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Bioaccumulation Patterns and Responses of Fleece-flower; *Persicaria thunbergii* to Cadmium and Lead

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ABSTRACT: Application of phytoremediation in the polluted area to remove undesirable materials is a complex and difficult subject without detailed investigation and experimentation. We investigated the accumulation patterns of cadmium and lead in plants naturally grown, the bioavailability of plants to accumulate these toxic metals and the responses of *P. thunbergii* to cadmium and lead. The soil samples contained detectable lead (<17.5μg/g), whereas cadmium was not detected in the soils of study area. The whole body of *Persicaria thunbergii* contained detectable lead (<320.8μg/g) but cadmium was detected only in the stem (<7.4μg/g) and root (<10.4μg/g) of *P. thunbergii*. Cadmium was not detected in *Trapa japonica* and *Nymphoides peltata*, whereas lead was detected in *T. japonica* (<323.7μg/g) and *N. peltata* (<177.5μg/g). Correlation coefficient between lead content in soil and in these plant samples represented positive correlation. The total content of lead in each plant sample increased in the order of *N. peltata* $\leq$*P. thunbergii*<*T. japonica*. For the confirmation of bioaccumulation and response of plant to heavy metals, we selected *P. thunbergii* out of tested plant species based on field data, and *P. thunbergii* was exposed to cadmium and lead. The analysis of cadmium and lead in *P. thunbergii* and pH measurement in the culture pot of *P. thunbergii* were accomplished during the experiment. The contents of heavy metals in *P. thunbergii* treated with 10mM metals were highly detected in more quantity than those in *P. thunbergii* treated with 5mM metals as follows: 1.7~5.6 times in cadmium and 1.3~4.3 times in lead, respectably. The contents of lead and cadmium in *P. thunbergii* tended to increase, whereas the pH in cultured pot contained cadmium and lead tended to decrease throughout the experimental period. In the leaf of *P. thunbergii* exposed cadmium and lead, copper/zinc-superoxide dismutase (Cu/Zn-SOD) activity was elevated and catalase (CAT) activity was reduced in the presence of cadmium. Our results provided useful information for the selection of plants suitable for phytoremediation of metal-contaminated soils.

Key words: Antioxidant enzyme, Heavy metal accumulation, Heavy metal stress, Phytoremediation

INTRODUCTION

Accumulation of heavy metals through the root systems of plants and subsequent release of metals during decomposition of plant materials represent a pathway for recycling of heavy metals in the ecosystem. Such a pathway could have an important effect on the level of toxic metals in surface soil and water. The presence of cadmium and lead in the ecosystem has increased in some areas to levels that threaten the health of aquatic and terrestrial organisms. The mode of accumulation of heavy metals by a variety of plant species and the effects of heavy metals on plants have been studied as a subject of concern among a number of investigators (Jackson *et al.* 1990, Somashekaraish *et al.* 1992, Luo and Rimmer 1995, Chaoui *et al.* 1997, Joner and Leyval 2001, Carmak *et al.* 1996, Hartley-Whitaker *et al.* 2001, Romero-Puertas *et al.* 2002, Ward and Savage 1994 and Fytianos *et al.* 2001). The majority of these studies involve the growth inhibition and even plant death, the relationship between the partitioning of heavy metals in lake sediment and their availability to the yellow water lily, the inhibition of chlorophyll biosynthesis, the correlation between the content of heavy metals in the soil and the amount absorbed by bush beans, an adverse effect on the synthesis and functioning of many biologically important compounds such ad enzymes, vitamins and hormones, the uptake patterns of heavy metals, the variation of antioxidative enzymes caused by heavy metal stress in plant cell and intact plant and heavy metal concentration in vegetable grown in uncontaminated or slightly contaminated area. Only recently, there has been considerable interest in the use of terrestrial plants as a green technology for the remediation of surface soil and water contaminated with toxic heavy metals, and the value of heavy metal accumulating plants for environ-

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mental remediation has been fully realized. This technology is termed phytoextraction (Nanda Kumar *et al.* 1995, Blaylock *et al.* 1996). This study aims to illuminate the relationship between the content of heavy metals accumulated by three plants (*Persicaria thunbergii, Trapa japonica* and *Nymphoides peltata*), naturally grown along riversides, and heavy metal content in the surface soil, to assess the bioavailability of heavy metals in *P. thunbergii*, and to determine the activity of antioxidant enzymes in *P. thunbergii* exposed to heavy metals, thus allowing assessment of the potential use of these plant for phytoextraction.

MATERIALS AND METHODS

Study area and sample collection

The Mankyung Rriver water system, one of the largest water systems in Korea, is located in the west of Korea, and includes Jeonju (26.3km), Samchon (10.4km), Bongdong (11.4km), Soyang (7.5km) streams, and the Mankyung (25.3km) River (Fig. 1). We selected this water system because there are various possible heavy metal contamination sources on one side and agricultural area on the other side of this water system, and many plant species showed various life-forms, namely, emergent, floating, floating leaved and submerged plants, are grown in this water system. We selected the total seven sampling stations, and each sampling station was consisted of three sampling sties. Plant samples, *Persicaria thunbergii, Trapa japonica* and *Nymphoides peltata*, were collected with the whole plant being carefully uprooted, and a sample of surface soil (after removal of the top 3cm) was collected from each plant' s habitat. The plant and soil samples were collected from each sampling station in every week from June to October 1994. Collected samples were immediately frozen with dry ice and transferred to the laboratory for heavy metal analysis. For a laboratory exposure experiment, plant samples of *P. thunbergii* were collected at Sanggwan myun (SM) streamsides, a relatively pristine area in this water system (Fig. 1), and acclimated in modified Hoagland' s solution for 2 weeks, before beginning the bioaccumulation experiment.

Analysis of heavy metals in plant and soil samples

Plant samples were dried at 180°C for 12 hours and ground with a mortar and pestle. The plant samples, 2g in dry weight, were decomposed by digestion with 20 ml nitric acid and 10 ml hydrogen peroxide, and impurities were filtered off. Soil samples were dried at 70°C for 24 hours and passed through a 600μm nylon sieve. Ten grams of dry soil were digested with 20 ml nitric acid, 10 ml hydrogen peroxide, 5 ml hydrochloric acid and 5 ml sulfuric acid, and impurities were removed by filtration. The final volume of each sample was made up to 50 ml with deionized water, and analyzed by a Trace Element Analyzer 3000 (TEA 3000) using the stripping voltametric method (Florence *et al.* 1987).

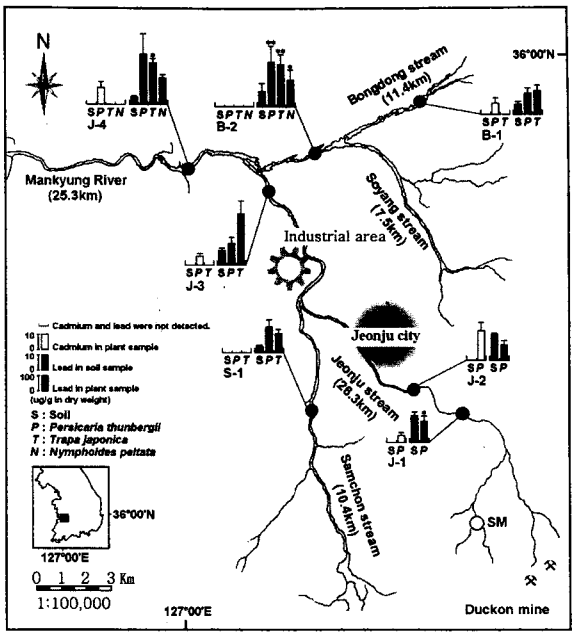

Fig. 1. Location of sampling stations (seven station; each station consists of three sites) within the Mankyung River water system, and the bar indicates lead content in surface soil and plant sample. J-, Jeonju stream; B-, Bongdong stream; S-, Samchon stream; SM, Sanggwan myun; * $p \leq 0.05$; ** $p \leq 0.01$.

Laboratory exposure experiment

To assess the bioaccumulation of heavy metals, *P. thunbergii* was grown using hydroponics in modified Hoagland' s solution [macronutrients: 5mM KNO_2; 5mM $Ca(NO_3)_2 \cdot 4H_2O$; 2mM $MgSO_4 \cdot 7H_2O$; 1mM KH_2PO_4; micronutrients: 2.4μM $MnCl_2 \cdot 2H_2O$; 9μM H_3BO_3; 1.5μM KI; 1.2μM $Na_2MnO_4 \cdot H_2O$; 13μM $Fe_2(C_4H_4O_6)_3$; pH 6.50] containing individual metals where cadmium [$Cd(NO_3)_2$] and lead [$Pb(NO_3)_2$] were added at nominal concentration of 5 and 10mM. Plants were also exposed to mixture of metals as follows: cadmium 5mM + lead 5mM and cadmium 10mM + lead 10mM. *P. thunbergii* was grown in a growth chamber (temperature, 25~30°C; relative humidity, 60~70%; illumination, 1,200~1,300μmol $\cdot m^{-2} \cdot s^{-1}$; daylight, 15 hours light/9 hours dark) for 6 days. Heavy metal content in *P. thunbergii* after metal treatment was recorded at intervals of 24 hours, and the variation of pH in a culture solution was measured at intervals of 12 hours by a BenchTop pH/ISE meter (ATI Orion).

Determination of antioxidant enzymes in the leaf of *P. thunbergii*

After *P. thunbergii* was exposed to 5mM cadmium and 5mM lead for 6 days, five grams of the fresh leaf of *P. thunbergii* were pulverized with a mortar and pestle using liquid nitrogen and then

homogenized in 5 ml extraction buffer (pH 7.8) composed of 50mM potassium phosphate, 0.1mM EDTA and homogenizing glass beads. The homogenate was centrifuged at 12,000 G for 20 min at 4°C, after which the supernatant was transferred to a new tube and kept at -20°C. This crude protein preparation was used for enzyme assays, and protein concentration was determined according to the method of Bradford (1976) using BioRad Bradford reagent and bovine serum albumin as a standard. Superoxide dismutase (SOD, EC 1.15.1.1) activity assay was based on the method of Beauchamp and Fridovich (1971), which measures the inhibition in the photochemical reduction of nitroblue tetrazolium (NBT) at 560 nm. Catalase (CAT, EC 1.11.1.6) activity assay was based on the method of Havir and McHale (1987), which measures the rate of decrease in absorbance of hydrogen peroxide (H_2O_2) at 240 nm. Each measurement was carried out using UV-spectrophotometer (Spectronic GENESYS 5) in triplicate. SOD and CAT activity on 10% nondenaturating polyacrylamide gels was visualized by using the slightly modified procedures of Laemmli (1970). SOD activity staining of the gel was performed by the method of Rao *et al.* (1996). The gels were stained by incubation in a solution containing 2.5mM NBT for 30 min, followed by incubation in 50mM potassium phosphate buffer (pH 7.8) containing 28μM riboflavin and 28mM TEMED for 20 min in the dark. The gels were then placed in distilled water and exposed on 40W fluorescent bulb for 50 min at room temperature. In some experiment, the gels were incubated in 50mM potassium phosphate buffer (pH 7.0) containing 3mM KCN or 5mM H_2O_2 for 30 min prior to the staining of SOD activity to visualize KCN- and H_2O_2-sensitivie isoforms (Rao *et al.* 1996). CAT activity staining of the gel was performed by the procedure of Anderson *et al.* (1995). The gels were soaked in 3.27mM H_2O_2 for 25 min, rinsed twice in distilled water, and stained in a freshly prepared solution consisting of 1% (w/v) potassium ferricynide and 1% (w/v) ferric chloride. Each staining reaction for SOD and CAT activities was stopped with 7.5% glacial acetic acid.

RESULTS AND DISCUSSOIN

Heavy metal content in surface soil

All soil samples from the Mankyung River water system had detectable lead (Pb^{2+}, 4.1 ±0.62 to 17.5 ±1.81μg/g), whereas cadmium (Cd^{2+}) was not detected in this region (Fig. 1). The lead contents in the surface soil of J-1, J-2, J-3 and J-4 along the Jeonju stream were 17.5 ±1.81μg/g, 16.4 ±0.95μg/g, 9.4 ±1.21μg/g and 4.7 ±0.59μg/g, respectively. These lead contents in the Jeonju stream were higher levels than that in the Bongdong stream (B-1, 6.5 ±1.64μg/g; B-2, 9.8 ±4.88μg/g) and the Samchon stream (S-1, 4.1 ±0.62μg/g). Therefore, these results indicate that the high levels of lead contents at the station J-1, J-2 and J-3 along the Jeonju stream are closely connected with the Duckon mine located above the Jeonju stream, and the primary source of lead in the Jeonju stream is thought to be effluent leached from this mine. These surface dispersion patterns of lead in the Jeonju stream are similar to the results in other mining area (Gratton *et al.* 2000).

Heavy metal content in plant samples

Whole plants of *P. thunbergii* throughout the Mankyung River water system contained detectable lead (<320.8μg/g), whereas cadmium was detected only in the stem (<7.4μg/g) and root (<10.4μg/g) of *P. thunbergii*. Cadmium contents varied appreciably among *P. thunbergii* samples collected at specific sampling stations, that is, J-1, J-2, J-3, J-4 and B-1. While cadmium was not detected in *T. japonica* and *N. peltata*, lead was detected in *T. japonica* (<323.7μg/g) and *N. peltata* (<177.5μg/g). The overall range of cadmium and lead contents in *P. thunbergii* was from 4.0 to 17.5μg/g (mean ±SD, 8.5 ±5.40μg/g) and 101.6 to 320.8 μg/g (183.3 ±84.38μg/g). The overall range of lead contents in *T. japonica* and *N. peltata* was from 118.1 to 323.7μg/g (218.5 ± 53.03μg/g) and 168.1 to 177.5μg/g (172.8 ±39.91μg/g), respectively. The total content of lead in each plant sample increased in the order of *N. peltata*< *P. thunbergii* ≤*T. japonica* (Fig. 2). These results are similar to the bioaccumulation patterns of heavy metals within various plant species, including *Agrostis gigantea, Phaseolus vulgaris, Lolium perenne, Lolium muliflorum* and *Triticum aestivum* (Hogen and Rauser 1981, Hardman *et al.* 1984, Kovacs 1992 and Nan *et al.* 1999). Correlation coefficients between lead content in surface soil and lead content accumulated by plant samples were calculated from the field data as follows: *P. thunbergii*; 0.584 (>t12, 0.05) at J-1 and 0.828 (>t12, 0.01) at B-2, *T. japonica*; 0.615 (>t12, 0.05) at J-4 and 0.737 (>t12, 0.01) at B-2, and *N. peltata*; 0.734 (>t12, 0.05) at B-2 (Fig. 1). Samecka-Cymerman and Kemper (1999) proved that the heavy metal contents in *Mimulus gutattus* were strongly correlated with heavy metal contents in sediment and Herawati *et al.*

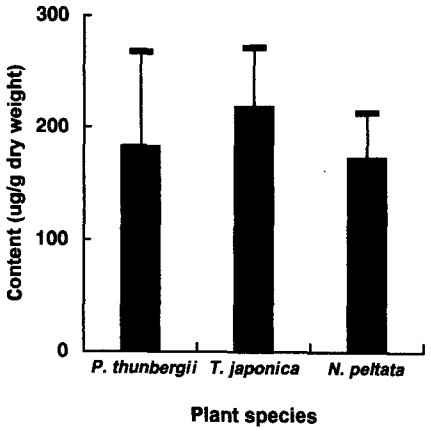

Fig. 2. The comparison of lead contents in each plant species.

(2000) reported a significant positive correlation between cadmium and zinc contents in rice and these metal contents in different type soil, and therefore we concluded that the relationship between lead contents in the Mankyung River water system and lead contents in plant samples naturally grown in this region was statistically positive correlation.

Heavy metal contents in *P. thunbergii* after metal treatment

To compare cadmium and lead contents in *P. thunbergii* exposed to these metals under laboratory experiment, *P. thunbergii* were selected for uniformity from Sanggwan myun (Fig. 1) streamsides, and were hydroponically grown in modified Hagland' s solution containing cadmium and lead for 6 days. Heavy metal content was recorded at intervals of 24 hours. *P. thunbergii* did not show visible phytotoxicity for the duration of the experiment. The variations in cadmium or lead contents in the *P. thunbergii* are shown in Table 1. The amount of lead accumulated in *P. thunbergii* was more than that of cadmium. This result similar to the bioaccumulation pattern of cadmium and lead in *P. thunbergii* collected from the Mankyung River water system. Cadmium and lead contents in *P. thunbergii* tended to increase with increase in the initial concentration in the culture solutions and with the passage time. The total amount of cadmium and lead accumulated during the multiple exposure experiment increases as cadmium and lead contents in the culture solution increase. The cadmium content in *P. thunbergii* exposed to cadmium and lead simultaneously tended to be lower than that of treated with cadmium alone. Similar results have been reported earlier by O' keeffe *et al.* (1984), Jain *et al.* (1990) and Nanda Kumar *et al.* (1995). However the lead content in *P. thunbergii* exposed to the mixture of 5mM lead and cadmium tended to be higher than that of treated with 5mM lead alone (Fig. 3). This result may indicate that the influence due to the presence of other ions and the physiological and biochemical mechanisms involved in root to shoot transport of lead in *P. thunbergii*. In present study, compare the contents of cadmium and lead accumulated by *P. thunbergii*, naturally grown, with the contents of these metals accumulated by *P. thunbergii* exposed to these metals, all

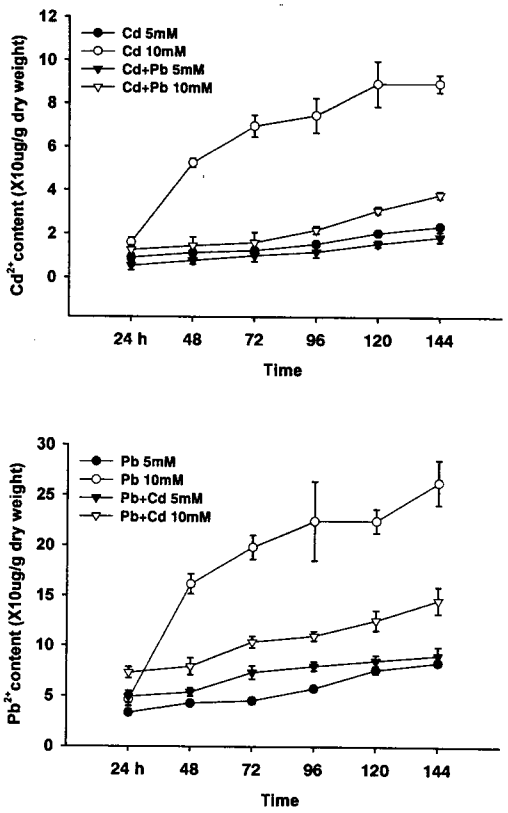

Fig. 3. Time course relationships of Cd^{2+} and Pb^{2+} contents in *P. thunbergii* exposed to cadmium and lead for 6 days.

the data from field and laboratory investigation showed that the amount of lead was higher than that of cadmium in *P. thunbergii*. Moreover, from the field investigation, the bioconcentration factor (BCF) of *P. thunbergii* for lead was 4.2 in the aboveground (leaf and stem) and 22.2 in the underground (root), and from the laboratory experimentation, the bioaccumulation coefficients of *P. thunbergii* for cadmium and lead were 2.0 (cadmium) and 3.2 (lead), respectively. These results illustrate that discrepancy between the ratio of absorbed metal content and the absorbancy

Table 1. Heavy metal contents in *P. thunbergii* grown using hydroponics in modified Hoagland' s solution contained 5 and 10mM cadmium, 5 and 10mM lead, 5mM cadmium + 5mM lead, and 10mM cadmium + 10mM lead for 6 days. The values are mean(standard deviation (n=3)

(Unit: μg/g dry weight)

Assay metal	Exposure condition	24 hours	48 hours	72 hours	96 hours	120 hours	144 hours
Cd^{2+}	Cd^{2+} 5mM	9.1±0.90	11.4±1.29	12.4± 0.92	15.4± 1.15	20.3± 0.75	23.4± 0.78
	Cd^{2+} 10mM	16.0±2.05	52.5±2.05	69.5± 4.96	74.3± 7.87	87.7±10.33	87.7± 4.03
	Cd^{2+} +Pb^{2+} 5mM	6.0±1.64	7.8±1.48	10.1± 2.67	11.8± 2.25	15.5± 2.39	18.4± 2.03
	Cd^{2+} +Pb^{2+}10mM	12.6±1.84	14.5±3.64	16.0± 4.30	21.8± 1.56	30.8± 2.01	37.8± 1.49
Pb^{2+}	Pb^{2+} 5mM	33.6±3.13	43.0±2.05	45.6± 3.40	57.8± 2.40	76.2± 3.79	83.5± 2.78
	Pb^{2+} 10mM	46.5±6.61	162.0±9.92	198.6±11.97	224.8±39.29	225.0±11.69	263.1±22.27
	Pb^{2+}+Cd^{2+} 5mM	49.3±6.13	54.0±4.06	73.6± 6.63	79.9± 4.58	85.4± 5.41	90.6± 8.74
	Pb^{2+}+Cd^{2+}10mM	73.2±5.61	79.5±8.52	104.1± 5.93	110.2± 4.86	125.9±10.22	146.0±13.29

of heavy metal in plant is because of the various concentration of heavy metal treatment, a kind of heavy metal and media, the part of plant and plant species.

pH variation in a *P. thunbergii* culture pot

The pH in a *P. thunbergii* culture solution (pH 6.50) containing cadmium and lead alone was measured at intervals of 12 hours for 6 days. In the case of the addition of cadmium, the pH in a culture solution was from 6.50 to 4.39 and in the addition of lead, from 6.50 to 4.05 with the lapse of time (Fig. 4). Thurman (1981) reported that various organic acids from plants offset as being binding to metal ion the toxicity of heavy metal on the one hand and decrease the pH in soil on the other hand. Sanders *et al.* (1986) reported to be the lower the pH in soil sample, the greater the extractable heavy metal in soil sample, and that the uptake of heavy metal from soil by plants may be influenced by the pH in soil as well as by soil texture and heavy metal addition. In present results, this pH decreasing in a *P. thunbergii* culture pot is explainable because of various organic acids produced from *P. thunbergii* by metallic stress.

Response of antioxidant enzyme activities to heavy metal exposure in *P. thunbergii*

Plants possess a number of antioxidant molecules and enzymes that protect them against oxidative damage. Superoxide dismutase (SOD), the first enzyme in the detoxifying process, converts reactive oxygen species ($O_2^{\cdot -}$) to hydrogen peroxide (H_2O_2) at a vary fast rate, subsequently, catalase (CAT) decomposes hydrogen peroxide to water (H_2O) and oxygen molecule (O_2). The induction of the activities of a particular group of enzymes is considered to play an important role in the cellular defense strategy against oxidative stress caused by toxic metal. *P. thunbergii* displayed increased SOD activity at 5mM concentration of cadmium and lead, whereas CAT activity decreased at 5mM concentration of cadmium (Table 2). The electrophoretic analysis also revealed remarkable difference in SOD and CAT activity in the leaf of *P. thunbergii* exposed to cadmium and lead for 6 days. The activity of SOD was increased in the leaf of *P. thunbergii* after treatment with cadmium and lead supply indicating an increased level of the superoxide radical, whereas the activity of CAT was diminished after cadmium treatment (Fig. 5). Three distinct types of SODs, that is, Cu/Zn-, Mn- and Fe-SOD, have been identified from a wide range organisms. It is known that Cu/Zn-SOD is inactivated by potassium cyanide and by hydrogen peroxide, whereas Fe-SOD is inhibited only by hydrogen peroxide. However, Mn-SOD is unaffected by either inhibitor. The electrophoretic pattern of SODs in the leaf of *P. thunbergii* showed several bands of activity, which were identified as Cu/Zn SODs, since they were inhibited by potassium cyanide and hydrogen peroxide (Fig. 6). These results are similar to the variation of antioxidant enzymes for heavy metals within various plant species, including *Glycine max, Phaseolus vulgaris, Alyssum argenteum, Alyssum maritimum, Quercus robur* and *Pisum sativum* (Cakmak and Horst 1991, Somashekaraish *et al.* 1992, Schickler and Hadar 1999, Racchi *et al.* 2001 and Romero-Puertas *et al.* 2002).

It may be concluded from this study that plants of *P. thunbergii*

Table 2. Activities of superoxide dismutase (SOD) and catalase (CAT) in the leaf of *P. thunbergii* exposed to 5mM concentration of cadmium and lead for 6 days. The values are mean(standard deviation (n=3)

(Unit: U mg^{-1} protein)

Treatment heavy metal	Enzyme activity	
	SOD	CAT
Cntrol (C)	48.9 ± 5.75	39.8 ± 5.45
Cadmium (Cd)	62.1 ± 7.28	9.7 ± 1.21
Lead (Pb)	74.5 ± 5.79	57.3 ± 4.32
Cd/C	127 %	24 %
Pb/C	152 %	144 %

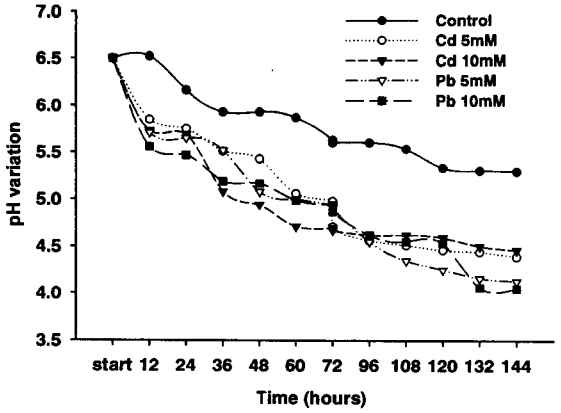

Fig. 4. Time course relationships of the pH variation in *P. thunbergii* culture solution containing cadmium and lead for 6 days.

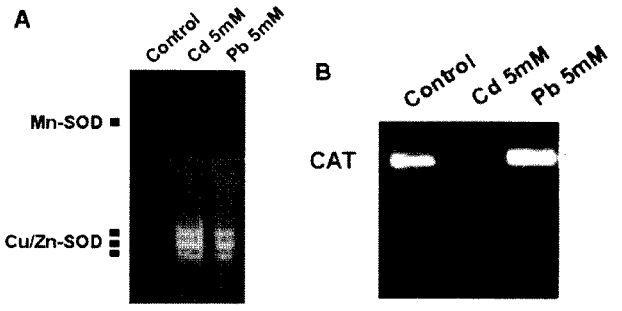

Fig. 5. SOD and CAT protein profiles on native PAGE of the leaf of *P. thunbergii* treated with cadmium and lead. A and B; 40 μg aliquots of crude soluble proteins were loaded onto a 10% acrylamide gel.

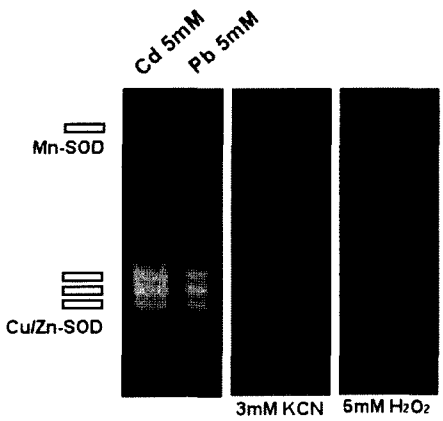

Fig. 6. SOD protein profiles on native PAGE of the leaf of *P. thunbergii* treated with cadmium and lead. The gels were incubated with 3mM potassium cyanide or 5mM hydrogen peroxide for 30 min before staining for SOD activity.

can accumulate heavy metals effectively and can more extract or stabilize heavy metals from soil by the organic acid of itself. The elevation of specific antioxidant enzymes in *P. thunbergii* reveals an activation of defense mechanism for toxic heavy metals. Therefore, we expect that the plants of *P. thunbergii* play an important role in the bioaccumulation and phytoextraction of metal ions in the region contaminated by toxic metals.

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