

# The Uptake, Accumulation, and Toxic Effects of Cadmium in Barnyardgrass (*Echinochloa crus-galli*)

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## Abstract

Cadmium (Cd) is regarded as one of the most toxic environmental pollutants. A better understanding of the growth response, uptake, and translocation of Cd in barnyard grass (*Echinochloa crus-galli*) would be helpful for evaluating the role of this grass species as a potential candidate to be used for phytoremediation. The studied plants were grown in treated soils with increasing doses of Cd (0, 0.3, 0.6, 0.9, and 1.5 mg·kg<sup>-1</sup>). The results showed that Cd contents in plant tissues were found to increase with increasing doses of Cd. The pattern of Cd accumulation in different parts of the plant were found as: root > aboveground part > seed. Translocation factor (TF) values were found to be less than 1, and translocation from root to aboveground part was found to be higher as compared to that of aboveground part to seed. There was no significant difference on the aboveground part fresh weight (FW) and tiller number per plant under different concentrations of Cd stress. The root FW and the lengths of roots and shoots were significantly decreased in response to Cd treatments at low concentrations. However, the 1.5 mg·kg<sup>-1</sup> Cd application didn't affect significant changes on the root and shoot length than that of the control. A high concentration of cadmium supply could promote chlorophyll content. SOD showed a similar trend to POD in Cd-exposed plants, with an increase at lower concentrations and a decrease at higher concentrations.

**Keywords:** cadmium, uptake, accumulation, physiological changes, barnyard grass

## Introduction

Cadmium (Cd) is one of the most toxic trace elements (No. 7 among the top 20 toxins), and is well known to be harmful to plants by inhibiting several plant physiological

processes such as oxidative reactions and nitrogen metabolism [1-2]. Cd-polluted soil in agricultural land, especially paddy fields, usually caused by irrigation water containing Cd running out from industrial and urban activity, transportation, and agricultural practices, has become a serious problem since Cd is also toxic to humans [3].

Root uptake and root-to-shoot translocation of Cd are two key physiological processes in Cd accumulation

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of plants [4]. It has been shown that plants are highly sensitive to Cd stress [5]. Cadmium stress in plants leads to a series of stress symptoms, including wilting, chlorosis, cell death, necrotic lesions, and growth reduction [6]. Moreover, Cd stress may disturb mineral nutrition [7] and carbohydrate metabolism [8], and may strongly reduce biomass production (Liu et al., 2010). The photosynthetic apparatus is particularly susceptible to Cd and a reduction of photosynthesis is a common response in plants exposed to Cd [9]. As Cd is unable to participate directly in biological redox reactions, it induces oxidative stress via different indirect mechanisms [10-11]. However, the toxic effects of Cd on plants depend on stress intensity and plant species [12-13]. Therefore, researching a plant's abilities for absorption, transfer, and accumulation of Cd and responses to toxicity induced by Cd is crucial for crop production, even for the protection of human health.

Barnyard grass (*Echinochloa crus-galli*) is a major weed of different field crops, especially rice. *E. crus-galli* can produce large number of seeds and grow better than crop plants in adverse environments, e.g., in nutrient-deficient and/or contaminated soil. It may cause a 21-79% yield loss in rice, depending upon the cropping system and management [14-15]. Interestingly, there is increased evidence showing that *E. crus-galli* may play an important role in improving heavy metal-contaminated soil [3, 17-18]. Therefore, a better understanding of the toxicity of Cd and the ability of *E. crus-galli* to remove Cd is important for evaluating the potential ability of this weed species for phytoremediation.

In the present study, we examined the effect of Cd on toxicity in barnyard grass. Our experimental aims were to determine: 1) the effect of different concentrations on Cd uptake and distribution in different parts and 2) plant physiological responses to Cd at different concentrations.

## Material and Methods

The experiments and analysis were respectively conducted at the experimental farm of Hunan Academy of Agricultural Sciences, China. *Echinochloa crus-galli* seeds from the city of Yuanjiang in Hunan Province were kept in 2% v/v KNO<sub>3</sub> solution overnight, then washed and grown on moistened filter paper for two days. The germinated seeds were transferred to cylindrical plastic pots of 28 cm diameter and 17 cm depth filled with 3.0 kg of sieved dry paddy soil (the contents of soil organic matter, total nitrogen, total phosphorus, alkaline hydrolytic nitrogen, effective phosphorus, and total cadmium were 3.72%, 1.20 g·kg<sup>-1</sup>, 1.02 g·kg<sup>-1</sup>, 164.35 mg·kg<sup>-1</sup>, 54.28 mg·kg<sup>-1</sup>, and 0.30 mg·kg<sup>-1</sup>, respectively, and soil pH was 5.7). Soil was spiked by adding a distilled water solution of Cd(NO<sub>3</sub>)<sub>2</sub> and mixed thoroughly. All pots were properly cared for as required according to conventional field management measures.

Treatments were set up with five levels of Cd contamination, i.e., 0-control, 0.3, 0.6, 0.9, and 1.5 mg Cd/kg soil dry weight. Four pots of barnyard grass plants

were considered one treatment. The entire experiment was performed according to a complete randomized design (CRD). Each experiment was replicated three times.

## Determining Cadmium in Plants

Harvested plants were thoroughly washed in distilled water and roots, seeds, and aboveground parts were separated, then oven dried at 80°C. Dried plant material (500 mg) was powdered and digested in an HNO<sub>3</sub>/HClO<sub>4</sub> (9:1, v/v) mixture using the wet digestion method. Cd concentration was routinely verified by inductively coupled plasma mass spectroscopy (ICP-MS, iCAP Qc, Thermo Fisher Scientific, USA). The cadmium translocation ability of *E. crus-galli* plants was assessed using the translocation factor (TF) calculated as follows.

$$TF = \text{Cd concentration in shoot} / \text{Cd concentration in root}$$

## Determination of Physiological Parameters

### Concentration of Photosynthetic Pigments

At tillering stage, the photosynthetic pigment concentrations were determined in the second leaves from the top by the extraction of leaf samples in 80% (v/v) acetone. The absorbance of the resulting extracts were measured at 663 nm, 646 nm, and 470 nm. The concentrations of chlorophyll a and b and total carotenoids (xanthophylls + carotenes) was determined according to the equations given by Lichtenthaler and Wellburn (1983) [19].

### Determination of Plant Growth

Plant growth was used as one of an important criterion to assess Cd toxicity. At mature stage, the plants were harvested, washed with tap water, rinsed with deionized water, and dried on absorbent towels. The tiller number per plant, the lengths of stems and roots, and the fresh weights of roots and aboveground parts were recorded.

### Activities of Antioxidant Enzymes

At tillering stage, plant material (500 mg, leaves) was homogenized in 100 mM potassium phosphate buffer (pH 7.0) containing 0.1 mM EDTA at 4°C. Homogenate was filtered through four layers of cheese cloth and centrifuged at 4,000 × g for 15 min at 4°C. Supernatant was used for enzyme activity and protein determinations. SOD activity was measured as the inhibition in the photochemical reduction of nitroblue tetrazolium (NBT) spectrophotometrically at 560 nm [20]. POD activity was measured following a protocol using guaiacol [21].

## Statistical Analysis

The experimental data reported were averages of three replicates. All statistical analyses were performed with data processing system software SPSS version 18.0

for Windows. Means and standard deviation were used for data that were analyzed using the least significant difference (LSD) method at the 5% level.

### Results and Discussion

Cadmium (Cd) is one of the most highly toxic trace pollutants for humans, animals, and plants. There are three transport processes most likely to mediate Cd accumulation into the shoots and, subsequently, into the seeds: (i) uptake by roots, (ii) xylem-loading-mediated translocation to shoots, and (iii) further translocation to seeds via the phloem [22].

#### Cadmium Accumulation of Different Plant Parts of *E. crus-galli*

In our study, data for Cd accumulation in *E. crus-galli* tissues indicated that most of the metal was accumulated in roots and a lower fraction was translocated to the aboveground parts, and values decreased in the order as roots > aboveground parts > seeds (Fig. 1). The concentrations of Cd in different parts of the plants were gradually increased with the increase of Cd in the soil. Cadmium contents were significantly increased with 0.6, 0.9, and 1.5 mg·kg<sup>-1</sup> Cd stresses in roots, indicating that uptake of Cd in roots is a key process in barnyard grass plant Cd accumulation. Similar results were reported in various plants [23-24]. Aboveground parts exposed to 0.9 and 1.5 mg·kg<sup>-1</sup> Cd showed a significantly higher Cd accumulation than that of the control. Plants grown in Cd-spiked soil had a higher Cd concentration in seed than that of the control, and the increases were 30.23%, 93.02%, 102.33%, and 162.79%, with 0.3, 0.6, 0.9, and 1.5 mg·kg<sup>-1</sup> Cd stresses, respectively. The significant increases of Cd content were not observed in roots and aboveground parts with 0.3 mg·kg<sup>-1</sup> Cd treatment, indicating that the presence of Cd at low concentration may not impact the Cd uptake of plants.

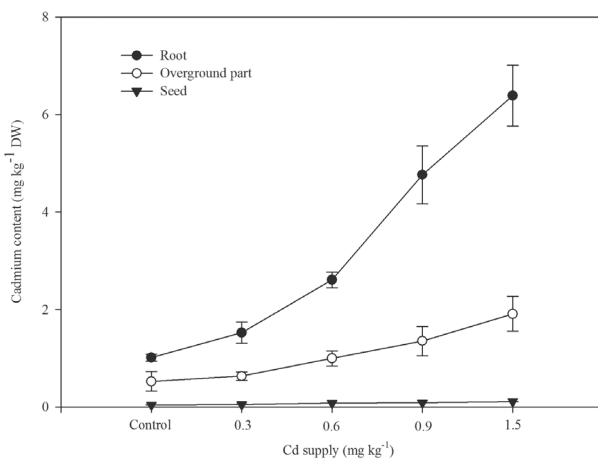


Fig. 1. Cadmium content in different parts of root, aboveground part, and seed with cadmium application.

#### Cadmium Translocation in Different Plant Parts of *E. crus-galli*

TF is the ratio of metal concentration in the aboveground plant part to that in root, being used to measure the effectiveness of a plant in translocating a metal from the roots to the aboveground plant parts [25]. The higher the TF value of a plant, the more the plant is suitable for phytoremediation through phytoextraction. The translocation factor (TF) of Cd from root to aboveground part and aboveground part to seed is presented in Table 1. It was clear that increased concentration of Cd significantly decreased the translocation of Cd from aboveground part to seed. Translocation from aboveground part to seed was found to be lower as compared to that of root to aboveground parts, which revealed that higher accumulation of Cd in root tissues and promotion of the aboveground part growth of *E. crus-galli* may be an effective route for soil remediation. All translocation factor values were found to be less than 1. The TF values of barnyard grass were found to be obviously decreased with the increase of Cd in the soil, which may be due to limited root-to-shoot transport when internal Cd concentration was high, which was similar to the previously reported results of cadmium accumulation in sunflower (*Helianthus annuus* L.) plants [26].

#### Effects of Cadmium Application on Changes in Plant Growth Parameters

Cd is one of the most commonly found metals in soil, which limits crop productivity worldwide as this metal tends to accumulate within plant organs and negatively interfere with essential physiological processes [27]. In order to examine the growth parameters of *E. crus-galli* in different treatments, the roots and aboveground part FW and length as well as tiller number per plant were determined (Table 2). There was no significant difference in the aboveground part FW and tiller number per plant under different concentrations of Cd stress. Compared with the control, 0.3 and 0.6 mg·kg<sup>-1</sup> Cd stress caused a significant decrease in root FW by

Table 1. TF of cadmium from root-to-aboveground part and from aboveground part-to-seed of *E. crus-galli*.

Treatments (mg·kg <sup>-1</sup> )	TF (From root to aboveground part)	TF (From aboveground part to seed)
0.3	0.445±0.023 a	0.088±0.008 a
0.6	0.374±0.007 b	0.083±0.004 b
0.9	0.252±0.017 c	0.064±0.005 c
1.5	0.323±0.015 b	0.059±0.005 d

Values are mean ± standard error (SE) of three replications. Different small letters within the same short columns indicate significant differences between treatments according to Duncan's multiple range test at *p*<0.05 level.

Table 2. Effects of cadmium application on the growth parameters of *E. crus-galli*.

Treatments (mg·kg <sup>-1</sup> )	FW (g per plant)		Organ length (cm)		Till number per plant
	Root	Aboveground parts	Root	Aboveground parts	
0.3	15.36±0.58 b	30.07±4.69 a	30.60±0.94 bc	122.83±2.02 b	6.00±0.58 a
0.6	17.48±0.58 b	24.97±2.01 a	29.17±1.43 c	110.40±3.65 b	5.33±0.33 a
0.9	23.97±0.58 a	45.84±1.98 a	35.67±2.41 a	112.80±1.31 b	5.67±0.89 a
1.5	21.72±0.58 a	43.56±5.79 a	34.93±1.21ab	109.53±2.28 a	6.67±0.65 a
Control	23.54±4.33 a	32.01±3.44 a	37.53±0.94 a	124.33±0.58 a	6.00±0.48 a

Values are mean ± standard error (SE) of three replications. Different small letters within the same short columns indicate significant differences between treatments according to Duncan's multiple range test at  $p < 0.05$  level.

34.75 and 25.74%, respectively. No significant differences in root FW were observed between 0.9 mg kg<sup>-1</sup> and 1.5 mg·kg<sup>-1</sup> Cd stress and the control. The root lengths of the 0.3 and 0.6 mg·kg<sup>-1</sup> Cd-treated plants were significantly reduced by 18.47 and 22.28%, than that of control. In addition, shoot lengths were severely decreased by 1.21, 11.20, and 9.28%, respectively, in response to 0.3, 0.6, and 0.9 mg·kg<sup>-1</sup> Cd treatments, which are similar to those obtained by Ullah et al. (2012) [28]. However, the 1.5 mg·kg<sup>-1</sup> Cd application didn't affect significant changes on root and shoot length compared to control. Similarly, in carrot and radish (*Raphanus sativus*) exposed to increasing Cd concentrations, there was no significant difference in the development of cotyledons or new leaves [29]. To a certain extent, we speculated that *E. crus-galli* had tolerant potential to Cd stress regarding growth parameters.

### Effects of Cadmium Application on Changes of Leaf Chlorophyll Content

In green plants' life activity, chlorophyll plays the role of absorbing, transferring, and transforming energy. Chlorophyll content was strongly reduced in tomato (*Lycopersicon esculentum*) [30] and garden cress [27]. However, the exposure of *E. crus-galli* to 0.9 and 1.5 mg·kg<sup>-1</sup> Cd significantly increased the concentration of chlorophyll a and b in comparison to the control plants (Figs 2a-b). The concentration of Chl a was similar to the control plants and in plants exposed to 0.6 mg·kg<sup>-1</sup> Cd. The decline of the Chl b concentration in the 0.6 mg·kg<sup>-1</sup> Cd-exposed plants reached 12.70% more than the control. The ratio of Chl a/b and the concentration of carotenoids was increased significantly in Cd-exposed plants compared

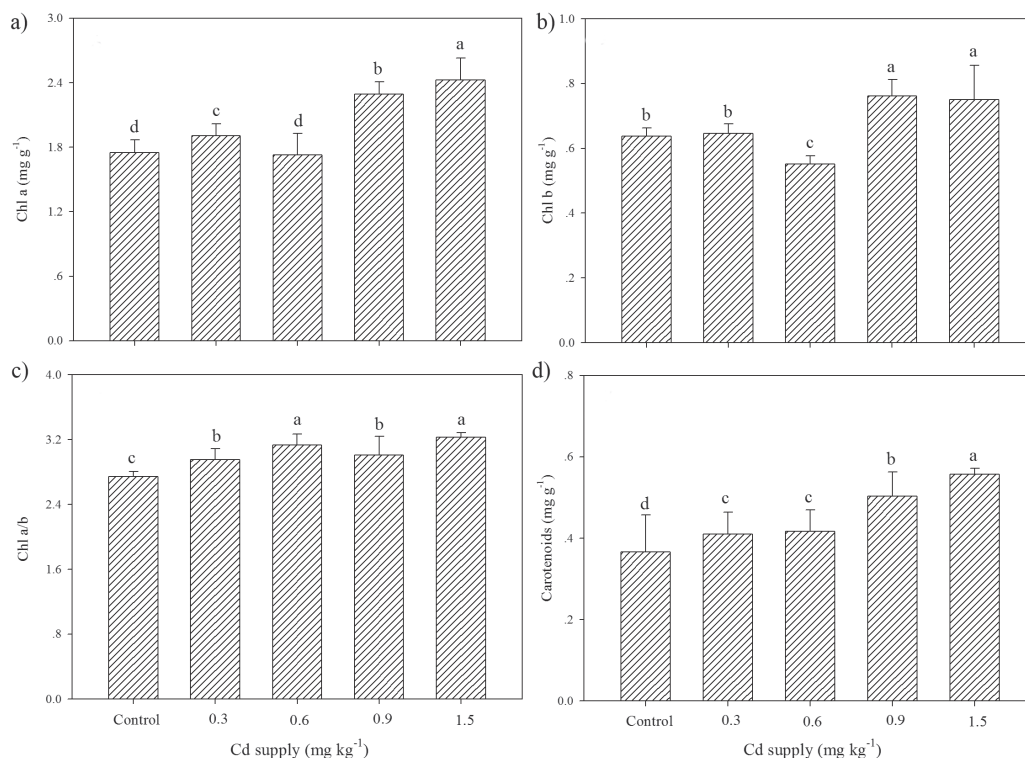


Fig. 2. Changes of leaf chlorophyll a), chlorophyll b), the ratio of chlorophyll a/b, and carotenoids with cadmium application.

to the control plants (Figs 2c-d), indicating that a high concentration of cadmium supply could promote the chlorophyll contents. Meanwhile, carotenoid increases in *E. crus-galli* plants exposed to different Cd concentrations may reflect an attempt to protect chlorophyll and/or the photosynthetic apparatus from the photooxidative destruction caused by Cd toxicity.

### Effects of Cadmium Application on the Changes of Leaf Antioxidant Enzyme Activities

In tolerant plant species, POD activity was found to be higher, enabling plants to protect themselves against oxidative stress [31]. In the present study, compared to the control, the addition of 0.3 or 0.6 mg·kg<sup>-1</sup> Cd caused an increase in the level of POD by 84.74% and 44.11%, respectively. On the contrary, the POD levels decreased by 50.66% and 54.10% under 0.9 and 1.5 mg·kg<sup>-1</sup> Cd stress, respectively (Fig. 3).

Activities of SOD and POD in Chinese cabbage roots were lower in the Cd treatment than the control [32]. In our study, SOD showed a similar trend to POD in Cd-

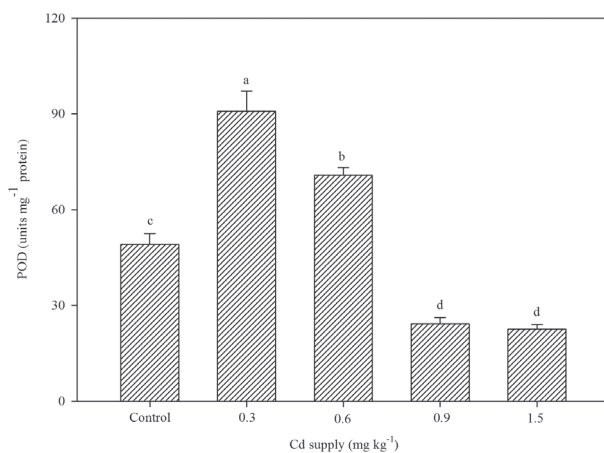


Fig. 3. Leaf POD activities with cadmium application.

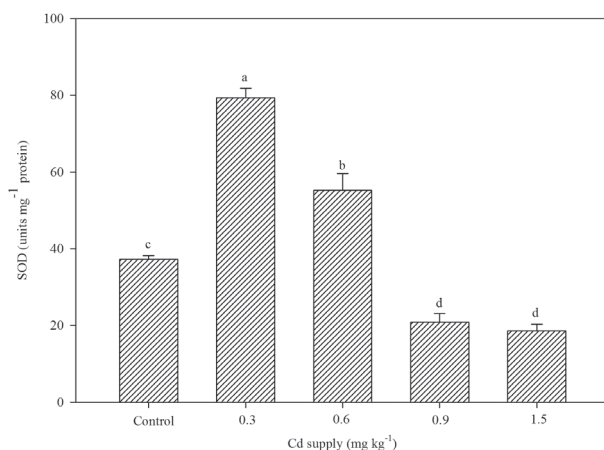


Fig. 4. Leaf SOD activities with cadmium application.

exposed plants, with increases at lower concentrations and decreases at higher concentrations (Fig. 4). Treatments with 0.3 or 0.6 mg·kg<sup>-1</sup> Cd resulted in 113.13% and 48.17% increases in SOD compared with that of the control. SOD activities were distinctly lower than that of the control by 43.96% and 50.11%, respectively. Antioxidant enzymes generally showed stimulation at lower cadmium-contaminated concentrations and decreased at higher cadmium-contaminated concentrations. Increases in SOD activity may be attributed to the increased production of active oxygen species or increased expression of genes encoding SOD [33]. A reduction in SOD activity at higher Cd concentrations may be attributed to an inactivation of enzymes by H<sub>2</sub>O<sub>2</sub>, which is produced in different cellular compartments and also from a number of non-enzymatic and enzymatic processes in cells.

### Conclusions

In conclusion, our results demonstrated that the uptake and translocation of Cd in barnyard grass (*Echinochloa crus-galli*) and the growth response at different Cd treatments is the physiological basis for evaluating the role of this grass species as a potential candidate to be used for phytoremediation.

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