

Cadmium accumulation and its effects on physiological characteristics of *Arundo donax* L. in a simulated wetland

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Abstract

A simulated wetland experiment was used to investigate the effect of cadmium (Cd) on the growth of *Arundo donax*, as well as its photosynthetic characteristics and antioxidant enzyme activities. Cd accumulation in the order of stem < roots < leaves increased gradually with increasing Cd concentrations. Due to the higher transport of Cd, its accumulation in the aboveground parts of *A. donax* was nearly 2 times that in belowground parts. There were no differences in physiological parameters, such as the relative chlorophyll content (SPAD), the potential (F_v/F_m) or effective (Y) photochemical efficiency of PSII, and photosynthetic rate (Pn). There were slight changes in transpiration rate (Tr), intercellular CO₂ concentration (C_i) and stomatal conductivity (G_s). The activity of superoxide dismutase (SOD) was stimulated by Cd treatments, and the decrease in catalase (CAT) was compensated by the induction of peroxidase (POD) suggesting these two enzymes function concurrently to remove H₂O₂. These results indicate that despite the oxidative stress involved in the mechanism of Cd toxicity associated with the high transport of Cd in *A. donax*, its photosynthetic system was not harmed. This suggests a strong tolerance of this species to increased Cd pollution and its potential use for phytoremediation purposes in wetland environment.

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

1. Introduction

Cadmium (Cd) is a typical, heavy metal pollutant with high toxicity and high solubility in water, soil and sediments; hence, it poses a serious environmental concern (Pinto *et al.*, 2004; He *et al.*, 2017). Cadmium, at trace levels, can undermine ecosystem functioning and human health (Zhi *et al.*, 2016). Therefore, how to effectively control cadmium pollution and restore the ecological environment is one of the most urgent environmental problems. Although the toxicity effects of Cd²⁺ in plants

have been well studied, some questions still remain unaddressed (He *et al.*, 2017).

In general, excess Cd accumulation in plants can profoundly interfere with a series of physiological processes such as uptake, transport, and assimilation of mineral nutrients and photosynthesis, and thereby lead to nutrient deficiency, disruption of ATPase activity, decrease of photosynthesis, inhibition of various enzyme activities, induction of oxidative stress and reduce genotoxicity (Metwally *et al.*, 2005; Imran *et al.*, 2008; Johna *et al.*, 2009; Chen *et al.*, 2012; Alshaal *et al.*, 2015; Çikili *et al.*, 2016; Moussa *et al.*, 2016; Shahid *et al.*, 2017; He *et al.*, 2017). These studies also suggested that the accumulation of and tolerance to Cd in plants varied greatly among plant species (Çikili *et al.*, 2016), cultivars (Chen *et al.*, 2012) and genotypes (Imran *et al.*, 2008). For example, Metwally *et al.* (2005) reported that, in the selected ten genotypes of *Pisum sativum* L., Cd toxicity expression has both similarities and distinct features. In addition, some important environment factors (such as soil PH, organic matter and microorganisms) have governed the sorption/desorption processes and chemical speciation of Cd in soils (Sappin-Didier *et al.*, 2005; Liu *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 due to the advantages of its environmental friendliness, safety, and cost-effectiveness (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), the process is often linked with slow growth rate and low biomass production. As a result, net removal of metals via phytoextraction is quite limited (Elhawat *et al.*, 2014). Therefore, the use of low-cost, fast-growing indigenous

plants with efficient biomass production, such as giant reed (*Arundo donax* L.), is 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 large biomass, high adaptability and unique physiological features, it readily absorbs and concentrates toxic chemicals from contaminated soil (Elhawat *et al.*, 2014). Furthermore, *A. donax* has gained a broad reputation as a good candidate for use in energy production, the paper industry, biofuels and the development of building materials (Nassi *et al.*, 2010; Elhawat *et al.*, 2014). As a C₃-grass, *A. donax* shows high photosynthetic rates and unsaturated photosynthetic potential compared to C₄ plants (Alshaal *et al.*, 2015). Previous studies have shown that, under high levels of cadmium, arsenic, plumbum, chromium and nickel stress, *A. donax* can growth and remain healthy, with its photosynthetic system unharmed (Alshaal *et al.*, 2015). This ability is based on its high antioxidant capacity, which allows its antioxidative enzymes to catalyse the dismutation of highly reactive O₂⁻ into non-toxic forms, such as O₂ and H₂O (Miao *et al.*, 2012). These results indicate that metals, such Cd, Ni, As, Cr and Pb, are most likely sequestered in a very effective manner within the *A. donax* plant, thus providing potent protection of the photosynthetic machine. There are several works concerning the promising phytoremediation potential of *A. donax* as applied to bauxite residue/red mud (Alshaal *et al.*, 2013), urban wastewater and saline lands (Williams *et al.*, 2008; Mirza *et al.*, 2010; Zema *et al.*, 2012; Elhawat *et al.*, 2014), marginal lands (Nassi *et al.*,

2013) and metals polluted soil (Papazoglou *et al.*, 2005; Miao *et al.*, 2012; Shabana *et al.*, 2012; El-Ramady *et al.*, 2014; Liu *et al.*, 2017). 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 aid in the recovery of Cd-contaminated wetlands.

Thus, our objective was to monitor the uptake and translocation of Cd in *A. donax* and to investigate plant growth responses, photosynthetic characteristics and antioxidant enzyme activities of *A. donax* under different levels of Cd stress.

2. Material and methods

2.1. Test soil and plant

A mixed medium of soil and water, collected from the garden of the Guangxi Institute of Botany, was used to simulate the wetland environment. The basic physiochemical properties of soil and water were analyzed (Table 1). The test soil (5 kg) was placed in plastic pots (base diameter × height × outside diameter: 26 cm × 75 cm × 38.5 cm) and the volume of the water was always maintained at 4 L above that of the soil (3.9 ± 0.3 cm). Test soil in each pot was homogeneously sprayed with aqueous solutions containing 0.00, 0.01, 0.025, 0.05, 0.125 and 2.5 mg Cd per liter of water that were prepared by dissolving salts of CdCl₂·2.5H₂O. The soil-grown plants were obtained from young meristematic buds in tissue culture grown in sterile aqueous medium.

Table 1. Basic physiochemical properties of tested soil and water

Soil	pH	TN (g kg ⁻¹)	TP (g kg ⁻¹)	TK (g kg ⁻¹)	AN (mg kg ⁻¹)	AP (mg kg ⁻¹)	Cd (mg kg ⁻¹)
	6.50	1.167	0.80	0.78	181.26	3.72	0.01
Water	pH	DO (mg L ⁻¹)	SpC (μs cm ⁻¹)	Ca ²⁺ (mg L ⁻¹)	Mg ²⁺ (mg L ⁻¹)	HCO ₃ ⁻ (mmol L ⁻¹)	Cd (μg L ⁻¹)
	7.38	1.56	235.00	53.00	7.63	1.70	<0.001

Note: TN, total nitrogen; TP, total phosphorus; TK, total potassium; AN, ammonia nitrogen; AP, available phosphorus; DO, dissolved oxygen; SpC, specific conductance

2.2. Culture and harvest of plant

A. donax was cultivated in a greenhouse with day/night temperatures of 28/20 °C and a relative humidity of 65%. During the growing period of *A. donax*, the volume of the water was always maintained at 4 L above that of the soil (3.9 ± 0.3 cm). After four months of cultivation, photochemical efficiency, photosynthetic gas exchange parameters and antioxidant enzymes activities were determined for each of the plants. Then, the plants were harvested and carefully washed with tap water and 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, cut with stainless steel scissor, and dried at 40 °C for 72 h for elemental analysis. Cd was determined in the soil and plants using the methods of Sasmaz *et al.* (2016) and Mazur *et al.* (2016) respectively. Soil samples were digested in a mixture of HNO₃:HCl:H₂O

(6 ml per 1.0 g of soil, v/v: 1:1:1) for 1 hour at 95 °C. The plant samples were digested in nitric acid by microwave reaction system (Multiwave PRO, Austria) and the temperature control settings were as follows: 5 min, 95 °C; 10 min, 180 °C; 45 min, 200 °C. (200 mg in 3 mL conc. HNO₃). Following digestion all the samples were determined by inductively coupled plasma-mass spectrometry (ICP-MS, PerkinElmer Instruments, USA). The phytoextraction ability of *A. donax* L. plants was assessed using a translocation factor (TF) and a bioaccumulation factor (BF) as follows:

$$TF = [Cd]_{shoot} / [Cd]_{root}$$

$$BF = [Cd]_{shoot} / [Cd]_{soil}$$

2.3. Photosynthetic parameters

Chlorophyll fluorescence parameters and photosynthetic gas exchange parameters were determined by the method described by Lichtenthaler *et al.* (2005) using LI-6400XT (Li-Cor, Lincon, USA) and portable fluorometer

(Monitoring-PAM, Walz, Germany). Photosynthetic rate (Pn), transpiration rate (Tr), intercellular CO₂ 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 μmol m⁻² s⁻¹, relative humidity of 40%, and CO₂ concentration of 370 μmol mol⁻¹. The top-most fully expanded leaves of treated and control plants were first light- and dark-adapted for 20 min to obtain the minimum (F₀) and maximum (F_m) chlorophyll fluorescence. The potential photochemical efficiency of PSII was calculated using the following formula:

$$F_v/F_m = (F_m - F_0)/F_m$$

where F_v is the variable fluorescence. The effective photochemical efficiency of PSII, Y, was determined according to Genty *et al.* (1989) using the following formula:

$$Y = (F_m - F'_0)/F'_m = \Delta F/F'_m$$

where F'₀ is the minimum fluorescence after dark adaptation, F'_m is the maximum fluorescence after dark adaptation and ΔF is the fluorescence spike at the end of the induction kinetic on top of the AL-induced kinetics. The relative chlorophyll content was estimated using a SPAD-502 Plus meter (Konica Minolta, Osaka, Japan) and is given as SPAD values, which are proportional to the chlorophyll content in leaves. All measurements were taken from five plants in each treatment replicate between 08:00 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 (0.3 g) were homogenized in 5 cm³ of ice-cold 50 mM phosphate buffer at a pH 6.5 (for POD, SOD) and a 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 SPSS (Version 18.0). Cd accumulation values are expressed as the means ± standard deviation (SD) of the four replicates. An ANOVA was applied to examine statistical significant differences among the 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 treatments showed no detrimental or toxic effects (Table 2). The morphological characteristics, as well as the leaf and root biomasses of *A. donax*, did not show statistically significant differences compared with control (Table 2). However, the stem biomass showed statistically significant differences between the control and

heavy-metal treated plants (Table 2). This indicated that the stems may be the Cd-sensitive organs in *A. donax*, which agrees with previous studies that showed that the reduction of shoot dry biomass caused by Cd application has been demonstrated in many species of 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 according to the scale suggested by Shahbaz *et al.* (2011) as tolerant for the reduction rate of <30%. Previous studies also showed that *A. donax* is only slightly affected by the presence of metals such as cadmium, nickel, arsenic and lead in the rhizosphere on soil medium. This trait makes the plant suitable for contaminated soils and polluted areas, where it is capable of high biomass production (Papazoglou *et al.*, 2005; Mirza *et al.*, 2010; Kausar *et al.*, 2012).

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

Concentration (mg L ⁻¹)	Height (cm)	Number of nodes	Biomass of <i>A. donax</i> (g)		
			Leaves	Stems	Roots
0.00	56 ^a	11 ^a	10.0 ^a	18.4 ^a	14.8 ^a
0.01	62 ^a	9 ^a	8.9 ^a	13.5 ^b	8.8 ^a
0.025	52 ^a	13 ^a	12.8 ^a	13.8 ^b	10.3 ^a
0.05	65 ^a	10 ^a	10.2 ^a	14.7 ^b	9.2 ^a
0.125	66 ^a	9 ^a	10.2 ^a	14.0 ^b	11.3 ^a
2.50	60 ^a	10 ^a	9.1 ^a	13.1 ^b	13.8 ^a

Note: Data represent average mean from four repetitions. Data with different superscript letters indicate a significant difference at *P* < 0.05

3.2. Accumulation of Cd in *A. donax*

The accumulation of Cd in the plants (including roots, leaves and stems) and soil all showed a positive correlation with Cd concentration, and the order of Cd accumulation was stem < roots < leaves (Table 3). The maximum accumulation of Cd was found in plants at 2.50 mg L⁻¹, at which the Cd concentrations in leaves, roots and stems were 83.63 ± 6.92 mg kg⁻¹, 56.88 ± 0.84 mg kg⁻¹ and 23.05 ± 1.73 mg kg⁻¹, respectively (Table 3). The TF and BF were all above the reference value (1.0) for hyperaccumulation, and with the increase of Cd concentrations, Cd uptake from the soil–water to plants decreased but its transport from root to shoots increased (Table 3). In general, Cd is accumulated more in the belowground parts (roots) than in the aboveground parts (shoots) of plants, such as soybean (Shamsi *et al.*, 2010), some Asteraceae plants (De Maria *et al.*, 2013) and Solanaceae plants (Çikili *et al.*, 2016). However, in present study, the accumulation of Cd in the aboveground parts was nearly 2 times that found in the belowground part (roots) (*P* < 0.05; Table 3). This may contribute to its higher translocation and bioaccumulation factor to Cd in the mixed medium of soil and water. Song *et al.* (2014) also found that, at solutions containing 200 mM Cd, the concentration of Cd in shoots was 3 times more than that of the roots of *Brassica Rapa*, which may contribute to its higher shoot-to-root metal accumulation ratio capacity. This suggests that *A. donax* has a higher capacity for uptake Cd and has different mechanisms of tolerance,

physiological transport, and accumulation of Cd in a mixed medium of soil and water, such as wetland. One possible reason may be that, under present conditions, Cd²⁺ was easily absorbed by plants because Cd²⁺ is of considerable importance to plants and animals due to its high water solubility, mobility, persistence, and toxicity even in

minute amounts (di Toppi and Gabbrielli, 1999). Sabeen *et al.* (2013) also found that Cd uptake in *A. donax* was higher in hydroponics cultures with Hoagland solution (Liu *et al.*, 2008), which showed higher bioaccumulation and translocation factor values, compared with soil-based environments.

Table 3. Concentrations (mean values) of Cd in *A. donax* plants after 4-month cultivation (mg kg⁻¹)

Concentration (mg L ⁻¹)	Soil (mg kg ⁻¹)	Aboveground (mg kg ⁻¹)		Belowground/(Roots, mg kg ⁻¹)	TF	BF
		Leaves	Stems			
0.00	0.011 ^f	0.1 ^e	0.1 ^e	0.2 ^e	1.0 ^c	18.2 ^a
0.01	11.7 ^e	46.0 ^d	6.2 ^d	36.8 ^d	1.4 ^b	3.1 ^b
0.025	19.2 ^d	53.2 ^{cd}	9.7 ^{cd}	42.7 ^c	1.5 ^b	2.3 ^c
0.05	34.1 ^c	60.8 ^{bc}	14.0 ^{bc}	46.7 ^c	1.6 ^{ab}	1.4 ^e
0.125	45.7 ^b	67.3 ^b	17.9 ^b	52.2 ^b	1.6 ^{ab}	1.9 ^d
2.50	58.7 ^a	83.6 ^a	23.1 ^a	56.9 ^a	1.8 ^a	1.8 ^d
PCC	0.71 ^{**}	0.57 [*]	0.69 ^{**}	0.45 [*]	0.53 [*]	-0.25

Note: TF: translocation factor; BF: bioaccumulation factor; PCC: Pearson correlation coefficient between Cd concentration and each parameter. Data with different superscript letters indicate a significant difference at $P < 0.05$ and asterisks indicate significant differences at $*P < 0.05$ and $**P < 0.01$

Table 4. Chlorophyll fluorescence parameters and gas exchange parameters of *A. donax* at different Cd treatment levels

Concentration (mg L ⁻¹)	SPAD	Yield	F _v /F _o	F _v /F _m	Pn	Tr	Gs	Ci
0.00	34.57 ^a	0.80 ^a	4.34 ^a	0.81 ^a	8.33 ^a	0.25 ^{ab}	3.90 ^a	1.73 ^a
0.01	35.13 ^a	0.76 ^a	3.50 ^b	0.78 ^a	6.42 ^a	0.13 ^b	2.00 ^b	1.64 ^{ab}
0.025	36.31 ^a	0.71 ^a	2.72 ^d	0.73 ^a	7.31 ^a	0.24 ^{ab}	3.05 ^{ab}	1.59 ^{ab}
0.05	37.63 ^a	0.78 ^a	3.65 ^b	0.79 ^a	10.68 ^a	0.36 ^a	4.52 ^a	1.39 ^b
0.125	35.24 ^a	0.68 ^b	2.06 ^c	0.67 ^b	9.72 ^a	0.32 ^a	4.54 ^a	1.53 ^{ab}
2.50	39.20 ^a	0.79 ^a	3.84 ^b	0.79 ^a	7.08 ^a	0.22 ^{ab}	3.38 ^a	1.77 ^a

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

3.3. Physiological parameters

Compared to the control treatment, there were no differences in the relative chlorophyll content (SPAD), or the effective (Y) and the potential (F_v/F_m) photochemical efficiency of PSII (except that at 0.125 mg L⁻¹) (Table 4). This result suggested that PSII reaction centers were not damaged because SPAD is a measurement of photosynthesis, F_v/F_m provides the most frequently applied chlorophyll fluorescence ratio and Y is a more realistic impression of the overall leaf photosynthetic condition (Wang *et al.*, 2016). However, F_v/F_o was inhibited by Cd application. The possible reason may be that the ratio F_v/F_o shows higher amplitude at stress conditions because all changes of F_v and/or F_o are immediately reflected by it. Thus, in leaves with partial photo inhibition the values of F_v/F_m changed very little, whereas F_v/F_o already exhibited a large significant decline (Lichtenthaler *et al.*, 1992). The present study showed that there were no differences in Pn, Tr (except that at 0.01 mg L⁻¹), Gs (except that at 0.01 mg L⁻¹), and Ci (except that at 0.05 mg L⁻¹), 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. The results agreed with previous studies, in which Pn, Gs, Tr, Ci and stomatal limitation (Ls) of *A. donax* were unaffected by irrigation with trace metal aqueous solution containing high concentrations of Cd (Prasad, 1995; Papazoglou *et al.*, 2005; Alshaal *et al.*,

2015). This finding probably contributed to the fact that *A. donax* possesses a very effective antioxidant system, which protects chloroplast and stomatal functioning (Alshaal *et al.*, 2015).

3.4. Antioxidant enzymes activities

Compared to control plants, SOD activity in the leaves of *A. donax* was significantly increased (from 0.01 to 0.125 mg L⁻¹) or slight increased (2.5 mg L⁻¹) under Cd stress (Figure 1a), suggesting that the conversion of O₂⁻ increased since SOD catalyzes the dismutation of O₂⁻ to H₂O₂ and O₂ (Han *et al.*, 2010). POD activity was significantly increased but CAT activity was significantly decreased at the mid-level Cd concentration (from 0.025 to 0.125 mg L⁻¹) compared with the control group (Figure 1b, c), suggesting that POD plays a critical role in converting H₂O₂ into water and molecular O₂ in the mid-level Cd treatment group. Meanwhile, CAT activity was higher in response to the highest Cd concentration (2.5 mg L⁻¹) and the lowest (0.01 mg L⁻¹) Cd concentrations, suggesting that CAT played an important role in converting H₂O₂ into water and molecular O₂ in the lowest and highest Cd concentrations. One of the possible explains could be related to the mixed medium of soil and water since Cd has high water solubility, mobility, persistence, and toxicity even in minute amounts (di Toppi and Gabbrielli, 1999). In the lowest Cd concentrations, SOD activity increased (Figure 1a), suggesting the

production of H_2O_2 . Although there was no significantly difference in POD activity (Figure 1b) between the control and the lowest Cd concentrations, there was significantly difference in CAT activity (Figure 1c). This result indicated that the deficiency of POD activity could be compensated by the induction of CAT activity. In general, SOD activity was increased with increasing concentration of Cd at earlier stages of plant growth in soil (Gonçalves *et al.*, 2007; Han *et al.*, 2008; Ahmad *et al.*, 2015), but decreased with the exposure time in *A. donax* (Han *et al.*, 2008). Thus, after a 4-month cultivation, SOD showed a low values in the highest Cd concentration, which weakened the capability of *A. donax* to detoxify ROS. However, SOD activity was still higher in the highest Cd concentrations than in the control, suggesting the production of H_2O_2 still increased. Although POD activity at this treatment was not changed, the increased CAT activity may scavenge the increased H_2O_2 . Previous study also showed that the CAT activities of *A. donax* increased resistance to the stress of multi-metals in soil, which plays an important role in countering As, Cd, Pb-induced oxidative stress (Miao *et al.*, 2012). These results indicated that the decrease in CAT was compensated for by the induction of POD activity and these two enzymes function concurrently to remove H_2O_2 in both soil medium and soil-water medium. This phenomenon is similar to the effect of As in *Pteris vittata* (Cao *et al.*, 2004). This might explain why we were unable to observe toxicity symptoms at the date of the experiment completion in the plants at all concentrations.

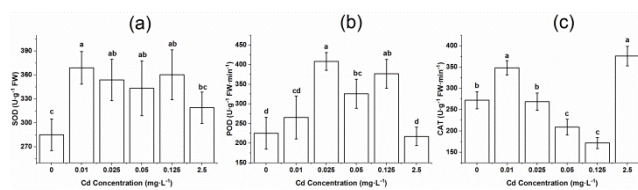


Figure 1. Effects of Cd stress on (a) superoxide dismutase (SOD), (b) peroxidase (POD) and (c) catalase (CAT) activities in the leaves of *A. donax*. FW: fresh weight. Different lower case letters on the top of the bars denote significant differences ($P < 0.05$) among different Cd treatments

4. Conclusion

In conclusion, although the accumulation of Cd in the aboveground parts (leaves and stems) was nearly 2 times that in the belowground part (roots) of *A. donax*, all treatment plants showed no detrimental or toxic symptoms or physiological disturbances, suggesting *A. donax* may have a higher capacity to uptake Cd in the simulated wetland without destroying its photosynthesis system. The oxidative stress may involve the mechanism of Cd toxicity with *A. donax* showing a strong tolerance to the increased Cd pollution. These findings indicate that *A. donax* possesses a very effective antioxidant system that protects chloroplast and stomatal functioning, and therefore, may have a potential use for phytoremediation in wetland environment.

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