

EFFECTS OF EXOGENOUS CADMIUM ON ACTIVITY OF ANTIOXIDANT ENZYME, CD UPTAKE AND CHEMICAL FORMS OF RYEGRASS

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Abstract. The cadmium (Cd) contamination in farmland soils in China has been becoming more and more serious because of the large amount of industrial waste residue, sewage irrigation and the unreasonable application of pesticides and fertilizers. Pot experiment was conducted to study the effects of different Cd levels (0, 75, 150, 300 and 600 mg·kg⁻¹) on dry weight, activities of antioxidant enzyme and Cd chemical forms of the two ryegrass varieties (Band and Arbde). The results showed that dry weights of shoot and plant of ryegrass (Band and Arbde) were firstly increased with the increase of Cd level, which reached the highest values respectively at 75 mg·kg⁻¹ and 150 mg·kg⁻¹, and respectively increased by 10.06% and 4.04%, 25.84% and 16.89% compared with the control. Shoot Cd concentrations of Band and Arbde were 171.827 mg·kg⁻¹ and 169.122 mg·kg⁻¹ respectively at 150 mg·kg⁻¹ Cd. The average Cd concentrations of chemical forms in shoots of the two ryegrass varieties were in order of NaCl extractable Cd (F_{NaCl}) > acetic acid extractable Cd (F_{HAC}) > water extractable Cd (F_W) > residual Cd (F_R) > HCl extractable Cd (F_{HCl}) > alcohol extractable Cd (F_E). Compared with the two ryegrass varieties, Arbde was more effective in remediation of cadmium contamination in soils due to higher plant biomass and greater Cd accumulation.

Keywords: *Cd contamination; ryegrass; activity of antioxidant enzyme; Cd uptake; Cd chemical forms*

Introduction

Cadmium (Cd) pollution in soil has become a global issue due to the emission of three types of industrial waste, i.e. mining, chemical fertilizers and pesticides used in agriculture and sewage irrigation (Ren et al., 2014; Hejazi et al., 2017). The Yangtze River Basin is located in cadmium ore resources enrichment area of China, and there were reports that Cd content in soils and agricultural products exceeds Chinese government standard (Sun et al., 2016). The soil Cd pollution around smelting plant is more serious, for example, in the investigation of the surrounding environment of a smelting plant in Northeast China, it is found that the content of Cd in collected soil samples is from 4.1 to 167.6 mg·kg⁻¹ (Chen, 2016; Hashemi, 2017). Cadmium is highly toxic to plants, water soluble and therefore promptly adsorbed in tissues and its presence greatly influences the entire plant metabolism (Li et al., 2016). Cadmium pollution has the characteristics of concealment, latency, accumulation and long term, which could access to biological body with long-term accumulation through the food chain, thus endangering human health, such as Japan's "Itai-Itai Disease" (Liu et al., 2015).

Phytoremediation as a kind of in-situ remediation technology with low cost and small environmental disturbance, is considered to be the best remediation method of heavy metal contaminated soil (Chen et al., 2015; Radan et al., 2017). At present, Cd enrich plants has been found included *Solanum nigrum* L., *Zygophyllum fabago* L., *Arabidopsis thaliana*. L., *Trifolium repens* L. and *Iris hex agona* L., etc (Chen, 2016). Unfortunately, most of the plants that were already found out to have lots of accumulation of Cd grow slowly, and have low biomass so that their restoration takes a long period, while they require grow in very restricted conditions. These conditions limit using phytoextraction technology at large scale. Ryegrass (*Lolium multiflorum* L.) is the annual or perennial herbs, and is a kind of forage grass with higher yield in gramineous plants. It has been reported that ryegrass is an ideal plant for phytoremediation of heavy metals in soils due to surviving in tailings with poor ecological environment, and fast vegetation construction and strong tiller ability, and rapidly covering the ground (Xu et al., 2007; Wu et al., 2014; Xiang, 2014). Perennial ryegrass have obvious remediation effect on Cd polluted soil (Feng et al., 2016; Sun et al., 2016; Vazdani et al., 2017). Studies on the growth and Cd uptake by ryegrass under Cd stress have been reported (Xu et al., 2007; Chen et al., 2015; Feng et al., 2016), however, there are few studies on difference of Cd resistance, Cd uptake and Cd chemical forms between ryegrass varieties. In order to further explore the effects of different Cd levels (0, 75, 150, 300 and 600 mg·kg⁻¹) on growth, activity of antioxidant enzyme and the Cd chemical forms in ryegrass, two ryegrass varieties (Band and Arbde) planted in pots containing Cd pollution and were then analyzed (Ahamed and Lognathan, 2017). This work will provide interesting data for studying on the expression difference of Cd accumulation /Cd tolerance related genes between ryegrass varieties, and breeding and cloning high biomass and high Cd-enrichment varieties in future research.

Materials and methods

Plant material, soil and Cd treatments

Two ryegrass (*Lolium multiflorum* L.) varieties (Band and Arbde) were used in the present study. The seeds were purchased from Jiangxi Scarecrow agricultural park. Purple soil based on Chinese Soil Taxonomy (CST) was collected from purple soil base of Southwestern University in Beibei region, Chongqing. The contents of organic matter, total nitrogen, alkali-degradable nitrogen, available P, available K, Cd in soil, and the soil pH were 8.87 g·kg⁻¹, 1.54 g·kg⁻¹, 74.60 mg·kg⁻¹, 65.07 mg·kg⁻¹, 38.59 mg·kg⁻¹, 0.110 mg·kg⁻¹, and 5.23, respectively.

The pot experiment was conducted with Cd concentrations of 0, 75, 150, 300 and 600 mg·kg⁻¹ (CdCl₂·2.5H₂O) from March 3, 2015 to May 5, 2015 in the greenhouse of College of Resources and Environmental Sciences at Southwest University, China. 5 kg of air-dried soil was passed through a 40-mesh screen and then treated with CdCl₂·2.5H₂O (0, 75, 150, 300 and 600 mg·kg⁻¹) solution and mixed to be homogeneously put in a plastic pot (diameter, 25 cm; height, 17 cm) and kept balance for two weeks. Thirty seedlings with the same growth potential were planted in each pot. The base fertilizer included P (NH₄H₂PO₄), K (KCl), and N (NH₄H₂PO₄ and urea) at concentrations of 100 mg·kg⁻¹, 150 mg·kg⁻¹, and 180 mg·kg⁻¹, respectively. The seedlings of ryegrasses (*Lolium multiflorum* L.) with 10 cm high were then transplanted 30 plant for each pot. The moisture content in soil was kept 60% of the maximum

moisture in the fields with deionized water. Fast- measurement of Soil Moisture (TZS-IW, Zhejiang Tuopu Instrument Co., Ltd., China) was used to determine the moisture content in soil. The experiment was performed in triplicate, and was randomized complete block arrangement. The plants were harvested on May 5, 2015, and were kept at 105 DEG C for 15 min for denaturing the enzymes, and then oven-dried at 60 DEG C until there was no further change in the weight of the sample.

Soil physical and chemical properties

Soil pH was determined by soil water ratio of 1:2.5; organic matter, total nitrogen, available nitrogen, available phosphorus and available potassium in soils were determined by conventional analysis methods (Yang et al., 2008; Issaka and Ashraf, 2017; Xiao et al., 2017).

Antioxidant enzyme activity and MDA content

Tissue samples of shoot and root in two ryegrass (*Lolium multiflorum* L.) varieties (Band and Arbde) were homogenized in ice-cold deoxygenated 20 mmol·L⁻¹ Tris-HCl buffer (pH 7.4, 1:9, W/V) and centrifuged at 3000 ×g for 10 min. Aliquots of 100 μL were used for enzyme activity measurement. The activity of catalase (CAT) was measured using a microtiter plate assay (Grant et al., 2008; Yang et al., 2017). The activity of peroxidase (POD) was determined by monitoring the increase in absorbance at 470 nm during the oxidation of guaiacol (Hemeda and Klein, 1990). The activity of superoxide dismutase (SOD) was determined by the method of Minami and Yoshikawa (1979) with 50 mmol·L⁻¹ Tris-Ca-codylic sodium salt buffer (pH 8.2) containing 0.1 mmol ·L⁻¹ EDTA.

Plant Cd fraction

The concentrations of Cd in its various chemical forms in ryegrass shoots were determined through continuous leaching method (Tessier et al., 1979). A flame atomic absorption spectrometer (Perkin SIMMA 6000 Elmer, Norwalk, USA) was used to determine Cd concentration. The detection limit was 0.005 mg·kg⁻¹. The plant reference material (GBW # 08513) provided by National Institute of Standards and Technology was adopted to monitor the quality of determination results. The recovery rates of cadmium in all plant samples were higher than 95%, and the accuracy of relative standard deviation (RSD) was less than 10%.

Soil Cd fraction

The concentrations of Cd in its various chemical forms in soil was conducted by five-step sequential extraction method proposed by Tessier et al. (1979), and the extracted Cd was determined by atomic absorption spectrophotometry (Perkin Elmer SIMMA 6000, Norwalk, USA). The detection limit was 0.005 mg·kg⁻¹. The plant reference material (GBW # 08303) provided by National Institute of Standards and Technology was adopted to monitor the quality of determination results. The recovery rates of Cd in all plant samples were higher than 95%, and the accuracy of relative standard deviation (RSD) was less than 10% (Zhao and Chen, 2017).

Statistical analysis

Three-way analysis of univariate ANOVA and correlation analysis were performed using SPSS version 21.0 package (SPSS, 2012). The effects of interaction among Cd levels on dry weight of plant, antioxidant enzyme activity, Cd concentration and Cd uptake in plant, and Cd fraction in plant and soil for different ryegrass varieties was subjected to a two-way analysis of variance (ANOVA; i.e., varieties and Cd treatments) followed by the least significant difference (LSD) test ($p < 0.05$) (Xu et al., 2010).

Results

Biomass

As shown in *Figure 1*, with the increase of Cd level in soils, shoot dry weight of Band and Arbde were increased. Highest shoot dry weight of Band was found in 75 $\text{mg}\cdot\text{kg}^{-1}$ Cd and increased by 10.06% compared with the control, while highest shoot dry weight of Arbde was observed at 150 $\text{mg}\cdot\text{kg}^{-1}$ Cd and increased by 25.84% compared with the control. At the same Cd level, shoot dry weight of Arbde was higher than that of Band.

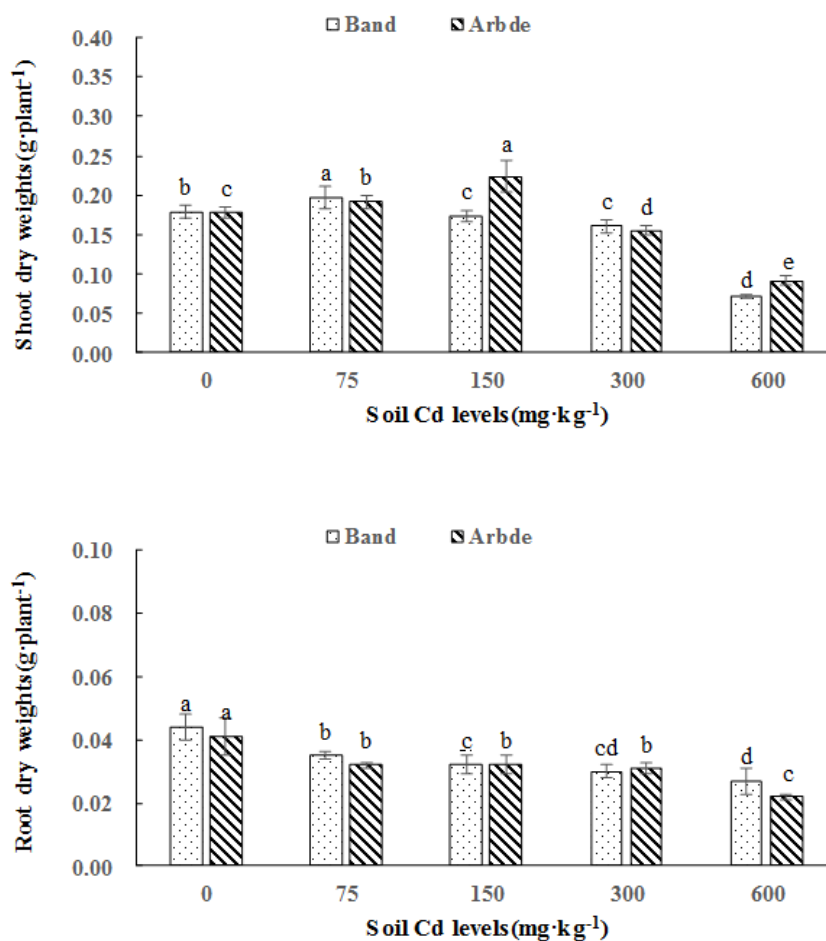


Figure 1. Effects of different Cd levels ($\text{mg}\cdot\text{kg}^{-1}$) on dry weight of ryegrass; different letters (a, b, c) indicate significant difference at $P \leq 0.05$ among different Cd levels in the same variety

Antioxidant enzyme activity

Significant difference of the activities of SOD, POD and CAT in shoots and roots of ryegrass were found between different varieties and among different Cd pollution levels ($P < 0.05$) (Figs. 2-4). As shown in Figure 2, the activity of SOD in shoots of Band significantly increased with the increase of Cd level in soil, with increases of 14.52%-68.38% compared with the control, while the activity of SOD in shoots of Arbde decreased with the increase of Cd level in soil, with the decrease of 4.17%-41.67% compared with the control. The activity of SOD in roots of the two varieties increased with the increase of Cd level in soils, and highest activities of SOD in roots of Band and Arbde were observed at the Cd level of $75 \text{ mg} \cdot \text{kg}^{-1}$ and respectively increased by 3.24% and 10.66% compared with the control.

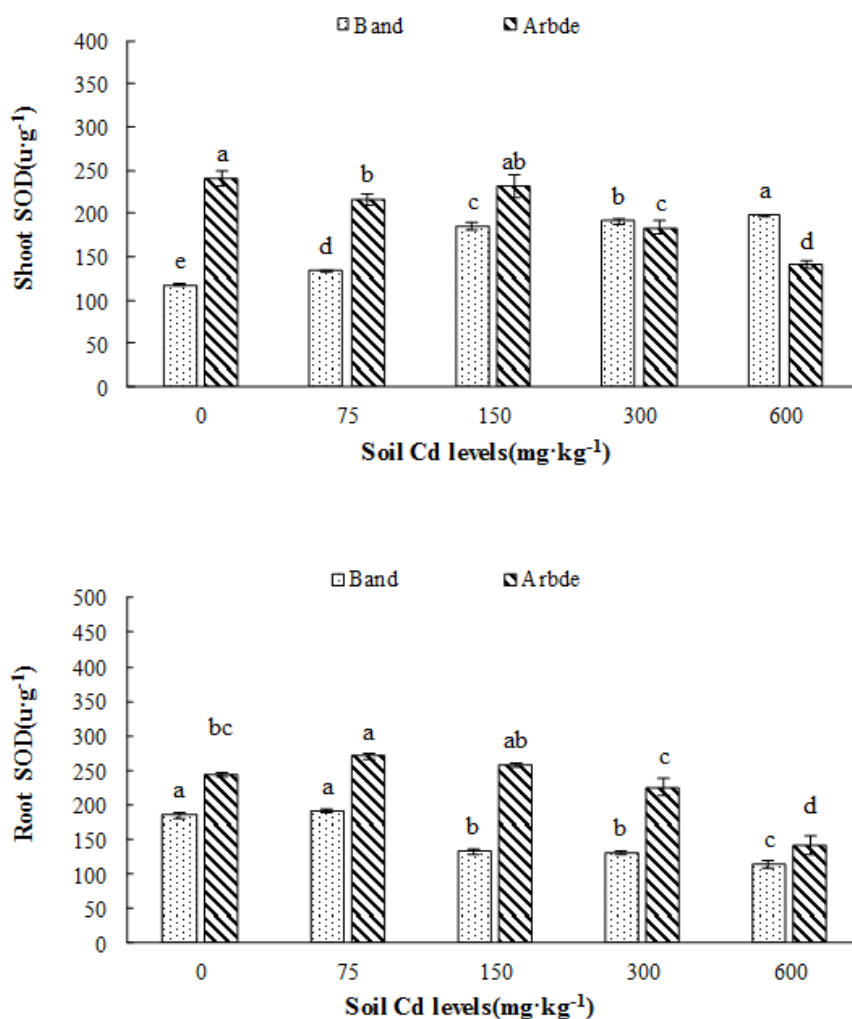


Figure 2. Effects of different cadmium levels on SOD activity in ryegrass; different letters (a, b, c) indicate significant difference at $P \leq 0.05$ among different Cd levels in the same variety

As shown in Figure 3, the activity of CAT in shoots and roots of Band and Arbde increased significantly with the increase of Cd level in soils. Highest activities of CAT

in shoots and roots were found in 150 mg·kg⁻¹ Cd, and respectively increased by 8.83% and 136.36% compared with the control. Under the same Cd level, the activity of CAT in roots of the two varieties ryegrass was lower than that in shoots.

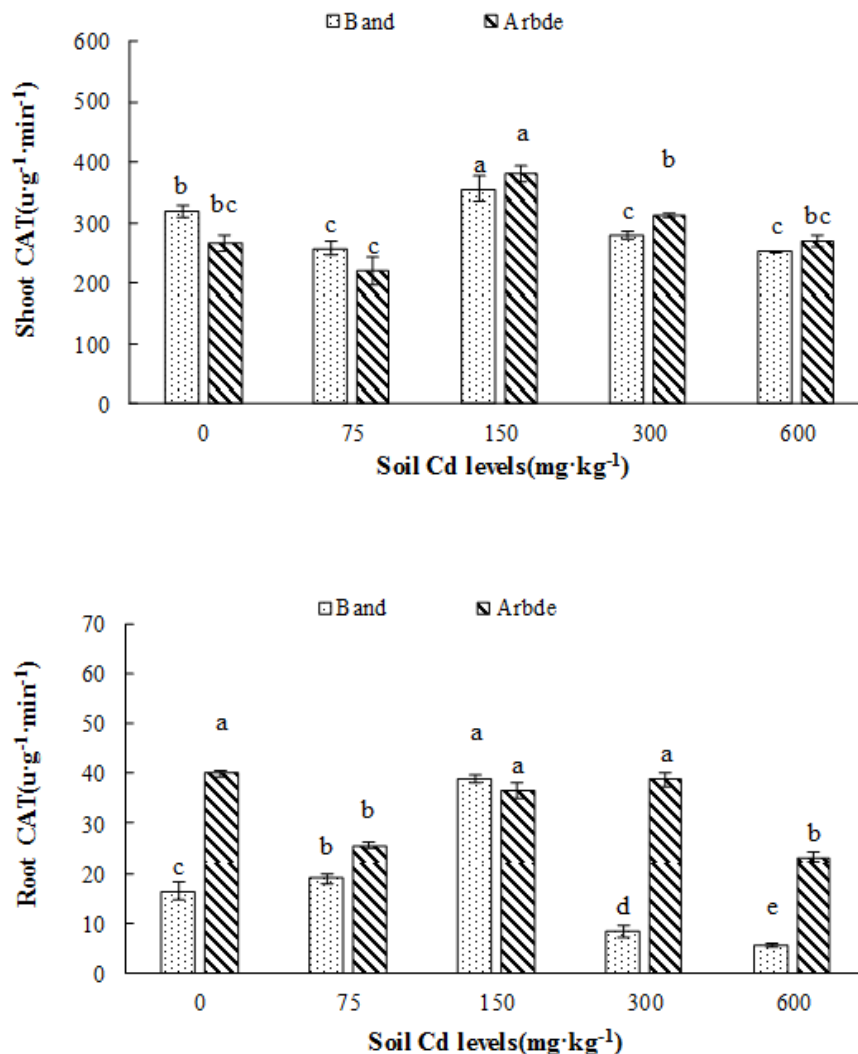


Figure 3. Effects of different cadmium levels on CAT activity in ryegrass; different letters (a, b, c) indicate significant difference at $P \leq 0.05$ among different Cd levels in the same variety

As shown in Figure 4, the activity of POD in shoots and roots of the two varieties of ryegrass increased significantly with the increase of Cd level. The activity of POD in shoots and roots of Band respectively increased by 23.50%-201.67% and 7.42%-20.01% compared with the control, respectively. The activity of POD in shoots and roots of Arbde respectively increased by 50.62%-232.06% and 4.58%-59.62% compared with the control.

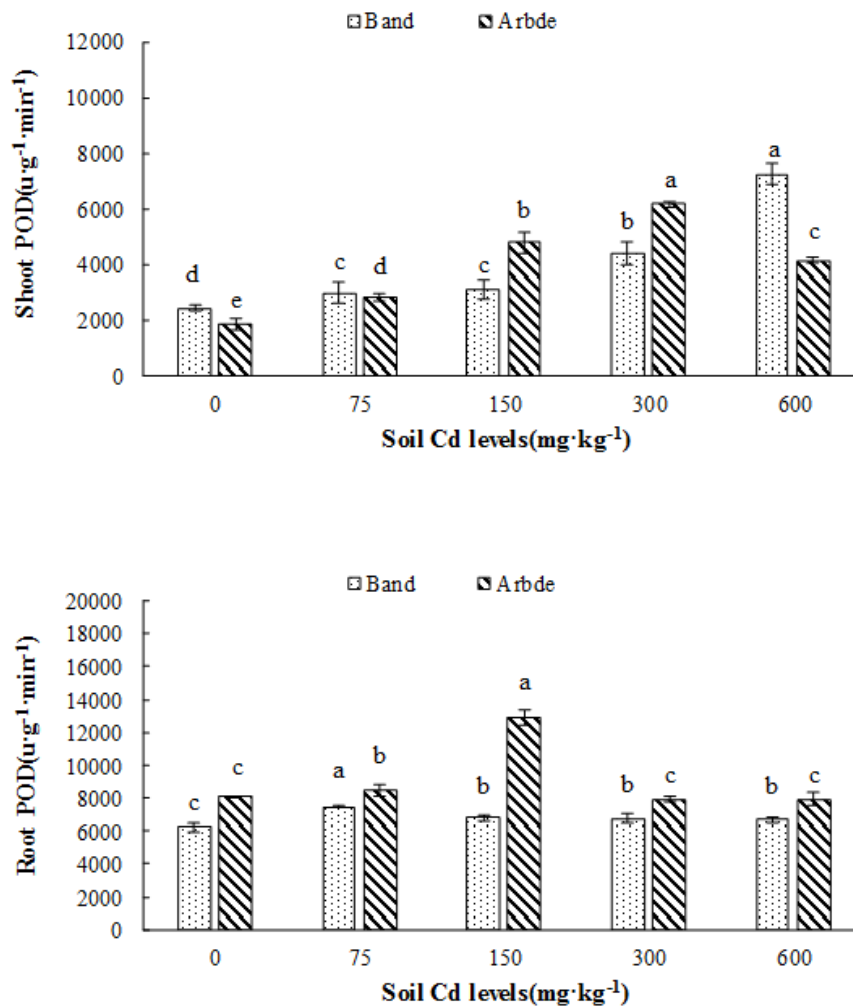


Figure 4. Effect of different cadmium levels on POD activity in ryegrass; different letters (a, b, c) indicate significant difference at $P \leq 0.05$ among different Cd levels in the same variety

MDA content

As shown in *Figure 5*, the MDA content in shoots and roots of the two varieties of ryegrass increased significantly with the increase of Cd level. Highest MDA contents in shoots and roots of Band significantly increased by 2.78% and 88.05% at 150 mg·kg⁻¹ and 75 mg·kg⁻¹ Cd respectively compared with the control. In the case of shoots and roots of Arbde, highest MDA contents in shoots and roots of Arbde significantly increased by 66.62% and 23.86% at 300 mg·kg⁻¹ and 150 mg·kg⁻¹ Cd respectively compared with the control.

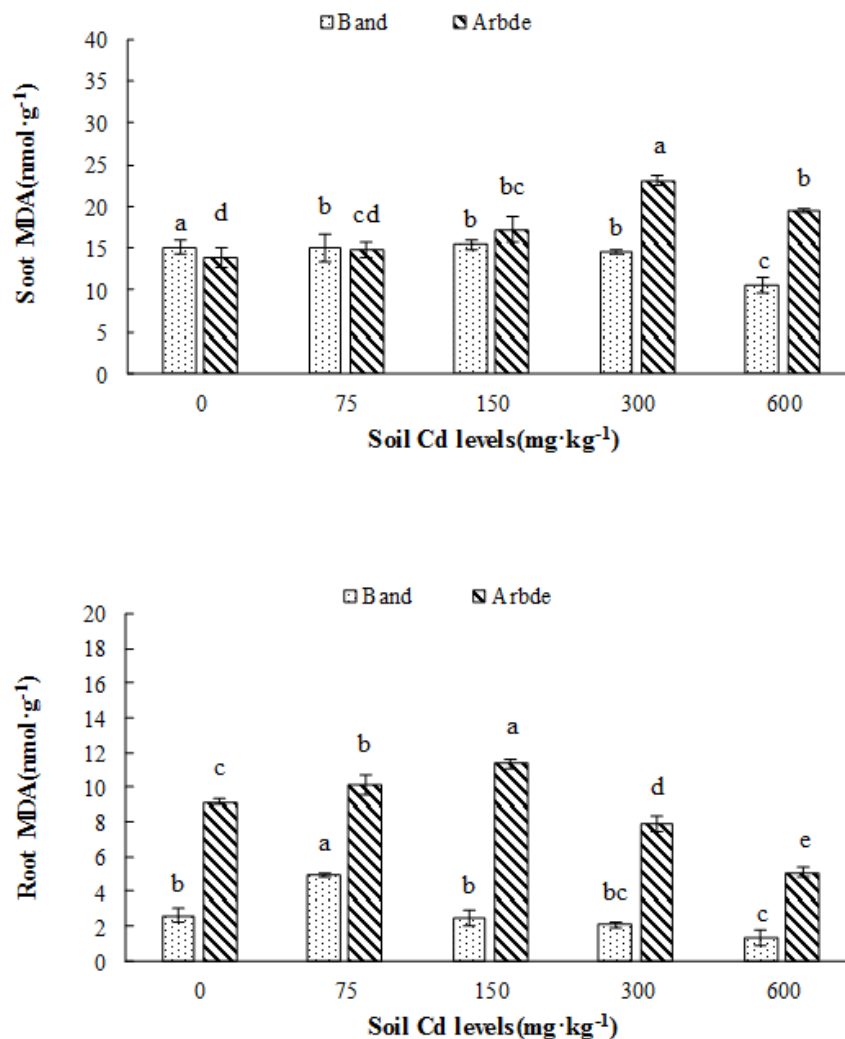


Figure 5. Effects of different cadmium levels on MDA content in ryegrass; different letters (a, b, c) indicate significant difference at $P \leq 0.05$ among different Cd levels in the same variety

Concentration of Cd in shoot and root

As shown in Figure 6, Cd concentrations in shoots and roots of the two ryegrass varieties increased significantly with the increase of Cd level ($P < 0.05$). When exposed to 150 mg·kg⁻¹ Cd, Cd concentrations in shoots and roots of Band and Arbde were respectively as 171.827 mg·kg⁻¹, 374.494 mg·kg⁻¹ and 169.122 mg·kg⁻¹, 229.676 mg·kg⁻¹, which exceeded the critical value of cadmium hyperaccumulator (100 mg·kg⁻¹ Cd). At the same cadmium level, Cd concentrations in roots of the two ryegrass varieties were higher than those in shoots, and Cd concentrations in shoots and roots of Band was lower than that of Arbde.

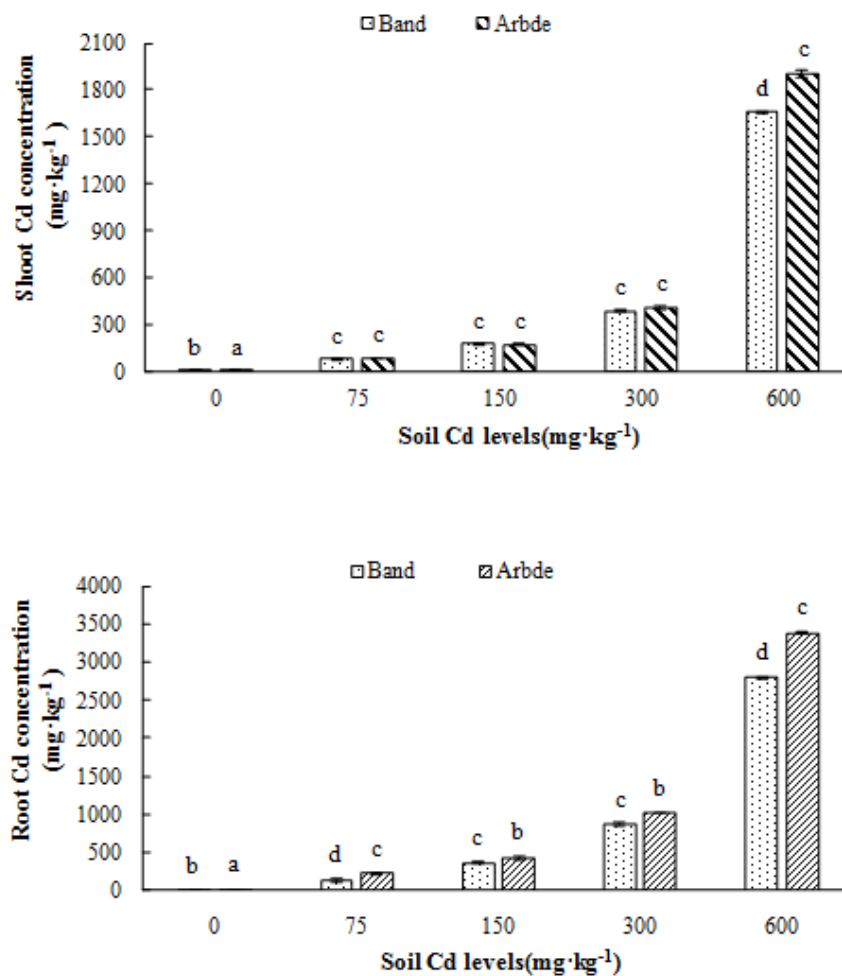


Figure 6. Effect of different Cd levels on Cd content in different parts of ryegrass; different letters (a, b, c) indicate significant difference at $P \leq 0.05$ among different Cd levels in the same variety

Cd accumulation in shoot and root

As shown in Figure 7, Cd accumulation in shoots and roots of the two ryegrass varieties increased with the increase of level of cadmium in soils, and significant differences were found between varieties and among Cd treatments ($P < 0.05$). When exposed to 75-600 mg·kg⁻¹ Cd, Cd accumulations in shoots and roots of Band were 53.84-422.93 times and 8.10-116.56 times that of the control. Highest Cd accumulation in shoots was observed at 600 mg·kg⁻¹ Cd by 118.977 ug·plants⁻¹; Cd accumulations in shoots and roots of Arbde were 25.72-304.23 times and 6.94-70.04 times that of the control. Under the same Cd level, the total amount of Cd accumulation in the plant (the sum of Cd accumulations in shoots and roots) of Arbde was greater than that of Band.

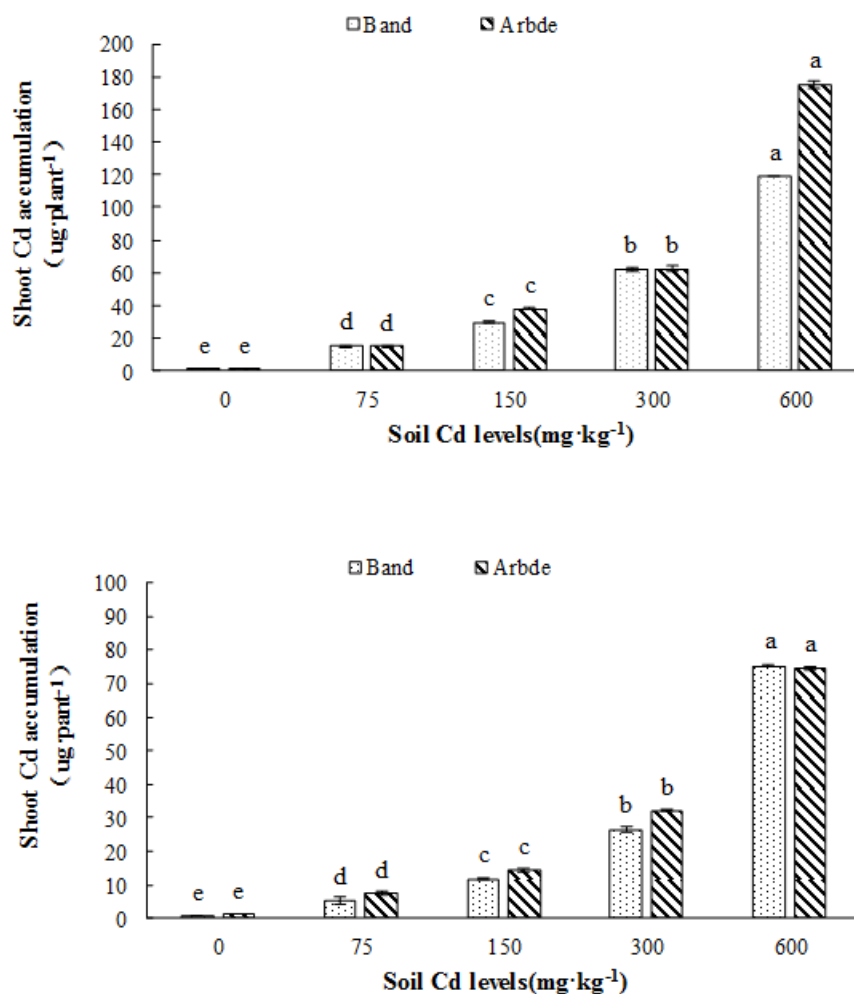


Figure 7. Effect of different cd levels on cd accumulation in different parts of ryegrass; different letters (a, b, c) indicate significant difference at $P \leq 0.05$ among different Cd levels in the same variety

Plant Cd fraction

As shown in *Figure 8*, significant differences of distribution proportion of cadmium with different forms (FDC) in shoots of the two ryegrass were found between varieties and among Cd treatments ($P < 0.05$). The concentrations of Cd chemical forms in shoots of the two ryegrass varieties were in order of sodium chloride extraction state (F_{NaCl}) > acetic acid extraction state (F_{HAc}) > ionized water extraction state (F_{W}) > residual state (F_{R}) > hydrochloric acid extraction state (F_{HCl}) > ethanol extraction state (F_{E}). F_{NaCl} , F_{HAc} and F_{W} of Band and Arbde respectively accounted for 40.7%-52.8% and 38.2%-50.0%, 19.2%-31.7% and 21.6%-36.3%, 12.5%-29.6% and 10.55%-27.17% of total amount of Cd extraction, while F_{E} with higher activity had the lowest content, and only accounted for 1.1%-3.6% and 1.4%-4.0% of total amount of Cd extraction.

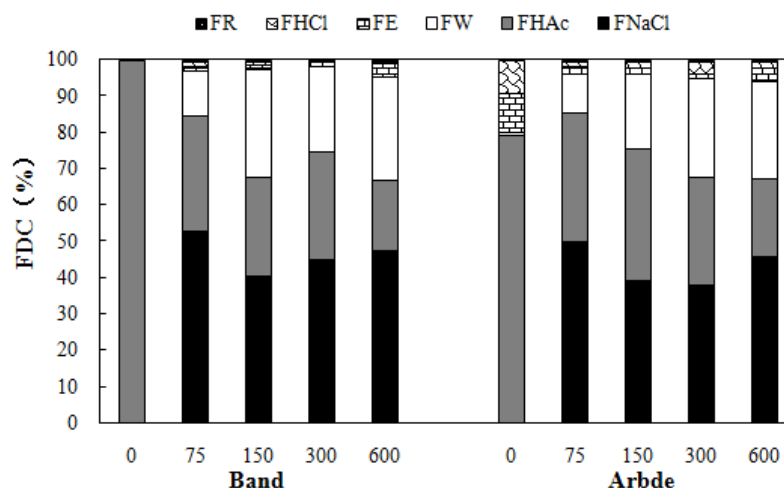


Figure 8. Effect of different Cd levels on the distribution of cadmium in ryegrass

Soil Cd fraction

The content and the distribution proportion (FDC) of Cd with different forms of the treated soils in the experiment were respectively shown in *Figure 9* and *Figure 10*. The contents of Cd with different forms (EXC-Cd, CAB-Cd, FeMn-Cd, OM-Cd and RES-Cd) in soils increased with the increase of Cd level ($P < 0.05$). The FDC of Cd with various chemical forms in soil was in order of EXC-Cd > CAB-Cd > FeMn-Cd > RES-Cd > OM-Cd. The FDC of EXC-Cd in soil of Band and Arbde were respectively as 61.42%-82.64% and 55.09%-83.23%. The FDC of OM-Cd was lowest with FDC of 0.87%-6.26%. Under the same cadmium level, soil EXC-Cd concentration of Arbde were lower than that of Band, but no significant differences were found in other soil Cd chemical forms.

Correlation between plant Cd concentration and soil Cd fraction

As shown in *Table 1*, significant correlations were observed among Cd concentration of shoots in Band and EXC-Cd, CAB-Cd, FeMn-Cd and RES-Cd in soil, whose correlation coefficients were respectively as 0.966, 0.943, 0.803 and 0.666. Moreover, significant correlations were also found among Cd concentration in roots of Band and EXC-Cd, CAB-Cd, FeMn-Cd and RES-Cd in soil, whose correlation coefficients were respectively as 0.983, 0.960, 0.838 and 0.687. As shown in *Table 2*, significant correlations were observed among Cd concentration of shoots in Arbde and CAB-Cd in soil, whose correlation coefficient was 0.847. Significant correlations were also found among Cd concentration in roots of Arbde and EXC-Cd and CAB-Cd in soil, whose correlation coefficients were respectively as 0.989 and 0.885.

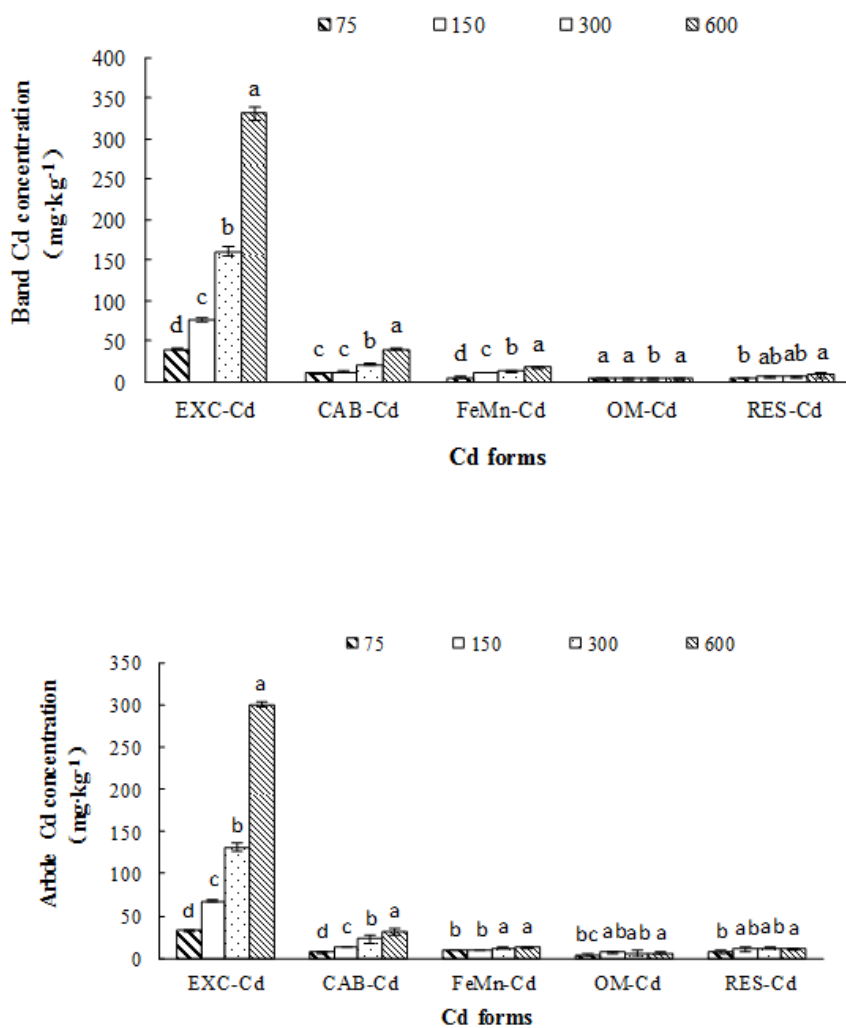


Figure 9. Effect of different Cd levels on concentration of Cd in soil; different letters (a, b, c) indicate significant difference at $P \leq 0.05$ among different Cd levels in the same variety

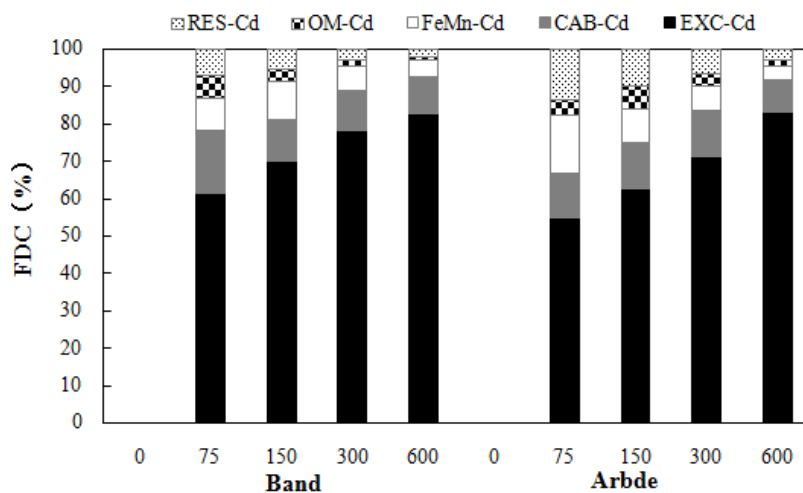


Figure 10. Effect of different Cd levels on Cd fraction distribution coefficient (FDC) in soils

Table 1. Correlation coefficient between Cd contents of Band and the concentration of Cd fractions in soil

	Shoot-Cd	Root-Cd	EXC-Cd	CAB-Cd	FeMn-Cd	OM-Cd	RES-Cd
Shoot-Cd	1						
Root-Cd	0.996**	1					
EXC-Cd	0.966*	0.983**	1				
CAB-Cd	0.943**	0.960**	0.988**	1			
FeMn-Cd	0.803**	0.838**	0.911**	0.930**	1		
OM-Cd	0.281	0.297	0.394	0.523*	0.615*	1	
RES-Cd	0.666**	0.687**	0.767**	0.812**	0.878**	0.706**	1

Table 2. Correlation coefficient between Cd contents of Arbde and the concentration of Cd fractions in soil

	Shoot-Cd	Root-Cd	EXC-Cd	CAB-Cd	FeMn-Cd	OM-Cd	RES-Cd
Shoot-Cd	1						
Root-Cd	0.996**	1					
EXC-Cd	0.973*	0.989**	1				
CAB-Cd	0.847**	0.885**	0.932**	1			
FeMn-Cd	0.555*	0.609*	0.697**	0.839**	1		
OM-Cd	0.405	0.449	0.546*	0.577*	0.698**	1	
RES-Cd	0.396	0.459	0.567*	0.730**	0.931**	0.793**	1

Discussion

Under Cd stress, plants are often shown with the toxicity symptom of Cd such as growth inhibition and so on, and the toxic effects increase with the increase of Cd level (Antoniadis et al., 2017). However, some studies have pointed out that low Cd stimulates and promotes plant growth (Shi et al., 2015). In this experiment, shoot dry weights of Arbde and Band firstly increased with the increase of Cd level in soil, and then decreased. 75 mg·kg⁻¹ Cd and 150 mg·kg⁻¹ Cd stimulate the growth of Band and Arbde, which was probably because the perennial ryegrass was likely to distribute Cd²⁺ to non metabolic active site such as vacuole, apoplast and so on in order to reduce the toxicity of Cd²⁺ to cells (Liu et al., 2013).

When exposed to Cd, large amount of reactive oxygen species and peroxides in plants are produced, such as O₂⁻, OH⁻, H₂O₂, etc., resulting in disruption of cell membrane integrity and stability, and peroxidation damage to the plant (Zhang et al., 2015). These free radicals and peroxides in plants are removed or reduced by increasing the activities of antioxidant enzymes of SOD, POD and CAT. SOD, as the first line of defense of plant antioxidants, can eliminate excess superoxide anion in cells, and POD and CAT can make H₂O₂ differentiate into non-toxic water and oxygen molecules so as to alleviate the peroxidation damage of Cd to the plant (Li et al., 2013; Wang et al., 2008). Malondialdehyde (MDA) is the product of membrane lipid peroxidation in plant cells subjected to oxidative stress under stress, which is usually regarded as physiological indexes of oxidative damage, and its content can reflect the intensity of

plant responses to stress conditions and the extent of cell membrane lipid peroxidation (Becana et al., 2000).

In the present study, the activities of SOD and POD enzymes in the two ryegrass varieties increased firstly with the increase of Cd in soil, and then decreased, which was in line with the typical defense characteristics of plants under Cd stress. This result was consistent with the result of Yang et al. (2000). The activity of CAT decreased with the increase of Cd level in soil. The reason may be that CAT is the most sensitive antioxidant enzyme to heavy metals, and its activity is firstly inhibited, causing the elimination of H₂O₂ in the body to be blocked and only to be conducted by POD, thus the activity of POD increased (Yu et al., 2010). The content of MDA increased firstly with the increase of Cd in soil, and then decreased, and can exhibit strong reactive oxygen tolerance, which was probably because the active oxygen in the body can be eliminated at high Cd stress (Xu et al., 2010).

Cadmium with strong mobility is easy to be absorbed by plants, and most plants can transfer Cd from roots to shoots (Zhang et al., 2013). In the present study, Cd concentrations in roots of the two ryegrass varieties were higher than those in shoots. It suggested that roots had stronger Cd enrichment ability, but the abilities to transfer Cd from root to shoot were weaker. This result is similar to the reports of Huang et al. (2016), Chen et al. (2014) and Sun et al. (2016). The Cd concentration in shoot of Cd hyperaccumulator with the critical content of 100 mg·kg⁻¹ was 100 times as much as common plants (Li et al., 2016). In this experiment, when exposed to high Cd (≥150 mg·kg⁻¹), Cd concentration in shoots of Band and Arbde both exceeded the critical value of hyperaccumulator, and the two ryegrass varieties with strong tolerance to Cd had no obvious toxic symptoms, which were similar to the findings of Sun et al. (2016). Under the same Cd level in soil, Cd concentration and Cd accumulation in shoots and roots of Arbde were higher than those of Band. It was suggested that Arbde was more effective in remediation of cadmium contamination in soils due to higher plant biomass and greater Cd accumulation.

There were obvious differences in Cd migration ability of different Cd chemical forms in plants, which further affects the transport and transfer of cadmium in plants and the toxicity of cadmium to plants (Li et al., 2015). In the present study, F_{NaCl} was dominant chemical form of cadmium in Band and Arbde due to Cd had a strong affinity to sulfhydryls in proteins or other organic compounds, which was consistent with the early report by Zhang et al. (2011). The F_E with higher activity in Band and Arbde only accounted for 1.1%-3.6% and 1.4%-4.0% of the total amount of Cd extraction respectively, thus greatly limiting the toxic effect of Cd on ryegrass growth.

The available cadmium in soil is one of important factors affecting Cd uptake by plants (Castaldi et al., 2005). In the experiment, the concentration of various Cd chemical forms in soils were significantly positively correlated with Cd concentrations of shoot and root in the two ryegrass varieties ($P < 0.01$) (excepted for OM-Cd), which were consistent with the study of Yang et al. (2011). EXC-Cd was dominant chemical form of cadmium in soils of the two ryegrass, and the FDC of Cd with various chemical forms in soil was in order of EXC-Cd > CAB-Cd > FeMn-Cd > RES-Cd > OM-Cd, which was consistent with the results of Chen (2016). With the increase of Cd level, the percentage of EXC-Cd accounting for the total cadmium was also increasing, which was consistent with the research results reported by Zhang et al. (2013). Cadmium concentrations of roots in the two ryegrass varieties had a very significant positive correlation with EXC-Cd, indicating the absorption of Cd concentration in roots of

ryegrass and the exchangeable cadmium content in soil, which was consistent with the results of (Yang et al., 2011). Under the same Cd level, the concentrations of EXC-Cd in soil of Band was higher than that of Arbde, corresponding to the result that Cd concentrations in shoots and roots of Arbde were higher than those of Band (excepted for shoot Cd concentration at 150 mg·kg⁻¹ Cd), which was probably caused by the difference between varieties, while the specific reasons need to be studied.

Conclusion

The shoot dry weight of the two ryegrass varieties (Band and Arbde) increased firstly and then decreased with the increase of Cd level in soil, and reached the maximum at 75 mg·kg⁻¹ and 150 mg·kg⁻¹ of Cd respectively. The activities of SOD, POD and the contents of MDA in shoots and roots of the two ryegrass varieties increased with the increase of Cd level in soil, while CAT activity decreased. The concentrations of Cd chemical forms in shoots of the two ryegrass varieties were in order of F_{NaCl}> F_{HAC}> F_w> F_R> F_{HCl}>F_E. The order of FDC of Cd in each form was described as EXC-Cd > CAB-Cd>, FeMn-Cd>, RES-Cd > OM-Cd. Except for OM-Cd. the concentration of various Cd chemical forms in soils were significantly positively correlated with Cd concentrations of shoot and root in the two ryegrass varieties (*P*<0.01) (excepted for OM-Cd). Compared with the two ryegrass varieties, Arbde was more effective in remediation of cadmium contamination in soils due to stronger tolerance to Cd, higher biomass and greater Cd accumulation. Recommendations for future studies are to found the plants or varieties with high Cd-accumulation are needed to be screened out and cultured, and their genes are induced into plants with great biomass, rapid growing and strong adaptability, so that ideal Cd super-accumulation plants.

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