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# Cadmium accumulation in *vetiveria zizanioides* and its effects on growth, physiological and biochemical characters

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ABSTRACT

Hydroponic experiment was carried out to investigate the effect of cadmium (Cd) on growth, Cd accumulation, lipid peroxidation, antioxidative enzymes, leaf chlorophyll, root activity, protein content and Cd uptake kinetics of vetiver grass. The results showed that 1 mg/L Cd in solution led to increased chlorophyll contents, root activity and enhanced the growth of vetiver grass after 15 days, with 2.2% biomass increased compared to the control. Malondialdehyde (MDA) contents were significantly enhanced by all Cd supply levels. The development of toxic symptoms corresponded to a high accumulation of Cd and to the decrease of water content, chlorophyll, protein content and root activity, but to high increase in catalase and peroxidase activities in plants. Cd concentration in shoots and roots increased with increasing Cd supply levels, and reached a maximum of 93 and 2232 mg/kg Cd dry weight at 30 mg/L treatment, respectively.



## 1. Introduction

Cadmium (Cd) is a potentially hazardous trace metal which causes environmental and human health problems due to its high mobility in the soil–plant system (Liu et al., 2007a,b). Cd can be taken up into plant root system through some other nutrient metabolic pathways such as zinc, iron and calcium (Cosio et al., 2004) and then it reaches root cell membrane via the apoplast, including cell wall continuum and intercellular space.

Cd induces complex changes in plants at genetical, biochemical and physiological levels, leading to phytotoxicity, the most obvious symptoms of which are reduction of tissue and growth, leaf roll and chlorosis, and leaf root necroses (Schutzendubel et al., 2001). One of the major consequences of Cd toxicity is the enhanced production of reactive oxygen species (ROS). These ROS include super oxide radical ($O_2^{\cdot-}$), hydroxyl radical ($OH^{\cdot-}$) and hydrogen peroxide ($H_2O_2$) that are produced as by products during membrane linked electron transport activities as well as by a number of metabolic pathways and in turn cause damage to the biomolecules such as membrane lipids, protein, chloroplast pigments, enzymes (Luna et al., 1994; Wang et al., 2007, 2008). The deleterious effects resulting from the cellular oxidative state may be alleviated by enzymatic and non-enzymatic antioxidant machinery of the plant that vary at various cellular and subcellular levels in different plants. Plants use a diverse array of enzymes like catalase (CAT), peroxidases (POD), ascorbate peroxidase (APX), superoxide dismutases (SOD) as well as several molecules like glutathione, carotenoids and ascorbic acid to scavenge different types of reactive oxygen species, thereby protecting potential cell injury against tissue dysfunction (Srivastava et al., 2004). CAT dismutates $H_2O_2$ into $H_2O$ and $O_2$, which is found in peroxisomes, cytosol and mitochondria. POD decomposes $H_2O_2$ by oxidation of co-substrates such as phenolic compounds and/or antioxidants (Dawes, 2000). Malondialdehyde (MDA), an indicator of lipid peroxidation, has also been used to assess oxidative stress (Polle et al., 1997). Given the above cited mechanisms utilized by plants to detoxify ROS, it is important to establish what effect enzyme activity has on this detoxification process.

Vetiver grass (*vetiveria zizanioides* (L.) Nash) is a tall (1–2 m), fast-growing, perennial tussock grass. It has a long (3–4 m), massive and complex root system, which can penetrate to the deeper layers of the soil. Owing to its unique morphological, physiological and ecological characteristics such as its massive and deep root system, and its tolerance to a wide range of adverse climatic and edaphic conditions, including elevated heavy metals, the interest in this grass has increased in recent years (Truong, 2000). In Australia, vetiver grass has been used successfully to stabilize highly saline, alkaline (pH 9.5) coal mined land, and highly acidic (pH 2.7) gold mined land. Pang et al. (2003) have investigated the physiological responses of vetiver grass to Pb, Zn and Cu. However, little data are available concerning the effect of different level cadmium on the growth response of vetiver grass. Hence, purpose of this study was to examine the effects of Cd on growth, protein,

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chlorophyll content, MDA, root activity, antioxidant enzyme activity and Cd accumulation in vetiver grass.

## 2. Methods

### 2.1. Plant materials and cadmium treatments

The experiments consisted of four Cd treatments in a randomized design. The Cd solution was freshly prepared by dissolving $Cd(NO_3)_2{\cdot}4H_2O$, in deionized water. One month-old vetiver seedlings were originally collected from Ningxiang county, Hunan Province, southern China. Plant roots were surface-sterilized in 0.3% hydrogen peroxide for 20 min, rinsed with distilled water, and then acclimatized in a hydroponic system with 12.5% Hoagland nutrient solution for 3 weeks. The pH was adjusted to 5.7 by the addition of small amounts of KOH (3 or 4 seeds per dish). Afterwards, the plants were treated in Hoagland's nutrient solutions which contained 0, 1, 7.5, 15, and 30 mg/L $Cd(NO_3)_2{\cdot}4H_2O$ for 15 days. Solutions were continuously aerated and changed regularly every 2 days.

### 2.2. Determination of Cd in plants

After 15-day's growth, plants of each treatment were harvested and washed thoroughly with distilled water, separated into roots and shoots (stems and leaves), dried at 70 °C for 48 h and weighted. Cd concentrations were determined by atomic absorption spectroscope (Analyst 300, Perkin–Elmer, Germany) after digesting the samples with $HNO_3$–$HClO_4$ (3:1).

### 2.3. Water content and translocation factor

The water content of the plants during treatment was determined using the following equation:

$$\mathrm{WC}\ (\%) = (\mathrm{FW} - \mathrm{DW})/\mathrm{DW} \times 100. \quad (1)$$

where WC (%) is the water content, FW (g) is the fresh weight of plants and DW (g) is the dry weight of plants.

Prior to the fresh weight measurement, the plants were removed from the medium and washed with deionized water, followed by proper blotting with filter papers and weighed. After drying for 48 h at 70 °C in an incubator, the dry weight (DW) was measured.

The translocation factor (TF) is defined as the ratio of the metal concentration in stems to that in the roots, which is used to measure the effectiveness of a plant in transporting heavy metal from roots to shoots.

### 2.4. Determination of lipid peroxidation and soluble protein

The level of lipid peroxidation was expressed as the content of malondialdehyde (MDA) and was determined as 2-thiobarbituric acid (TBA) reactive metabolites. Fresh roots (0.5 g) were homogenized in a ceramic mortar in 10 mL 10% (w/v) trichloroacetic acid (TCA) with a pestle and mortar. Samples were incubated at 70 °C for 30 min, then chilled on ice and centrifuged at 10,000 rpm for 10 min at 4 °C. The absorbance of the resulting chromophore was measured at 532 nm and 600 nm wavelengths. The concentration of lipid peroxidation was expressed as mmol/g FW.

The total protein content was determined by the method of Bradford using bovine serum albumin (BSA) as standard (Bradford, 1976).

### 2.5. Determination of chlorophyll content and root activity

Chlorophyll content was determined spectro-photometrically on the supernatant at 646 nm and 663 nm as described by Lichtenthaler (1987).

Root activity was determined using the triphenyl tetrazolium chloride (TTC) method. Fresh roots (0.5 g) were cut into small pieces and placed into beakers followed with the addition of 0.4% TTC and phosphate buffer (1/15 mol/L, pH 7.0). After incubation at 37 °C for 2 h, 2 mL of 1 M $H_2SO_4$ was added to end the reaction. Afterwards the roots were taken out and homogenized in 4 mL of 95% (v/v) ethanol to extract the triphenyl tetrazolium formazane (TTF). The total extract was transferred into a graduated test tube and residues were washed three times with extraction buffer which was then transferred into same tube to get a constant volume. The absorbance of the color was measured at 485 nm. Root activity was expressed as on fresh weight basis.

### 2.6. Determination of antioxidative enzyme

The fresh leaves or roots (about 0.5 g) from each treatment were homogenized in an ice cold mortar using 50 mmol/L phosphate buffer (pH 7.0) containing 1 mmol/L EDTA and 1% polyvinylpyrrolidone (PVP). The homogenate was filtered through four layers of cheesecloth and centrifuged at 20,000 rpm for 20 min at 4 °C. The supernatant was used to determine enzyme activities, which were measured at 25 °C.

Catalase (CAT) activity was determined by monitoring the decrease in absorbance at 240 nm as a consequence of $H_2O_2$ consumption (Aeby, 1984). The assay mixture contained 50 mmol/L phosphate buffer (pH 7.0), 15 mmol/L $H_2O_2$, and 0.5 mL leaf extract in a 3 mL volume. POD activity was measured by following the change in absorption at 470 nm due to guaiacol oxidation in a reaction solution (3 mL final volume) composed of 50 mmol/L phosphate buffer (pH 7.0) 20 mmol/L guaiacol, 10 mmol/L $H_2O_2$ and 0.5 mL of crude extract.

### 2.7. Cadmium uptake kinetics

In order to determine the kinetics of Cd uptake from the medium solution, experiments were conducted at different initial concentrations ranging from 0.05 to 30 mg/L maintained in 500 mg/L $Ca(NO_3)_2$ solution for 4 h. After treatment for 4 h, plant roots were harvested and weighted. Cd concentration of substrates was determined by atomic absorption spectroscope. Rate of Cd uptake into roots of vetiver plants was used to calculate the initial rate of Cd uptake into seedlings, and kinetic constants were derived from these rates using a non-linear curve fitting algorithm.

Uptake kinetics are typically described using the analogous enzyme kinetic parameters: the maximum reaction rate, which is determined as the mean of uptake rates obtained at saturating substrate concentrations, and the Michaelis–Menten constant, which is the heavy metal concentration at which the uptake rate is half of the maximum. The rate equation is given by the Michaelis–Menten equation:

$$V = (V_{\max} \cdot S)/(K_m + S), \quad (2)$$

where $V$ is the initial velocity when no product is formed, $V_{\max}$ is the maximum reaction rate, $K_m$ is the Michaelis–Menten constant, and $S$ is the substrate concentration.

### 2.8. Statistical analysis

Each treatment was replicated three times for statistical validity. The data were analyzed through analysis of variance (ANOVA) using SPSS 11.5 and origin pro 7.5 statistical package. Student's *t*-

test was applied to determine the significance of results between different samples. Statistical significance was set at the $p < 0.05$ confidence level.

## 3. Results

### 3.1. Effect on dry biomass production and water content

The effects of Cd on plant growth were evaluated by examining the biomasses of vetiver grass after they had grown for 15 days. In this study, effects of Cd on root and shoot growth varied depending on concentrations of Cd. Compared to the control, a moderate Cd supply (1 mg/L) in the solution enhanced the growth of vetiver. After 15 days of growth, the biomass of roots and shoots increased by 1.7% and 2.6%, respectively (Table 1). However, the beneficial effects of Cd were not observed at high level of Cd. When the Cd concentrations were >7.5 mg/L, the plant biomass decreased as solution Cd concentration increased for both the roots and shoots. Compared to the control plant, the root and shoot biomass at 30 mg/L Cd declined by 48% and 44%, respectively. Table 1 shows the water content (WC) value of plants under different Cd stress. The water content of plants decreased gradually with increasing Cd level in the medium. At 30 mg/L Cd treatment, water content decreased to 85.12% of control. The significant reduction of dry biomass and water content with increasing concentration of Cd indicated that high Cd concentration produced toxic effects within 15 days of growth.

### 3.2. Cd accumulation in vetiver grass

The uptake and accumulation of Cd in vetiver roots and shoots varied with Cd concentration. As shown in Fig. 1, Cd concentrations in roots and shoots were positively correlated with Cd concentration in the solution. Cd concentration in roots ranged from 263 to 2232 mg/kg DW while ranged from 28 to 93 mg/kg DW in shoots. Most of Cd taken up by vetiver accumulated in the roots and small amounts of Cd were transferred to shoots. The presence of different Cd levels did not significantly affect the capability of plants to transfer Cd from roots to shoots. Concerning the translocation factor (TF), a trend to a lower percentage of Cd accumulation in shoots was noted with increasing Cd level in the medium. Translocation factor in vetiver were 0.112, 0.075, 0.062 and 0.043, respectively, all being <1.0.

### 3.3. Effects of Cd on lipid peroxidation in roots

Fig. 2A demonstrates the relation between MDA content and concentrations of Cd in growth medium. MDA content in roots of vetiver increased dramatically with increasing Cd supply levels ($p < 0.001$), and reached its maximum at 30 mg/L Cd, 45.1% higher than that of control ($p < 0.001$).

### 3.4. Effects of Cd on protein contents in vetiver

The protein contents in vetiver grass with different Cd are given in Fig. 2B. Initially up to 1 mg/L Cd resulted in enhancement of protein content in leaves. Soluble protein content in roots and shoots tended to decrease with increasing Cd concentrations ($p < 0.001$), and the decrease was greater in roots than in shoots. The minimum

**Table 1**
Transfer factors of Cd and water contents in vetiver.

| | Transfer factor (TF) | Water content (%) | Root biomass (g/plant) | Shoot biomass (g/plant) |
|---|---|---|---|---|
| Control | – | 83.23 ± 0.31 | 1.79 ± 0.039 | 1.9 ± 0.041 |
| 1 mg $L^{-1}$ | 0.112 | 83.82 ± 0.16* | 1.82 ± 0.009* | 1.95 ± 0.032 |
| 7.5 mg $L^{-1}$ | 0.075 | 75.82 ± 0.16* | 1.56 ± 0.009 | 1.59 ± 0.032 |
| 15 mg $L^{-1}$ | 0.062 | 73.74 ± 0.16* | 1.02 ± 0.039* | 0.93 ± 0.065* |
| 30 mg $L^{-1}$ | 0.045 | 70.85 ± 0.45* | 0.86 ± 0.002* | 0.85 ± 0.004* |

* $p < 0.001$, Significant differences compared to control.

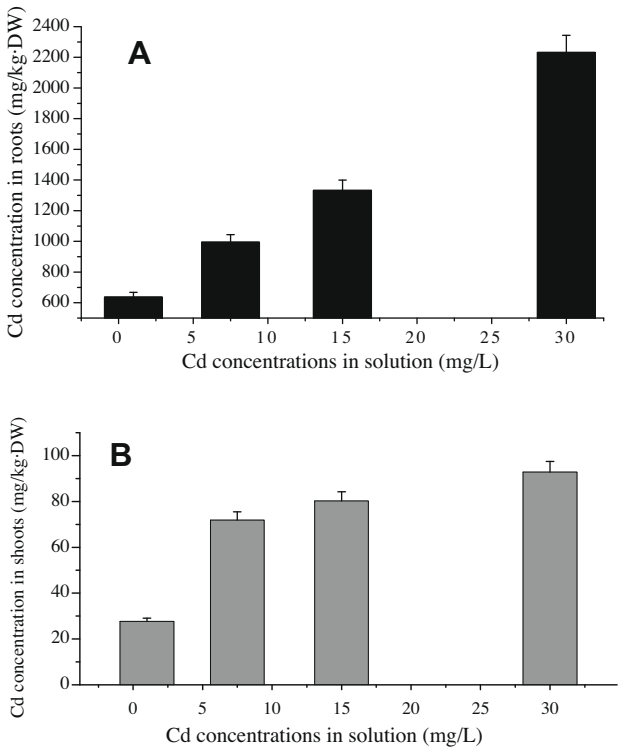


**Fig. 1.** Cd accumulation in roots (A) and shoots (B) of vetiver after 15 days of treatment period. (All of values are mean ± SD based on three independent observations, and bars indicate standard deviations.)

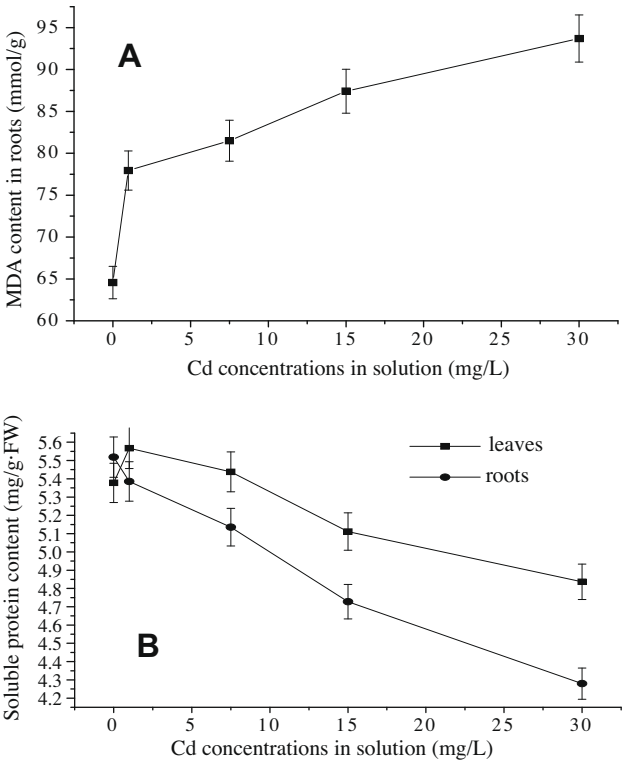


**Fig. 2.** MDA (A) and soluble protein (B) content in vetiver.

value was recorded in the roots and shoots exposed to 30 mg/L Cd (89.9% and 77.5% of control, respectively, $p < 0.001$).

### 3.5. Effects of Cd on chlorophyll contents in leaves

Both chlorophyll *a* and *b* contents increased at lower concentration (Fig. 3) and reached the highest level (1.903 ± 0.09 and 0.309 ± 0.15 mg/g FW of Chl *a* and Chl *b*, respectively) at 1 mg/L of Cd and then decreased slightly ($p < 0.05$). A concentration-dependent decline of Chl *a* and Chl *b* occurred as a consequence of exposure to Cd concentrations of 15 mg/L and 30 mg/L, and the values of Chl *a* and Chl *b* content were 88.7% and 87.01% of the control level at the highest Cd concentration in the solution, respectively ($p < 0.001$).

### 3.6. Effects of Cd on root activity

Cadmium toxicity on root activity of the vetiver is presented in Fig. 4. Root activity reached its highest value at 1 mg/L of Cd (13.53 ± 0.67 TTF/(g h) FW), where it was significantly higher than the control (148.02% of the control, $p < 0.001$). The root activity of control plants was 9.12 ± 0.46 TTF/(g h) FW ($p < 0.001$). The root activity decreased with the Cd concentration in medium solution increasing, but still higher than the control at 7.5 mg/L of Cd (10.31 ± 0.52 TTF/(g h) FW, $p < 0.001$). The lowest root activity was observed in plants exposed to 30 mg/L of Cd (5.49 ± 0.28 TTF/(g h) FW, 60.19% of the control, $p < 0.001$).

### 3.7. Effects of Cd on antioxidant enzyme activities in vetiver

The differences in antioxidant enzyme activities (CAT and POD) are given in Fig. 5A and B. Cd can cause production of superoxide radicals in plants and induced oxidative stress in plant tissues. In our experiment we observed that different Cd treatment levels caused considerable enhancement in the activities of POD and CAT which could scavenge superoxide radicals and alleviate their deleterious effects. This may imply that higher accumulation of ROS stimulate activity of antioxidative defense enzymes. In all of Cd treatment, leaves exhibited higher CAT activity than roots. On the contrary, the activities of POD in roots were higher than in leaves. POD activity in roots and leaves increased to about 337.12% and 190.12% of the control value at the highest Cd concentration, respectively ($p < 0.001$). Activity of CAT increased significantly with the maximum increase being 348.7% and 481.4% higher than control in roots and leaves at 30 mg/L Cd ($p < 0.001$), respectively.

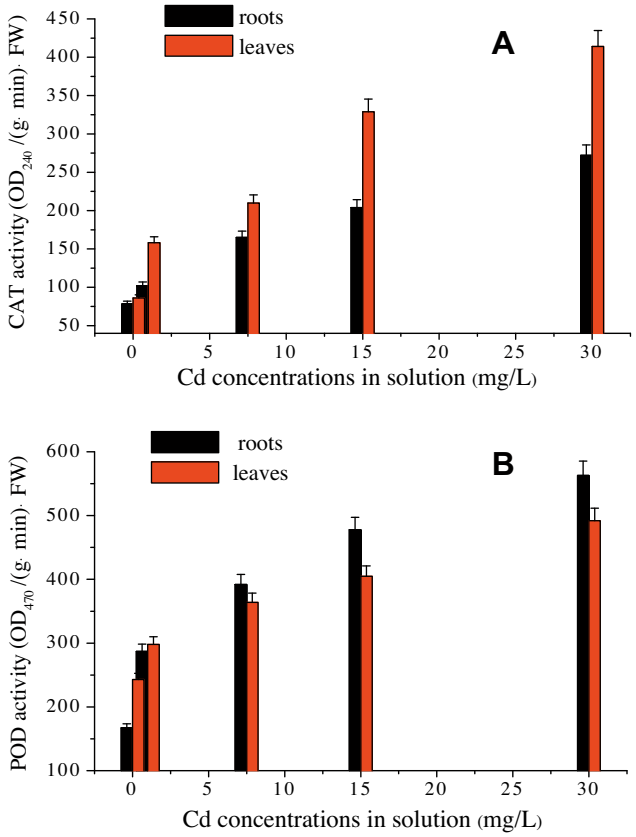


**Fig. 5.** Enzymatic activities of CAT (A) and POD (B) in vetiver grown in different Cd concentrations.

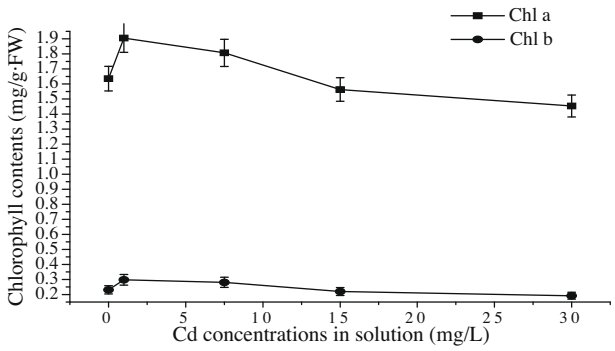


**Fig. 3.** Chlorophyll content in leaves of vetiver.

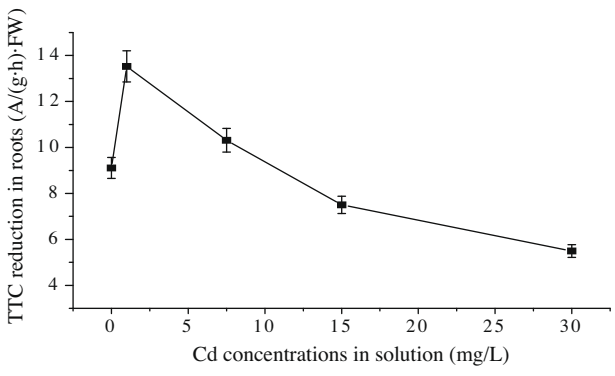


**Fig. 4.** The root activity of vetiver.

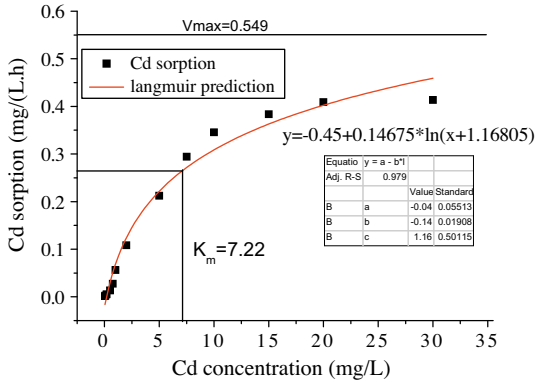


**Fig. 6.** Cd uptake kinetics by roots in medium after 4-h treatment.

### 3.8. Cadmium uptake kinetics in vetiver

Cd uptake by vetiver was dependent on Cd concentration in the solution. The Cd uptake by roots increased rapidly with the increase in Cd concentrations in medium solution up to 15 mg/L, and thereafter changed little with further increase. The uptake kinetics could be very well described by the Michaelis–Menten equation ($R^2 = 0.918$). The maximum uptake rate $V_{max}$ was 0.549 mg/(L h) with a half-saturation constant $K_m$ of 7.22 mg/L. The saturation kinetics of Cd uptake in vetiver seeds suggest that this process is mediated by saturable transport system (Fig. 6).

## 4. Discussion

### 4.1. Plant dry biomass and cadmium tissue concentration

Excessive application of Cd usually leads to inhibition of biomass production and phytotoxicity both in hyperaccumulators and non-hyperaccumulators is well documented. Nevertheless, the vetiver grass treated with 1 mg/L Cd showed moderate stimulation in biomass production, in accordance with previous finding that there is some potentially positive influence of Cd on plant growth at lower concentration. It seems that plants are able to defend and protect their integrity against mild environmental damaging stress. Various mechanisms have been suggested to explain the stimulatory effect, and one of the explanations is that metal ions may serve as activators of enzyme(s) in cytokinin metabolism, which accelerates the growth of plants (Péter et al., 2003). Second, low dose stress may cause changes in plant hormones and cytokinins that regulate plant growth and development. Hormones and cytokinins have been shown to cause an increase in chlorophyll accumulation; cytokinins also facilitate the synthesis a stabilization of LHC II (light-harvesting chlorophyll *a*/*b*-protein complex of photosystem II) and LHC I (light-harvesting chlorophyll *a*/*b*-protein complex of photosystem I), alter the relative distribution of CPCs (chlorophyll-protein complex) of thylakoid membranes, and increase photosynthetic activity (Nyitrai, 1996). Third, low molecular weight Cd-binding proteins might be synthesized in response to low stress of heavy metal by plants, and this type of protein has been shown to play a role in metal tolerance (Shahabad and Anjum, 1997). Therefore it could be suggested that vetiver grass must be considered to be a species of great potential for phytoextraction purposes in pollutant soils.

In present study showed that shoot and root growth of vetiver decreased significantly with high level of Cd treatment. The growth reduction observed in plants subjected to heavy metals often results from direct effects (toxicity of heavy metals accumulated in tissue) or from indirect effects (limitation of mineral and water acquisition). A decrease of biomass production was associated with accumulation of Cd in root and shoot tissues. Cd content in both roots and shoots increased significantly with exposure to increasing Cd concentrations. The accumulation of Cd was higher in roots than in shoots. Retention or immobilization of high amount of Cd in the root tissue, typically of several plants, can be regarded as one important protection mechanism against the diffusion of this heavy metal in plants (Verkleij and Schat, 1990). For this reason, Cd concentration in vetiver roots reaches 96% of the total metal taken up at 30 mg/L Cd treatment. Similarly, a survey on heavy metal distribution in various tissues of vetiver (Yang et al., 2003) reported that highest levels of Zn and Pb were accumulated in roots. The phenomenon of high Cd accumulation in roots is probably because of the absorption of Cd onto the negatively charged surface of the root cell wall or sequestering within the xylem in the root (Solís-Domínguez et al., 2007). It was reported that vetiver accumulated low concentrations of Cd in roots (Yang et al., 2003). Adversely, according to our present study, vetiver exhibited high rate of Cd accumulation in roots at all treatment levels. It seems that little Cd transported from root to shoot in this study in considering the TF < 0.2. This may be because that most of the Cd in the medium-root interface tends to be immobilized by root exudates in root zone and little Cd were available for the upward transport (Wang et al., 2008). Solís-Domínguez et al. (2007) estimated that normal concentration of Cd in leaf tissue range 0.05–0.2 mg/kg and 5–10 up to 30 mg/kg can be considered excessive or toxic. In our experiment, almost all treatment levels of vetiver showed Cd concentration higher than the normal or phytotoxic levels. These results may indicate that vetiver grass growing on the site contaminated with high level of Cd were tolerant of Cd. Restriction of upward movement from roots into shoots can be considered as one of the tolerance mechanism.

A prerequisite for hyperaccumulators is ability to efficiently tolerate high concentrations of metals within the plant tissues and cells. Basically, phytoremediation depends on high concentrations of the metal in plant biomass and production of a relatively large biomass (Zhao et al., 2002). Compared to the standard of Cd hyperaccumulation-100 mg/kg DW of Cd in shoots, vetiver accumulated 93 mg/kg DW of Cd in shoots and very closed to the standard of 100 mg/kg DW. Owning to its high tolerance to heavy metals and high biomass production, vetiver grass has a potential commercial and large scale to remediation and treatment of contaminated sites by Cd. Chen et al. (2000) found that the total above ground uptake of Cd by vetiver was even greater than that of the hyperaccumulator *Tlaspi caerulescens* owning to the former's high biomass.

### 4.2. Chlorophyll content and soluble protein

Photosynthesis, an important process for plant growth and biomass production, is also significantly reduced by increasing Cd concentrations in the growth medium. It is known that interventral chlorosis of leaves is one of the firstly visible symptoms of heavy metal toxicity. However, there was no chlorosis phenomenon in plants treated with Cd in this experiment. In tolerant plants, however, it has been reported that chlorophyll content increases or does not significantly change in response to treatment with heavy metals (Stiborova et al., 1986). In present study, chlorophyll content was higher than control at lower Cd treatments. Thus, we conclude that slightly lower concentrations of Cd could stimulate chlorophyll content in leaves. Similar results were obtained after exposing ramie seedlings to lower Cd concentration (Liu et al., 2007a,b). The chlorophyll content in vetiver and Cd concentration in solution were highly correlated ($R^2 = 0.907$ and 0.919 for Chl *a* and Chl *b*, respectively). Two possible mechanisms of Cd toxicity on photosynthesis have been proposed to explain the decrease in chlorophyll content with higher Cd level treatment. Cd can alter both chlorophyll biosynthesis by inhibiting protochlorophyllide reductase and the photosynthetic electron transport by inhibiting the water-splitting enzyme located at the oxidizing site of photosystem II (Van Assche and Clijsters, 1990).

Soluble protein content in organisms, is an important indicator of reversible and irreversible changes in metabolism, and is known to respond to a wide variety of stressor such as natural and xenobiotic (Singh and Tewari, 2003). The soluble protein content in leaves treated with Cd mainly displayed biphasic responses with increasing Cd concentration while protein content steadily decline ($R^2 = 0.984$) in roots. When vetiver was faced with low-level metal stress, protein content in leaves increased distinctly while decreased ($R^2 = 0.968$) under high Cd level stress. The functionality of protein can be affected by ROS either by oxidation of amino acid side chains or by secondary reactions with aldehydic products of lipid peroxidation (Reinheckel et al., 1998). Therefore, decreased protein content, in the present study, indicated that the Cd-induced oxidative stress appeared obvious in vetiver.

### 4.3. Root activity and water content

Root activity is regarded as a general indicator of root capability for water and nutrients uptake. Application of higher Cd level to vetiver caused root color changes, from white to brownish, which became thinner and also showed strong inhibition of branching and growth. Interestingly, the highest root activity was observed in the low Cd concentration treatment, and new roots emerged. Based on the date concerning root activity provide in this paper, it is concluded that Cd exerts its toxicity in a concentration-dependent manner by inducing a low toxic, hormetic effect at relatively low concentration and a high toxic, inhibitory effect with higher concentrations. With higher Cd concentrations in the solution, root activity decreased progressively ($R^2 = 0.953$) and root hair growth was relatively poor, which is supposed to be a consequence of restricted function of the protective mechanisms in vetiver. Similar results have been observed for ramie (Wang et al., 2008).

To examine the osmotic effect of abiotic stress on plant tissues, the water content or relative water content was frequently measured, and it was observed that plant water status was highly affected by heavy-metal stress (Ahsan et al., 2007). The water content in vetiver decreased gradually with increasing cadmium concentrations in medium, as compared to the control. Decreased in water content indicated a loss of turgor that resulted in limited water availability for cell extension process in vetiver. This decrease could be because of lower water availability under stress condition, or root systems which are not able to compensate for water lost by transpiration through a reduction of the absorbing surface (Gadallah, 2000). This result indicated that the excess level of Cd has a toxic and an osmotic effect on plants.

### 4.4. MDA and antioxidative stress

Oxidative stress often results in a radical chain reaction that degrades membrane lipids by peroxidation. It has been suggested that lipid peroxidation is a sensitive measure of oxidative damage and thus useful as a biomarker for oxidative stress (Polle et al., 1997). The primary lipid peroxidation by-product, malondialdehyde (MDA), was used for estimating the level of lipid peroxidation, and high accumulation of MDA often indicates severe lipid peroxidation. Various studies showed that the application of heavy metals will increase the MDA content in plan tissues. In a study of rice, Shah et al. (2001) have found that after 20 days of Cd exposure, the MDA content in *Ratna* increases from 96.78 nmol/g FW (control) to 135.48 nmol/g FW (500 μM Cd). Even in hyperaccumulators, such as *Pteris vittata*, the MDA content increases with increasing level of As concentration (Singh et al., 2006). In our experiment, MDA contents increased significantly in roots, which were significantly and positively correlated with Cd concentration in solution ($R^2 = 0.973$). This suggests that Cd leads to production of superoxide radicals, resulting in increased lipid peroxidative products and oxidative stress in vetiver. Increase of MDA content with an increase of Cd concentration in solution, suggests that higher Cd exposure level leads to more ROS in vetiver. ROS can rapidly attack all types of biomolecules such as nucleic acids, proteins and amino acids, leading to irreparable metabolic dysfunction and cell death. Therefore, the induction of antioxidant enzymes such as CAT and POD is an important protective mechanism to minimize oxidative damage in polluted environments (Luna et al., 1994). CAT belongs to most important enzymes scavenging the active oxygen species in plant cells. CAT participates in the main defense system against accumulation and toxicity of hydrogen peroxide and can play the role in controlling $H_2O_2$ level in cells. It acts on $H_2O_2$ and converts it to water and oxygen. It has been suggested that superior antioxidative defense, particularly CAT activity, may play an important role in the Cd-hyperaccumulator *T. caerulescens* (Boominathan and Doran, 2003). In our results showed that Cd-induced increasing activity of CAT in vetiver indicated that vetiver has a great ability to cope with oxidative stress caused by Cd.

POD is widely distributed in the plant kingdom and is one of the principle enzymes involved in the elimination of active oxygen species (AOS). POD can catalyze $H_2O_2$-dependent oxidation of substrates, is involved in removing oxygen radicals formed in plant tissues due to exposure to chemicals or heavy metals in soils and can thus take part in improving mechanical protection in plant tissues. In tolerant plant species, POD activities were found to be higher, enabling plants to protect themselves against the oxidative stress (Scalet et al., 1995). Our results showed that vetiver was able to maintain high levels of POD activity at higher concentrations of heavy metals. Moreover, POD participating in lignin biosynthesis can build up a physical barrier against toxic heavy metals. This also indicates that vetiver can efficiently avoid damage from heavy metals. In conclusion, higher concentrations of Cd caused oxidative damage as evidenced by increased lipid peroxidation, and decreased chlorophyll and protein contents. However, to cope with Cd toxicity, the vetiver grass is able to protect against Cd involving activation of various enzymatic antioxidants serve as important components of antioxidant defense mechanism.

### 4.5. Cadmium uptake kinetics

From a mechanistic point of view to interpret the kinetic experimental data, the prediction of the rate-limiting step is an important factor to be considered in the uptake process (Vadivelan and Kumar, 2005). Kinetic model adequately describes the bioaccumulation of Cd for vetiver plants over a wide range of exposure concentrations from 0.05 to 30 mg/L (Fig. 6). The concentration dependency of the cadmium uptake rates for vetiver plants was well-explained by a Michaelis–Menten-type uptake model. This model gives a mechanistic description of the uptake process characterized by a transport system with a maximum rate of transport ($V_{max}$) and a half-saturation constant $K_m$. A lower $K_m$, which indicates a measure of the affinity of the transporter for the metal species, indicates a higher affinity of this transport system (Redeker and Blust, 2004). To the best of our knowledge, no previous information about the kinetic parameters of Cd uptake in vetiver has been reported. Liu et al. (2007a,b) described the Cd and As uptake pattern of the wheat seedlings. $K_m$ and $V_{max}$ values obtained for Cd in vetiver are significantly higher than those described for Cd in wheat.

## 5. Conclusion

Exposing vetiver grass to Cd concentrations from 1 to 7.5 mg/L did not cause obvious toxic symptoms. A low level of Cd even slightly stimulated the growth of vetiver and concomitantly induced the level of chlorophyll and root activity in plants. Antioxidant enzyme activity was significantly induced with increasing Cd concentration in medium. Changes in biochemical parameters would occur before any visible symptom of toxicity appears, and the endpoint based on these parameters might more sensitive or indicative than morphological observation in revealing eco-toxicity of Cd. Our results showed that vetiver tolerated high concentrations of Cd and have an unusual ability to take up heavy metals from treatment medium. In view of their fast growth, high biomass, and adequate Cd tolerant system, vetiver appear to have great potential for remediation purposes. Hydroponic experiments can be criticized because they represent much simpler conditions than plants experience under field conditions. Future research is needed to analyze the potential of vetiver to accumulate Cd from contaminated soils in non-laboratory environments. Furthermore,

a better insight into the mechanistic details of Cd detoxification in vetiver may lead to engineering of these plants to enhance their Cd phytoremediation capacity.

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