Journal of Plant Biology, July 2008, 51(4) : 284-290

Effects of Lead on Bioaccumulation Patterns and the Ecophysiological Response in *Monochoria korsakowi*

In Sung Kim[1], Young Hwa Hong[2], Kyung Hong Kang[3], and Eun Ju Lee[1]*
[1]School of Biological Sciences, Seoul National University, Seoul 151-742, Korea
[2]Department of Chemical Engineering, Sungkyunkwan University, Suwon 560-759, Korea
[3]Research Institute of Bioresources, Jeonju University, Jeonju 560-759, Korea

Phytoextraction is a feasible method for eliminating heavy metals from contaminated soil. However, only a few plant species can hyperaccumulate toxic metals. We examined bioaccumulation patterns and the ecophysiological responses (i.e., photosynthetic pigments, total antioxidative capacity, and variations in antioxidant enzymes) in plants of *Monochoria korsakowi* that were exposed to various concentrations of lead. Contents increased significantly when more lead was applied, with most of this metal being found in the roots. Biomass for each organ type decreased with higher lead concentrations. Chlorophyll *a* contents declined in proportion to lead amounts, whereas those of chlorophyll *b* were not significantly diminished. Carotenoid contents were not influenced by such treatment. Total antioxidant capacity in the leaf blades of treated plants increased greatly with higher lead concentrations. We conclude that *M. korsakowi* could be used as a potential phytoremediator of heavy metals in contaminated soils.

Keywords: *antioxidant capacity, bioaccumulation, lead, Monochoria korsakowi, photosynthetic pigment, phytoextraction*

Heavy metals are elements that cannot be degraded by microbial or chemical processes. They tend to accumulate in soils and aquatic sediments. This problem is not restricted to soils with high metal contents, such as in mining areas, but also includes sites with moderate to low contamination. Absorption of toxic metals through root systems and the release of these metals during the decomposition of plant materials represents a recycling pathway in ecosystems that can have an important effect on the level of toxic metals. Many plants grow on contaminated sites; most can effectively adapt to this toxicity through various defense mechanisms, e.g., adsorption of metal ions to cells, enhancement of active efflux, and compartmentation of toxic metals in vacuoles (Tung and Temple, 1996), or through the induction of metal-chelates such as phytochelatins, organic acids, and inorganic complexes (Baker, 1987; Sanitá di Toppi and Gabbrielli, 1999; Hall, 2002). However, toxic metal ions absorbed through the root system can induce oxidative stress and cause the formation of reactive oxygen species, such as superoxide radicals, hydroxyl radicals, and hydrogen peroxide. These can then damage many cellular components including proteins, membrane lipids, nucleic acids, and chlorophyll (Gallego et al., 1996; Rucińska et al., 1999; Manios et al., 2003; Karuppanapandian et al., 2006).

Toxic heavy metals also influence the activity of iron enzymes, such as Fe (III) reductase, in the roots (Alcantara et al., 1994) as well as enzymes involved in photosynthesis and the photosynthetic apparatus (van Assche and Clijsters, 1990; Siedlecka and Krupa, 1996; Ewais, 1997), and antioxidative defense systems (Iannelli et al., 2002). Several wetland plants, e.g., *Iris pseudoacorus* (Samecka-Cymerman and Kempers, 2001), *Typha latifolia* (Manios et al., 2003), *Polygomum thunbergii* (Kim et al., 2003), and *Phragmites australis* (Ait et al., 2004), have been studied for their phytoextraction of toxic metals. *Monochoria korsakowi* Regel et Maack is another wetland species that naturally inhabits swamps and paddy fields. For phytoextraction to be effective, one must identify the quantitative relationships between toxic metal contents in soils or sediments and those in plants, and the physiological responses of plants to toxic metals. Using a long-standing, relevant bio-assay for assessing toxicity, we investigated patterns for the bioaccumulation of lead and the ecophysiological responses of *M. korsakowi* plants exposed to various Pb concentrations.

*Corresponding author; fax +82-2-872-1993
e-mail ejlee@snu.ac.kr

MATERIALS AND MEHODS

Plant Materials

Samples of *Monochoria korsakowi* plants were collected from an abandoned paddy field (Wanju, Korea: 36° 30′ N, 126° 30′ E) and transplanted to a 10 x 10 m artificial wetland. These plants were then cultured hydroponically and their seeds were obtained from ripened capsules. Three seedlings (10 to 15 cm tall) each were transplanted into pots (ϕ150 mm ×H200 mm) containing 3 kg of experiment soil (25% v/v sand, 25% v/v clay, and 50% v/v soil conditioner). All plantlets were acclimated and cultured under a hydroponic system (in 1 to 2 cm of water), being irrigated with modified Hoagland's solution for 2 months before the lead treatments began.

Lead Treatment

Plants were exposed to various lead concentrations in a greenhouse with ambient temperature ranging from 28 ± 3°C (day) to 17±3°C (night). Treatment concentrations were 0, 24.1, 48.3, 96.5, 241.3, or 482.6 μM $Pb(NO_3)_2$ (equivalent to 0, 0.5, 1.0, 2.0, 5.0, or 10.0 mg Pb^{2+} ions), which were applied a total of 10 times in 100 mL of modified Hoagland's solution at 3-d intervals. Three replicates were used for each treatment.

Biomass and Lead Analysis

After 30 d of treatment, plants were uprooted, blotted dry, and separated into organ types (leaves, petioles, capsules, and roots). Biomass (fresh weight) was determined for each portion. To analyze their lead contents, tissue samples were dried at 70°C for 48 h, then pulverized and decomposed via acid digestion. Lead concentrations were determined by an inductively coupled plasma emission spectrophotometer (ICPS-1000 IV, Shimazu). The BCF (bioconcentration factor) was defined in terms of $[Pb_{plant}]/[Pb_{soil}]$ and the TF (translocation factor) as $[Pb_{aerial}]/[Pb_{root}]$.

Photosynthetic Pigment Assay

To extract their photosynthetic pigments at the end of the treatment period, four 8-mm foliar discs (2 cm^2 total surface area) were cut from nourished leaves with a punch. These discs were placed immediately into 5 mL of dimethylsulfoxide (DMSO), and total pigments were extracted for 24 h at 60°C in the dark. The extracts were then centrifuged at 1,500*g* for 20 min and the supernatants were carefully collected. To assay for chlorophyll *a* and *b* and total carotenoid contents, we measured the absorption spectra of each extract at 665, 649, and 480 nm, respectively, using a UV-Vis spectrophotometer (Spectronic GENESIS 5, Milton Roy). Contents were then calculated by the equation of Wellburn (1994).

Total Antioxidant Capacity Assay

Total antioxidant capacity was measured in terms of hydrogen-donating; that is, the ability of all antioxidants in the leaf extract to reduce Cu^{3+} to Cu^{2+} was selected as an index of this capacity. Fresh leaves were pulverized with liquid nitrogen and homogenized in 50 mM potassium phosphate buffer (pH 7.2). The homogenate was centrifuged at 12,000 ×g for 20 min at 4°C, and the supernatant was transferred to a new tube. These aqueous extracts were used in measurements of total protein content and total antioxidant capacity. The former was obtained with a commercial protein assay kit (Bio-Rad Laboratories, Inc. # 500-0001; Bradford, 1976) while the latter was determined with a commercial colorimetric microplate assay kit (Oxford Biochemical Research, Inc. # TA 01; Oxford Biochemical Research, 2001). Total protein and antioxidant contents were calculated by extrapolation on a standard curve developed from known concentrations of bovine serum albumin (BSA) and glutathione (GSH), respectively.

Determination of SOD and CAT Activities

To assay SOD and CAT activities in plants exposed to various lead concentrations, 700 mg of leaves was ground in liquid nitrogen, then homogenized in 3 mL of cold 20 mM Tris buffer (pH 7.0). After the homogenate was centrifuged at 12,000 ×g for 30 min at 4°C, total protein content in the supernatant was determined at 595 nm by the dye-binding method (Bradford, 1976), using the Bio-Rad protein assay dye reagent with bovine serum albumin as a standard. In each extract, total SOD activity was determined with a SOD assay kit (Dojindo Molecular Technologies, Inc., Product Code S311) as described by Ukeda et al. (1999), whereas CAT activity was assayed according to the method of Kraus et al. (1995) and Azevedo et al. (1998), with some modifications. CAT activity was determined spectrophotometrically at 25°C in a reaction mixture that comprised 980 μL of 50 mM potassium phosphate buffer (pH 7.5) containing 500 μL of 50 mM hydrogen peroxide solution prepared immediately before use. The reaction was initiated by the addition of 20 μL of plant extract, and activity was determined by following the decomposition of hydrogen peroxide based on the change in absorbance at 240 nm for 2 min against a hydrogen peroxide-free mixture.

Polyacrylamide Gel Electrophoresis

Electrophoresis was carried out under non-denaturing conditions in 10% polyacrylamide (SOD) and 7.5% polyacrylamide (CAT), with a 4% stacking gel. Electrophoretic separation was performed at 4°C in a refrigerated vertical mini-slab gel cell (SE 450; Hoefer, USA). A constant current of 20 mA per plate in the stacking gel and 40 mA per plate in the separating gel was applied for 2 to 3 h. Electrophoresis buffers and gels were prepared as described by Laemmli (1970), except that SDS was excluded. Equal amounts of total protein (plant extract) were loaded onto the gels, using 20 μg per lane for SOD and 10 μg per lane for CAT.

SOD and CAT Staining

To stain total SOD isoforms, the non-denaturing PAGE slab-gel was rinsed in distilled deionized water, and incubated in 2.5 mM nitroblue tetrazolium solution for 30 min. The slab-gel was washed again with distilled deionized water, then incubated in the dark for 30 min at room temperature in a reaction mixture containing 50 mM potassium phosphate buffer (pH 7.0), 28 μM riboflavin, and 28 mM tetramethyl ethylene diamine. Afterward, the reaction mixture was poured off and the slab-gel was rinsed with distilled deionized water before being illuminated at 200 μmol m^{-2} s^{-1} in water until colorless bands of SOD isoforms appeared on the purple-stained slab-gel (Rao et al. 1996). This development of CAT isoforms was performed according to the procedure of Anderson et al. (1995). To activate CAT isoforms, the slab-gel was soaked in 3.27 mM hydrogen peroxide for 30 min, and rinsed with distilled deionized water. The activated slab-gel was then stained in a freshly prepared solution containing 1% ferric chloride and 1% potassium ferricyanide. After the CAT isoforms were identified, the slab-gel was washed with water and transferred to 7.5% acetic acid to arrest the staining process.

Statistical Analysis

We used the SAS statistics software package (SAS 9.1.3). All data were expressed as means ± standard errors (SE) of three independent replicates, and were based on two-tailed *t* tests. Significant differences were established with *P* values 0.05. An index of the association of two quantitative variables was expressed by a Pearson product-moment correlation coefficient (*r*).

Table 1. Lead contents in organs from *Monochoria korsakowi* plants exposed to lead for 30 d, and the positive correlation coefficient (*r*) values ($P<0.01$; $n=6$) between lead contents in plant samples and treatment concentrations. Values are means ± SE (μg Pb^{2+} g^{-1} dry weight; $n=3$). ND: not detected.

Organ (Correlation coefficient)	Lead treatment (mg Pb^{2+} per pot)					
	0	5	10	20	50	100
Capsule ($r=0.9939$)	ND	1.1± 0.66	2.8± 0.84	3.7± 0.52	14.0± 1.11	34.0± 1.04
Leaf blade ($r=0.9318$)	11.9± 2.35	29.9± 5.92	21.1± 1.05	54.9± 1.75	51.9± 8.79	92.6± 4.08
Petiole ($r=0.9924$)	5.4± 1.82	20.8± 1.31	52.5± 4.48	94.2± 2.89	309.3± 7.37	498.4±15.56
Root ($r=0.9915$)	14.0± 1.53	83.4± 2.41	103.7± 6.04	140.6± 3.33	556.1±12.61	1372.5±47.03
Whole plant ($r=0.9973$)	31.3± 5.70	135.2±10.30	180.1±12.41	293.4± 8.49	931.3±29.88	1997.5±67.71

RESULTS

Lead Accumulation and Relative Growth Rates

Accumulation patterns in *Monochoria korsakowi* plants treated with lead for 30 d are summarized in Table 1. In each organ type, lead contents were significantly increased as concentration rose, with the highest levels being found in the roots. This pattern occurred in the sequence of capsule < petiole < blade < root for treatments with 0 or 5 mg Pb^{2+}, and capsule < blade < petiole < root for 10, 20, 50, or 100 mg Pb^{2+}. The correlation between treatment concentration and lead contents in each organ or in the whole plant was strongly positive (Table 1). However, a significantly negative correlation was found between lead content and whole-plant biomass ($r= -0.8615$, $P<0.05$; $n=6$). The relative growth rate, based on organ biomasses, also was reduced as treatment concentration increased, especially in the capsules (Fig. 1). The bioconcentration factor value was highest (81.1) when plants were treated with 5 mg of Pb^{2+}, whereas the translocation factor value was greatest (1.1) at a treatment level of 20 mg of Pb^{2+} (Fig. 2).

Effect of Lead on Photosynthetic Pigments

Chlorophyll *a* (Chl *a*) contents in leaf blades of plants exposed to 5 or 10 mg Pb^{2+} were not altered greatly. In contrast, at 20, 50, or 100 mg Pb^{2+}, those contents declined to 66.4%, 68.4%, or 53.8% of the control value, respectively. Interestingly, Chl *b* contents were not significantly diminished, and carotenoid contents were not influenced by the lead (Fig. 3). However, correlations were significantly negative between blade lead levels and total photosynthetic pigment contents (i.e., Chl *a*: $r=-0.9597$, $P<0.05$, $n=6$; Chl *b*: $r=-0.8929$, $P<0.05$, $n=6$). The ratio Chl *a*/Chl *b* was significantly diminished by an increase in lead concentration, with values of 2.36 (0 mg Pb^{2+}), 2.01 (5 mg Pb^{2+}), 1.88 (10 mg Pb^{2+}), 1.83 (20 mg Pb^{2+}), 1.73 (50 mg Pb^{2+}), and 1.54 (100 mg Pb^{2+}).

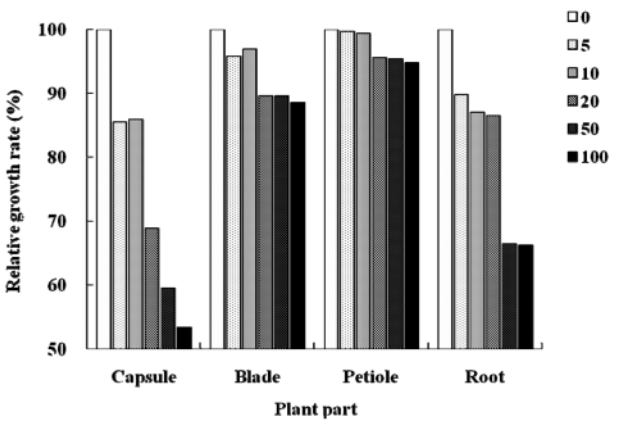

Figure 1. Relative growth rate based on biomass of each organ type from *Monochoria korsakowi* plants exposed for 30 d to lead (5 to 100 mg Pb^{2+}).

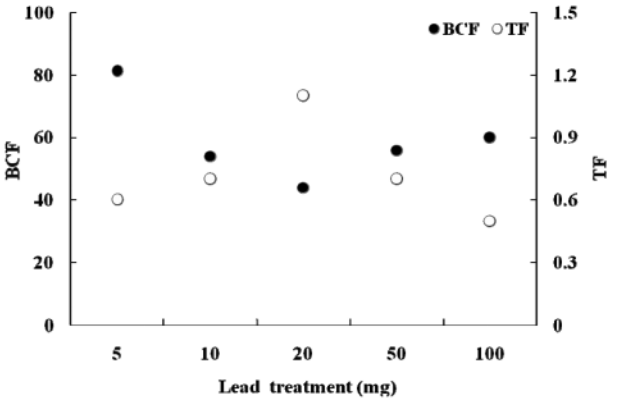

Figure 2. Bioconcentration factor (BCF) and translocation factor (TF) values for *Monochoria korsakowi* exposed for 30 d to 5 to 100 mg Pb^{2+}.

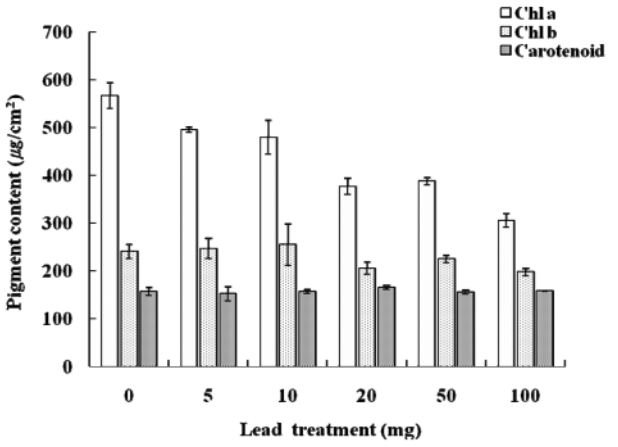

Figure 3. Variations in pigment contents from leaf blades of *Monochoria korsakowi* plants exposed for 30 d to lead (5 to 100 mg Pb^{2+}). Vertical bars indicate standard errors.

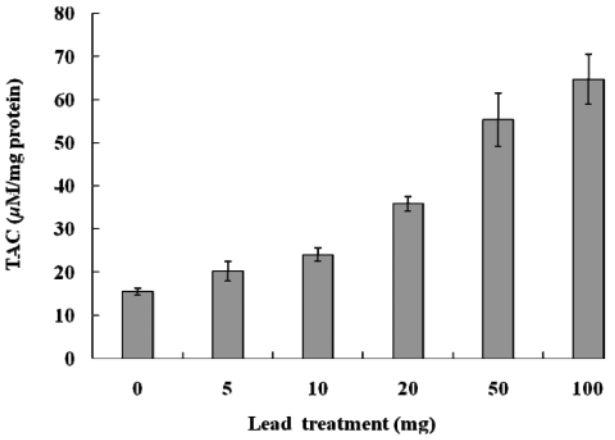

Figure 4. Variations in total antioxidative capacity (TAC) from leaf blades of *Monochoria korsakowi* plants grown for 30 d in lead-treated soil (5 to 100 mg Pb^{2+}). Vertical bars indicate standard errors.

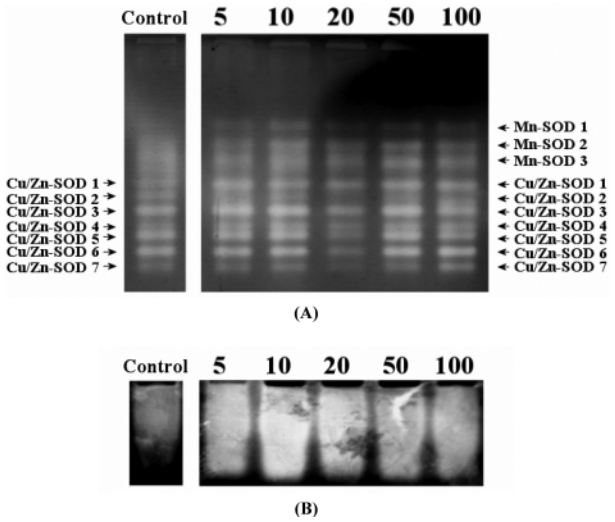

Figure 5. Native PAGE of SOD (A) and CAT (B) from *Monochoria korsakowi* plants grown for 30 d in lead-treated soil (5 to 100 mg Pb^{2+}). Numbers on top of each slab-gel represent total amounts of lead applied.

Effect of Lead on Total Antioxidant Capacity

Total antioxidant capacity in the blades of plants exposed to 5, 10, 20, 50, or 100 mg Pb^{2+} was 130%, 155%, 231%, 357%, or 417% higher, respectively, than that of the control (Fig. 4). We also noted a significant positive correlation between total lead contents in blades and total antioxidant capacity (r=0.9191, P<0.01; n=6).

Effect of Lead on SOD and CAT

Compared with the control plants, lead exposure distinctly enhanced total SOD and CAT activities (Table 2). For example, when foliar extracts were separated on native PAGE and monitored for SOD activity, only seven Cu/Zn-SOD isoforms were observed in the controls versus seven Cu/Zn-SOD isoforms and four Mn-SOD isoforms in the treated plants (Fig. 5A). Lead also directly heightened the intensity of the existing SOD isoforms, and caused the synthesis of new ones, i.e., Mn-SOD isoforms. Slab-gels stained for CAT activity revealed an enhancive CAT band in all lead treatments (Fig. 5B). A significant positive correlation was also observed between blade lead content and CAT activity (r=0.9086, P<0.05; n=6). However, the relationship between SOD activity enhancement and the increase in blade lead content showed no statistical correlation (r=0.7797, P<0.07; n=6).

DISCUSSION

Plants with a high capacity for metal accumulation may be used for phytoextraction in order to reduce the levels of heavy metals in ecosystems. Many wetland species have previously been assessed for their phytoextraction potential. Patterns of bioaccumulation depend upon plant species and the type and concentration of the heavy metal (Ye et al., 1997; Kim et al., 2003; Lim et al. 2006). Therefore, to evaluate the usefulness of *Monochoria korsakowi*, the first step in our study was to quantify its capacity for Pb accumulation when each accession was grown in lead-treated soils. Here, accumulation by each organ type increased in the order of capsule < blade or petiole < root (Table 1). Ye et al. (1997) have reported that cadmium, lead, and zinc accumulate in a natural population of the aquatic macrophyte *Typha latifolia* in the order of leaf < rhizome < root. We previously demonstrated that the underground portions of *Polygonum thunbergii* (Kim et al. 2003) show higher heavy-metal contents than the aboveground organs. The particular bioaccumulation pattern for lead in *M. korsakowi* plants is explained by the translocation of heavy metals from below ground (i.e., the roots) upward to the petioles, leaf blades, and capsules (Hardiman et al., 1984; Sinicrope et al., 1992). We found a positive correlation between lead treatment concentration and Pb contents in the capsules, blades, petioles, and roots. Field-grown plants of various species also show a significant positive correlation between heavy metal levels in habitat soils and in tissue samples (Samecka-Cymerman and Kempers, 2001); Herawati et al., 2000). In our experiment, the relative growth rate, based on biomass, decreased as the lead treatment concentration was increased (Fig. 1). Interestingly, during the 30-d experiment, plants of *M. korsakowi* survived in soil treated with up to 100 mg Pb^{2+}, and exhibited no visual metal-toxicity symptoms such as necrosis or

Table 2. Variations in SOD and CAT activities in *Monochoria korsakowi* plants exposed to lead for 30 d. Values are means ± SE (unit mg^{-1} protein; n=3).

Antioxidative enzyme	Lead treatment (mg Pb^{2+} per pot)					
	0	5	10	20	50	100
SOD	16.4±0.88	24.6±1.15	26.4±2.60	28.4±1.67	36.4±4.91	33.9±3.61
CAT	53.3±0.33	58.7±0.33	69.1±0.52	116.9±1.51	87.9±0.58	122.4±2.33

whitish-brown chlorosis.

High concentrations of phytotoxic metals may be more harmful to plant tissues than low levels. Toxic metal ions interfere with nitrogen assimilation in cells (Kevresan et al., 1998; Singh et al., 2002), probably by irreversibly substituting other micronutrients in critical enzymes. Those ions also inhibit chlorophyll formation by interfering with protochlorophyllide reduction and the synthesis of aminolevulinic acid (Stobart et al., 1985). Changes in photosynthetic pigments in response to heavy-metal stress not only indicate damage to the photosynthetic apparatus and its capacity, but also have consequences for reduced carbon assimilation, growth, survival, and reproduction (Vangronsveld and Clijsters, 1994). Sharma and Gaur (1995) and Ewais (1997) have proposed that, when evaluating the effect of heavy metals, one should consider the change in Chl *a* and *b* contents, and, especially, the parameter of fluctuations in the ratio of Chl *a*/Chl *b*. Toxic heavy-metal accumulation, responsible for the reduction in total chlorophyll contents, has a similar negative effect on that ratio. In the present study, lead treatment influenced the content of Chl *a* more than Chl *b* (Fig. 3), and the ratio of Chl *a*/Chl *b* was reduced from 2.36 (at 0 mg Pb^{2+}) to 1.54 (at 100 mg Pb^{2+}). Moreover, significantly negative correlations were calculated between leaf-blade lead contents and photosynthetic pigment contents.

Cheng et al. (2002) have reported that, in *Canna indica*, Chl *a* declines with rising cadmium stress, while Chl *b* is unaffected. This might result from a faster hydrolysis rate by the former when plants are under toxic heavy-metal stress (Abdel-Basset et al., 1995). Heavy metals induce oxidative stress by generating reactive oxygen species, e.g., superoxide radicals ($O_2^{\bullet -}$), hydroxyl radicals ($OH^{\bullet}$), and hydrogen peroxide (H_2O_2) in plants, which then protect themselves by stimulating their antioxidative defense systems (Foyer et al., 1994). Those systems involve several enzymes and low-molecular-weight quenchers present in cells, and play an additive role in the detoxification of ROS (Dixit et al., 2001). Our results demonstrated that an increase in blade lead content caused a significant enhancement of total antioxidant capacity (Fig. 4).

Generally, SOD converts $O_2^{\bullet -}$ into H_2O_2 and O_2, while CAT decomposes hydrogen peroxide into water and oxygen. (Bowler et al. 1994). In leaf blades of plants exposed to 5 to 100 mg Pb^{2+}, total SOD and CAT activities were apparently enhanced (Table 2; Fig. 5). Rucińska et al. (1999) have reported that this increasing pattern for the full SOD isozyme that is induced by lead (II) in the root tips of *Lupinus lutenus* exposed to 150 mg Pb^{2+} L^{-1} appears on native PAGE after 6 and 36 h. Moreover, our previous study (Kim et al. 2002) found similar variations in the activities of total SOD and CAT in *Persicaria thunbergii* when exposed to 5 mM Pb^{2+}.

Hyperaccumulating plants are defined by the following characteristics: 1) metal concentrations in the aerial portions are >10,000 mg kg^{-1} dry matter for Zn and Mn; >1,000 mg kg^{-1} for Co, Cu, Ni, As, and Se; and >100 mg kg^{-1} for Cd (Baker and Brooks, 1989; Ma et al., 2001); 2) the bioconcentration factor (BCF) is >1.0, and sometimes reaches 50 to 100 (Cluis, 2004); and 3) the translocation factor is >1.0 (Wei and Zhou, 2004). When we administered 5, 10, 20, 50, or 100 mg of lead, the mean values for Pb contents in the aerial portions were 51.8, 76.4, 152.8, 375.2, and 625.0 μg Pb^{2+} g^{-1} dry matter, while values for the roots were 83.4, 103.7, 140.6, 556.1, and 1372.5 μg Pb^{2+} g^{-1} dry matter, respectively (Table 1).The bioconcentration factor is defined as the ratio of metal concentration in the plant to metal concentration in the soil. Here, the BCF was highest (81.1) when plants were exposed to 5 mg of Pb^{2+}, whereas the translocation factor value was greatest (1.1) in response to 20 mg of Pb^{2+} (Fig. 2). BCF values can be indexed to estimate the capacity for plants to accumulate heavy metals. Based on many studies, BCF values differ with the type and concentration of heavy metals, plant species, and environmental conditions (Mattina et al., 2002; Kim et al., 2003). Here, although *M. korsakowi* plants took up the most lead when Pb was applied at 100 mg, the BCF was not at its maximum then. This may have been due to the different treatment concentrations used, the physiological characteristics of *M. korsakowi* plants, and culturing conditions.

The translocation factor (TF) describes the movement and distribution of heavy metals in plants, and depends on many factors. For treatments with 5, 10, 20, 50, or 100 mg Pb^{2+}, TF values were 0.6, 0.7, 1.1, 0.7, and 0.5 (Fig. 2). A positive correlation coefficient was found between lead contents in the roots and the aerial portions ($r=0.9781$, $P<0.01$; $n=5$). Hart et al. (1998) have reported that cadmium is translocated from the roots to the shoots in several species, and have suggested that this movement likely occurs via the xylem and is driven by leaf transpiration. Campbell et al. (1985) and Kim et al. (2003) have found statistically significant relationships between heavy-metal contents in the aerial portions and the roots of *Nuphear vearigatum* (copper in stems: $r=0.72$, $P<0.005$; $n=21$) and *Polygomum thunbergii* (lead in leaves: $r=0.5529$, $P<0.001$, $n=80$; lead in stems, $r=0.5425$, $P<0.001$, $n=80$). These phenomena are probably due to complex interactions between different kinds of metal ions and plant species, leading to variability in heavy-metal absorption and assimilation. Such uptake from the soil occurs either passively, with the mass flow of water into the roots, or through active transport, across the plasma membrane of the root epidermal cells. In fact, under normal growing conditions, plants can potentially accumulate certain metal ions to an order of magnitude greater than in the surrounding soil.

In conclusion, we have demonstrated that plants of *M. korsakowi* can be effective tools in the phytoremediation of wetlands contaminated with heavy metals. This is based on the following factors: 1) their massive accumulations of lead, and 2) the stimulation of specific antioxidative enzymes and total antioxidant capacity, which reveal a defense mechanism against reactive oxygen radicals that is induced by lead treatment. Therefore, we suggest that *M. korsakowi* has great potential for phytoextraction.

ACKNOWLEDGEMENTS

We wish to thank Ms. Suzanne Diamond for her helpful comments on the manuscripts. This study was supported by

the Brain Korea 21 Program of the Ministry of Human Resources.

Received April 16, 2008; accepted May 26, 2008.

LITERATURE CITED

Abdel-Basset R, Issa AA, Adam MS (1995) Chlorophylase activity: Effect of heavy metals and calcium. Photosynthetica 31: 421-425

Ait AN, Pilar BM, Ater M (2004) Tolerance and bioaccumulation of cadmium by *Phragmites australis* grown in the presence of elevated concentrations of cadmium, copper and zinc. Aquat Bot 80: 163-176

Alcantara E, Romera FJ, Cañete M, De La Guardia MD (1994) Effects of heavy metals on both induction and function of root Fe (III) reductase in Fe-deficient cucumber (*Cucumis sativa* L.) plants. J Exp Bot 45: 1893-1898

Anderson MD, Prasad TK, Stewart CR (1995) Changes in isozyme profiles of catalase, peroxidase, and glutathione reductase during acclimation to chilling in mesocotyls of maize seedlings. Plant Physiol 109: 1247-1257

Azevedo RA, Alas RM, Smith RJ, Lea PJ (1998) Response of antioxidant enzymes to transfer from elevated carbon dioxide to air and ozone fumigation, in the leaves and roots of wild-type and a catalase-deficient mutant of barley. Physiol Plant 104: 280-292

Baker AJM (1987) Metal tolerance. New Phytol 106: 93-111

Baker AJM, Brooks RR (1989) Terrestrial higher plants which hyperaccumulate metallic elements: A review of their distribution, ecology and phytochemistry. Biodiscovery 1: 811-826

Bowler C, van Camp W, van Montagu M, Inze D (1994) Superoxide dismutase in plants. Crit Rev Plant Sci 13: 199-218

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248-254

Campbell PGC, Tessier A, Bisson M, Bougie R (1985) Accumulation of copper and zinc in yellow waterlily, *Nupher verigatum*: Relationships to metal partitioning in the adjacent lake sediment. Can J Fish Aquat Sci 42: 23-32

Cheng S, Ren F, Grosse W, Wu Z (2002) Effects of cadmium on chlorophyll contents, photochemical efficiency, and photosynthetic intensity of *Canna indica* Linn. Intl J Phytoremed 4: 239-246

Cluis C (2004) Junk-greedy greens: Phytoremediation as a new option for soil decontamination. Biol Tech J 2: 60-67

Dixit V, Pandey V, Shyam R (2001) Differential antioxidative responses to cadmium in roots and leaves of pea (*Pisum sativum* L. cv. Azad) J Exp Bot 52: 1101-1109

Ewais EA (1997) Effects of cadmium, nickel and lead on growth, chlorophyll content and proteins of weeds. Biol Plant 39: 403-410

Foyer CH, Descourvieres P, Kuneert KJ (1994) Protection against oxygen radicals: An important defense mechanism studied in transgenic plants. Plant Cell Environ 17: 507-523

Gallego SM, Benavídes MP, Tomaro ML (1996) Effect of heavy metal ions excess on sunflower leaves: Evidence for involvement of oxidative stress. Plant Sci 121: 151-159

Hall JL (2002) Cellular mechanisms of heavy metal detoxification and tolerance. J Exp Bot 53: 1-11.

Hardiman RT, Jacoby B, Banin A (1984) Factors affecting the distribution of cadmium, copper and lead, and their effect upon yield and zinc content in bush beans (*Phaseolus vulgaris* L.). Plant Soil 81: 17-27

Hart JJ, Welch RM, Norvell WA, Sullivan LA, Kochian LV (1998) Characterization of cadmium binding, uptake, and translocation in intact seedlings of bread and durum wheat cultivars. Plant Physiol 116: 1413-1420

Herawati N, Suzuki S, Hayashi K, Rivai IF, Koyoma H (2000) Cadmium, copper and zinc levels in rice and soil of Japan, Indonesia and China by soil type. Bull Environ Contam Toxicol 64: 33-39

Iannelli MA, Pietrini F, Fiore L, Petrilli L, Massacci A (2002) Antioxidant response to cadmium *Phragmites australis* plants. Plant Physiol Biochem 40: 977-982.

Karuppanapandian T, Sinha PB, Haniya AMK, Manoharan K (2006) Differential antioxidative responses of ascorbate-glutathione cycle enzymes and metabolites to chromium stress in green gram (*Vigna radiata* L.) leaves. J Plant Biol 49: 440-447

Kevresan S, Petrovic N, Popovic M, Kandrac J (1998) Effect of heavy metals on nitrate and protein metabolism in sugar beet. Biol Plant 41: 235-240

Kim IS, Kang KH, Lee EJ (2002) Bioaccumulation patterns and responses of fleece-flower, *Persicaria thunbergii*, to cadmium and lead. Kor J Ecol 25: 253-260

Kim IS, Kang KH, Johnson-Green P, Lee EJ (2003) Investigation of heavy metal accumulation in *Polygonum thunbergii* for phytoextraction. Environ Pollut 126: 235-243

Kraus TE, McKersie BD, Fletcher RA (1995) Paclobutrazol-induced tolerance of wheat leaves to paraquat may involve increased antioxidant enzyme activity. J Plant Physiol 145: 570-576

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685

Lim YH, Kim IS, Shim HJ, Kang KH, Lee EJ (2006) Bioaccumulation patterns and ecophysiological responses of *Monochoria korsakowi* exposed to cadmium. Integrat Biosci 10: 125-130

Ma LQ, Komar KM, Tu C, Zhang W, Cai Y (2001) A fern that hyperaccumulates arsenic. Nature 409: 579

Manios T, Stentiford EI, Millner PA (2003) The effect of heavy metals accumulation on the chlorophyll concentration of *Typha latifolia* plants, growing in a substrate containing sewage sludge compost and watered with metaliferus water. Ecol Engr 20: 65-74

Mattina MI, Lannucci-Berger W, Musante C, White JC (2002) Concurrent plant uptake of heavy metals and persistent organic pollutions from soil. Environ Pollut 124: 375-378

Oxford Biomedical Research Inc. (2001) Colorimetric microplate assay for total antioxidant power product No. TA 01. Oxford Biomed Res 1-5

Rao MV, Paliyath G, Ormrod DP (1996) Ultraviolet-B- and ozone-induced biochemical changes in antioxidant enzymes of *Arabidopsis thaliana*. Plant Physiol 110: 125-136

RucińskaR, Waplak S, Gwóź dź EA (1999) Free radical formation and activity of antioxidant enzymes in lupin roots exposed to lead. Plant Physiol Biochem 37: 187-194

Samecka-Cymerman A, Kempers AJ (2001) Concentrations of heavy metals and plant nutrients in water, sediments and aquatic macrophytes of anthropogenic lakes (former open cut brown coal mines) differing in stage of acidification. Sci Total Environ 281: 87-98

Sanità di Toppi L, Gabbrielli R (1999) Response to cadmium in higher plants. Environ Exp Bot 41: 105-130

Sharma SS, Gaur JP (1995) Potential of *Lemna polyrrhiza* for removal heavy metals. Ecol Engr 4: 37-43.

Siedlecka A, Krupa Z (1996) Interaction between cadmium and iron, and its effects on photosynthetic capacity of primary leaves of *Phaseolus vulgaris*. Plant Physiol Biochem 34: 833-841

Singh DB, Varina S, Mishra SN (2002) Putrescine effect on nitrate reductase activity, organic nitrogen, protein, and growth in

heavy metal and salinity stressed mustard seedlings. Biol Plant 45: 605-608

Sinicrope TL, Langis R, Gersberg RM, Busnardo MJ, Zedler JB (1992) Metal removal by wetland mesocosms subjected to different hydroperiods. Ecol Engr 1: 309-322

Stobart AK, Griffiths WI, Ameen-Bukhari I, Sherwood RP (1985) The effect of Cd^{2+} on the biosynthesis of chlorophyll in leaves of barley. Physiol Plant 63: 293-298

Tung G, Temple PJ (1996) Uptake and localization of lead in corn (*Zea mays* L.) seedlings: A Study by histochemical and electron microscopy. Sci Total Environ 188: 71-85

Ukeda H, Kawana D, Maeda S, Sawamura M (1999) Spectrophotometric assay for superoxide dismutase based on the reduction of highly water-soluble tetrazolium salts by xanthine-xanthine oxidase. Biosci Biotechnol Biochem 63: 485-488

van Assche F, Clijsters H (1990) Effects of metals on enzyme activity in plants. Plant Cell Environ 13: 195-206

Vangronsveld J, Clijsters H (1994) Toxic effects of metals, *In* ME Farago, ed, Plants and the Chemical Elements. Biochemistry, Uptake, Tolerance and Toxicity. VCH, Weinheim, pp 149-177

Wei S, Zhou Q (2004) Identification of weed species with hyperaccumulative characteristics of heavy metals. Prog Nat Sci 14: 495-8-503

Wellburn AR (1994) The spectral determination of chlorophyll *a* and *b*, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J Plant Physiol 144: 307-313

Ye ZH, Baker JM, Wong MH, Willis AJ (1997) Zinc, lead and cadmium tolerance uptake and accumulation by *Typha latifolia*. New Phytol 136: 469-480