50 <sup>b</sup>	0.78 <sup>a</sup>	6.42 <sup>a</sup>	0.13 <sup>b</sup>	2.00 <sup>b</sup>	1.64 <sup>ab</sup>
0.025	36.31 <sup>a</sup>	0.71 <sup>a</sup>	2.72 <sup>d</sup>	0.73 <sup>a</sup>	7.31 <sup>a</sup>	0.24 <sup>ab</sup>	3.05 <sup>ab</sup>	1.59 <sup>ab</sup>
0.05	37.63 <sup>a</sup>	0.78 <sup>a</sup>	3.65 <sup>b</sup>	0.79 <sup>a</sup>	10.68 <sup>a</sup>	0.36 <sup>a</sup>	4.52 <sup>a</sup>	1.39 <sup>b</sup>
0.125	35.24 <sup>a</sup>	0.68 <sup>b</sup>	2.06 <sup>c</sup>	0.67 <sup>b</sup>	9.72 <sup>a</sup>	0.32 <sup>a</sup>	4.54 <sup>a</sup>	1.53 <sup>ab</sup>
2.50	39.20 <sup>a</sup>	0.79 <sup>a</sup>	3.84 <sup>b</sup>	0.79 <sup>a</sup>	7.08 <sup>a</sup>	0.22 <sup>ab</sup>	3.38 <sup>a</sup>	1.77 <sup>a</sup>

Note: Data with different superscript letters indicate a significant difference at  $P < 0.05$ .



**Figure 1.** Effects of Cd stress on SOD (a), POD (b) and CAT (c) activity in the leaves of *A. donax*. Different lower case letters on the top of the bars denote significant differences ( $P < 0.05$ ) among different Cd treatments.

Fv/Fm changed very little, whereas Fv/Fo exhibited already a large significant decline (Lichtenthaler *et al.* 1992). The present study shows that there were no differences on Pn, Tr (except that at 0.01 mg/L), Gs (except that at 0.01 mg/L), and Ci (except that at 0.05 mg/L), indicating that the photosynthetic system was not harmed and showed a strong tolerance of this plant to the increased heavy metal concentrations in the soil-water medium. This results agree with previous studies, in which Pn, Gs, Tr, Ci and Ls of *A. donax* were unaffected by irrigation with trace metal aqueous solution containing higher level Cd concentrations (Prasad 1995; Papazoglou *et al.* 2005; Alshaal *et al.*, 2015). These findings probably indicate that *A. donax* possess a very effective antioxidant system, that protects chloroplast and stomatal function (Alshaal *et al.*, 2015).

### 3.4. Antioxidant enzymes activities

In plants exposed to Cd, significant increase was found in SOD (except 2.5mg/L treatment) compared with control (Fig. 1a). Moderate levels of Cd (from 0.025 to 0.125 mg/L) significantly increased the activities of POD (Fig. 1b) while significant decrease was also found in CAT in the 0.05 and 0.125 mg/L treatments in comparison to control (Fig. 1c). Especially, the activity of CAT was stimulated by both 0.01 and 2.5 mg/L Cd treatments (Fig. 3c). The activity of SOD increased under Cd stress indicating that the conversion of  $O_2^-$  increased since SOD catalyzes the dismutation of  $O_2^-$  to  $H_2O_2$  and  $O_2$ . This

result agrees with previous studies, in which the activities of SOD and CAT of *A. donax* increased resistance to the stress of multi-metals in soil, especially CAT played a role in countering As, Cd, Pb-induced oxidative stress (Miao *et al.* 2012). The present study indicated that although the activity of POD was decreased at 2.5 mg/L, and CAT decreased at 0.05 and 0.125 mg/L, while the activities of CAT and POD were increased at 2.5 mg/L, and at 0.05 and 0.125 mg/L, separately. This result indicates that the decrease in CAT is compensated by the induction of POD activity, and shows that these two enzymes are functioning concurrently to remove  $H_2O_2$ , which is similar to the effect of arsenic (As) on *Pteris vittata* (Cao *et al.*, 2004).

### 4. Conclusion

Taken together, these results show that the highest determined Cd concentrations were in the leaves of *A. donax*, and the content of Cd in aboveground parts (leaves and stems) was nearly 2 times than that in belowground part (roots), indicating *A. donax* could have a higher capacity to uptake Cd in the wetland stimulated body almost without destroying its photosynthesis system. The oxidative stress may involve in the mechanism of Cd toxicity and *A. donax* showed a strong tolerance to the increased Cd pollution. These findings probably indicate that *A. donax* possess a very effective antioxidant system, that protects chloroplast and stomatal function, and may have a potential use for phytoremediation purposes in wetland environment.

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