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Bioaccumulation Patterns and Ecophysiological Responses of *Monochoria korsakowi* Exposed to Cadmium

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Abstract: We have examined the bioaccumulation patterns and the ecophysiological responses (photosynthetic pigment and total antioxidative capacity) of *Monochoria korsakowi* exposed to various cadmium concentrations, one of major environmental pollutants. Cadmium ion contents in *M. korsakowi* increased significantly with higher cadmium concentration, and most of the accumulated cadmium was found in the root parts. Biomass of each part decreased with higher cadmium concentration. As cadmium treatment concentration was increased, chlorophyll *a* content was decreased, whereas chlorophyll *b* content was increased. However, the variations of total chlorophyll and carotenoid contents were not evident. Total antioxidative capacity in the leaves of cadmium treated *M. korsakowi* increased greatly with higher cadmium concentration. We considered these results as indicative of the ability of *M. Korsakowi* plants to take up cadmium from wetlands.

Key words: Bioaccumulation, cadmium, *Monochoria korsakowi*, carotenoid, chlorophyll, antioxidative capacity

It is generally assumed that heavy metals can not be degraded by biological or chemical processes, and these metals tend to amass in soils and aquatic sediments. Absorption of toxic metals through the plant root systems and release of these metals during the decomposition of plant materials represent a recycling pathway of heavy metals in the ecosystem. Such a pathway could have an important effect on the level of toxic metals in soil and aquatic sediments. Although most of plants absorb toxic metals, such as cadmium, to various degrees, *Thlaspi caerulescens* plants can actively accumulate cadmium up to 100 μg/g in a whole plant without suffering toxic effects (Brooks and Robinson, 1998). In contrast, *Pisum sativum* plants show alteration of leaf physiology and metabolism at much lower cadmium concentration (Sandalio et al., 2001).

Plants respond in different ways to toxic heavy metals in most environmental conditions. Cadmium reduces the absorption of nitrate and its transport from roots to shoots by inhibiting the nitrate reductase activity in the shoots (Hernandez et al., 1996) and growth both in roots and in stems by the suppression of the elongation growth-rate of cells, especially in the stem, because of an irreversible inhibition exerted by cadmium on the proton pump (Aidid and Okamoto, 1993). Cadmium also influences the activity of iron enzymes, such as Fe (III) reductase, in roots (Alcantara et al., 1994), enzymes involved in photosynthesis (Van Assche and Clijsters, 1990), the photosynthetic apparatus (Siedlecka and Krupa, 1996), chlorophyll content (Ewais, 1997), and antioxidative defense system (Gallego et al., 1996; Iannelli et al., 2002).

During the last few years, several wetland plants, such as *Phragmites australis* (Ait Ali et al., 2004), *Polygonum thunbergii* (Kim et al., 2003), *Iris pseudoacorus* (Samecka-Cymerman and Kempers, 2001) and *Thyha latifolia* (Manios et al., 2003) have been studied for phytoremediation of toxic heavy metals. *Monochoria korsakowi* is a plant species in family Pontederiaceae, naturally inhabites in wetland habitats. However, its ability to accumulate heavy metals and responses to toxic metals are not well known. Therefore, the objective of this studied is to investigate the bioaccumulation patterns and ecophysiological responses of *M. korsakowi* plants exposed to various cadmium concentrations.

MATERIALS AND METHODS

Plant samples

Individuals of *Monochoria korsakowi* were collected from abandoned paddy fields (Wanju-gun, Jeonbuk, Korea) and

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transplanted to an artificial wetland (10 m × 10 m). Transplanted individuals were cultured under a hydroponic system with Hoagland's solution and seeds were collected from ripen capsules. Three individuals of *M. korsakowi* plantlets from seeds were selected (10-15 cm in height) and transplanted in each pot (ϕ150 mm × D200 mm) containing 3 kg of experiment soil (sand 25% v/v, clay 25% v/v and soil conditioner 50% v/v). All sample plants were acclimated and cultured under a hydroponic system (1-2 cm deep in water table) with modified Hoagland's solution for 2 months before the cadmium treatment.

Cadmium treatment

Monochoria korsakowi plant samples were exposed with various cadmium concentrations in a greenhouse with ambient temperature ranging from 28 ± 3°C (day) to 17 ± 3°C (night). Cadmium treatment concentrations per each time were 0 μM, 44.6 μM, 89.2 μM, 178.3 μM, 445.8 μM, and 891.7 μM $Cd(NO_3)_2 \cdot 4H_2O$ (equivalent to 0, 0.5, 1.0, 2.0, 5.0, and 10.0 mg Cd, respectively) in 100 ml modified Hoagland's solution, and cadmium treatment was done 10 times with an interval of 3 days. Three replicates were used for each cadmium treatment.

Biomass and Cd analysis

At the end of 30 days of cadmium treatment, plants were uprooted from each pot, washed, blotted dry, and divided into different portions (leaves, petioles, capsule, and roots). The biomass of each plant part was determined. To analyze cadmium content in the plant sample, each part of plant samples was dried at 70°C for 48 h, pulverized, and decomposed by acid digestion. Cadmium concentrations were determined by an inductively coupled plasma emission spectrophotometer (ICPS-1000 IV, Shimazu).

Photosynthetic pigment

To extract photosynthetic pigments in the leaves, 4 foliar discs (ϕ8 mm per disc; total surface area, 2 cm^2) were cut from the nourished leaf of *M. korsakowi* with an 8 mm diameter punch at the end of cadmium treatment. These leaf-discs were placed immediately into 5 ml dimethyl-sulfoxide (DMSO), and total leaf-pigments were extracted at 60°C for 24 h in the dark. The extracts were centrifuged at 1,500 × g for 20 min and the supernatants were collected carefully. For the assay of chlorophylls *a*, *b* and total carotenoid contents, the absorption spectra of each extract were measured at 665, 649 and 480 nm by UV-VIS spectrophotometer (Spectronic GENESIS 5, Milton Roy). The chlorophylls *a*, *b* and total carotenoids of each extract were calculated by the equation of Wellburn (1994).

Total antioxidative capacity

The total antioxidative capacity of *M. korsakowi* leaf extract was measured in terms of hydrogen donating, that is, the ability of all the antioxidants in the leaf extract to reduce Cu^{2+} to Cu^{+} was applied as an index of the leaf extract's antioxidative capacity. The fresh leaves were pulverized with liquid nitrogen and homogenized in 50 mM potassium phosphate (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. The aqueous extracts were used in measurement of total protein content and total antioxidative capacity. Total protein content was measured by a commercial protein assay kit (Bio-Rad laboratories, Inc. #500-0001; Bradford, 1976), and total antioxidative capacity was determined by a commercial colorimetric microplate assay kit (Oxford Biochemical Research, Inc. #TA 01; Oxford Biochemical Research, 2001). The total protein and antioxidant contents of the samples were calculated by extrapolation from a standard curve developed from known concentrations of bovine serum albumin (BSA) and glutathione (GSH), respectively.

Statistical analysis

We used the SAS statistics software package (SAS 9.1.3) for statistical analysis. The all data were expressed with mean ± standard error (SE) of 3 independent replicates, and based on two-tailed *t*-tests. Significant differences were established with p values less than or equal to 0.05. An index of the association of two quantitative variables was expressed by Pearson product-moment correlation coefficient (r).

RESULTS

Cadmium accumulation and biomass

The visible phytotoxicity in *M. korsakowi* was not shown during the cadmium treatment period (30 days), and cadmium contents in *M. korsakowi* increased significantly with higher cadmium treatment concentrations. Most of the cadmium accumulated by *M. korsakowi* was found in the root (Table 1). On the other hand, biomass of each part, especially capsule, was decreased with higher cadmium treatment concentrations (Fig. 1). Compared with control pots, the biomass of capsule, root, leaf and petiole in 100 mg cadmium treatment pots decreased up to 47.4, 29.3, 20.8, and 19.1%, respectively. A negative correlation between capsule biomass and cadmium treatment concentration was shown ($r = -0.905$, $p < 0.0131$, $n = 6$).

Chlorophylls *a*, *b* and carotenoid assay

Total chlorophyll contents (Chl *a* + Chl *b*) in the leaves of *M. korsakowi* exposed to different cadmium concentrations ranging from 5 to 100 mg were not changed greatly. However, as cadmium treatment concentration was increased, chlorophyll *a* (Chl *a*) content was decreased (negative

Table 1. Cadmium contents of *Monochoria korsakowi* plant parts grown in different cadmium concentrations for 30 days

Plant parts	Total amount of cadmium treatment in pot (mg Cd/pot)					
	0	5	10	20	50	100
Capsule	ND	1.1 ± 0.66	3.1 ± 0.22	7.6 ± 0.20	17.5 ± 0.54	24.3 ± 0.67
Leaf	3.7 ± 0.61*	5.3 ± 0.34	8.2 ± 0.44	35.7 ± 1.40	46.7 ± 1.07	86.7 ± 9.01
Petiole	ND	39.7 ± 3.32	31.6 ± 1.32	87.0 ± 2.14	268.0 ± 8.22	388.6 ± 9.73
Root	1.0 ± 0.58	123.4 ± 3.15	106.0 ± 6.45	112.8 ± 2.83	383.7 ± 8.69	504.9 ± 15.34

ND: not detected.
Mean ± SE (μg Cd/g dry weight, $n = 3$)

correlation; $r = -0.792$, $p < 0.0604$, $n = 6$), and chlorophyll *b* (Chl *b*) content was increased (positive correlation; $r = 0.763$, $p < 0.0774$, $n = 6$) (Fig 2). Moreover, the ratio of Chl *a*/Chl *b* was decreased significantly by the increase of cadmium treatment concentration, and the values were 2.36 (0 mg Cd), 1.90 (5 mg Cd), 1.47 (10 mg Cd), 1.35 (20 mg Cd), 1.27 (50 mg Cd) and 1.21 (100 mg Cd). In the cadmium exposed leaves, the variations of total carotenoid contents were small. However, in 50 mg Cd and 100 mg Cd treatment pots, the total carotenoid contents were increased a bit more than those of control (Fig. 2).

Total antioxidative capacity

Total antioxidative capacity in the leaves of cadmium treated *M. korsakowi* was higher than that of control (Fig. 3). The total antioxidative capacities in 20, 50, and 100 mg Cd treatment were significantly increased to 2.5, 3.2, and 5.6 times, respectively, compared with control. A significant positive correlation between cadmium treatment concentration and total antioxidative capacity in the leaves of *M. korsakowi* was confirmed ($r = 0.997$, $p < 0.0001$, $n = 6$).

DISCUSSION

The bioaccumulation pattern of heavy metals is different in each case depending on plant species, kinds of heavy metal, and the variation of heavy metal concentration (Ye et al., 1997; Kim et al., 2003). In this study, the cadmium accumulation pattern of *M. korsakowi* was shown in the sequence of capsule < leaf < petiole < root (Table 1). In

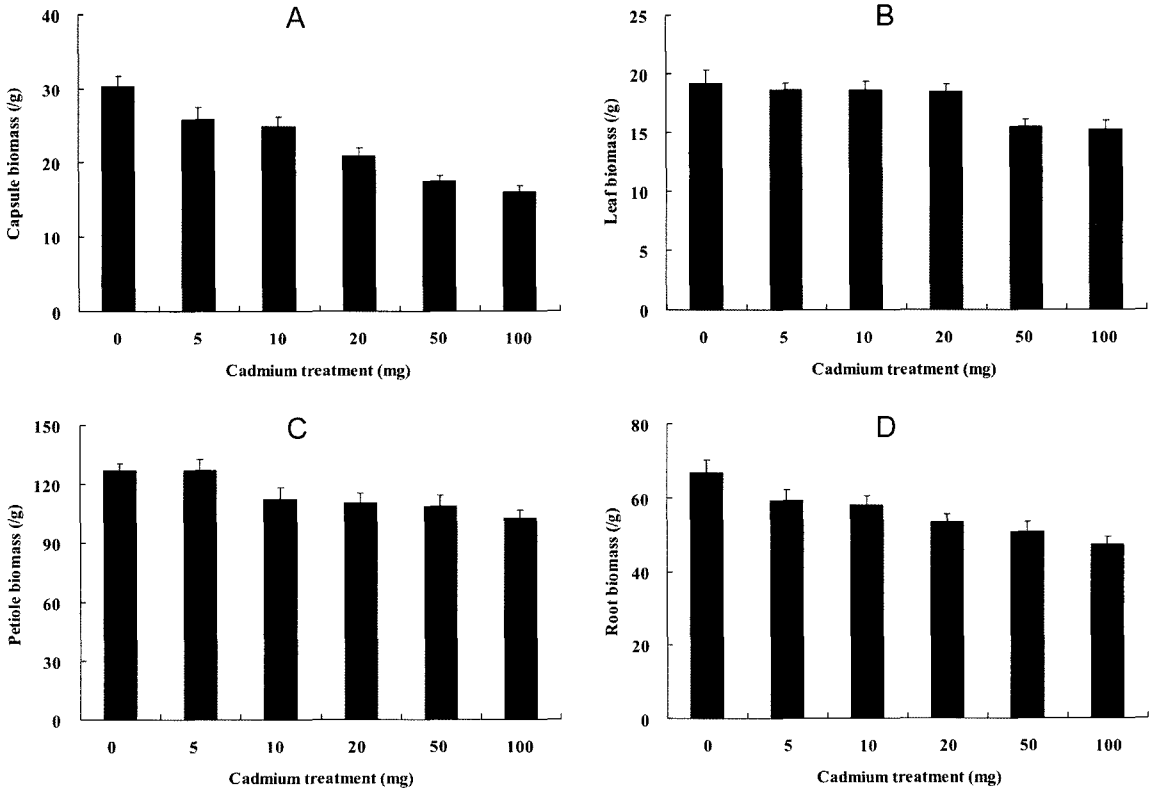

Fig. 1. Biomass variations of *Monochoria korsakowi* grown in different cadmium concentrations for 30 days: capsule (A), leaf (B), petiole (C), and root (D). Vertical bars represent standard error ($n = 3$).

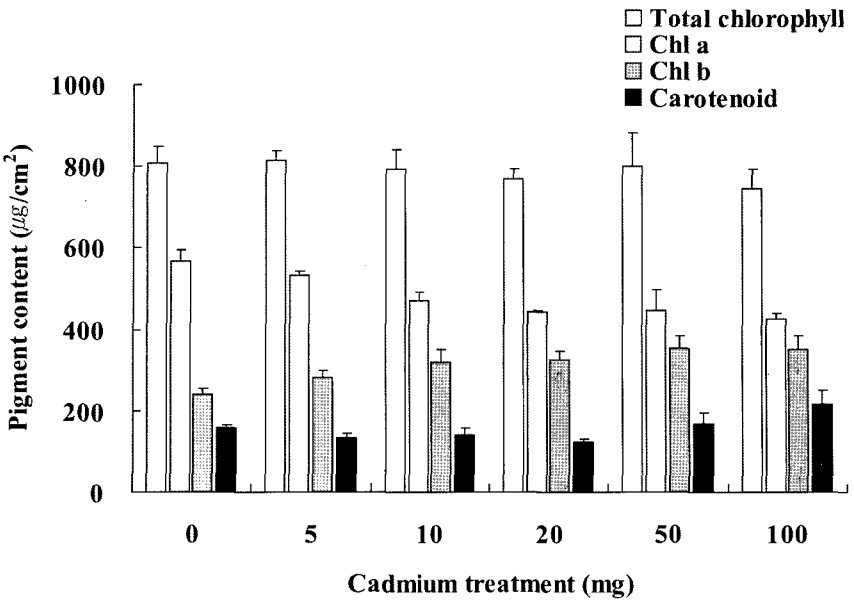

Fig. 2. Photosynthetic pigments in the leaves of *Monochoria korsakowi* grown in different cadmium concentrations for 30 days. Vertical bars represent standard error (n = 3).

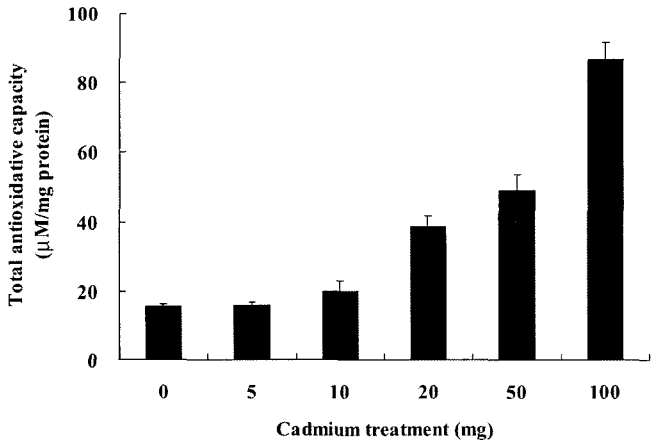

Fig. 3. Total antioxidative capacity in the leaves of *Monochoria korsakowi* plants grown in different cadmium concentrations for 30 days. Vertical bars represent standard error (n = 3).

addition, a positive correlation was found between cadmium treatment concentration and the cadmium contents accumulated in a whole *M. korsakowi* plant ($r = 0.977$, $p < 0.0007$, $n = 6$). In the highest cadmium concentration treatment (100 mg Cd), the mean values of cadmium contents in leaf, petiole, root and capsule were 86.7 ± 9.01 μg, 388.6 ± 9.73 μg, 504.9 ± 15.34, and 24.3 ± 0.67 μg Cd/g dry weight, respectively. Iannelli et al. (2002) reported that the amount of cadmium in the roots (3,690 μg/g dry weight) of *Phragmites australis* during hydroponic growth in the presence of 50 μM $CdSO_4$ are remarkably higher than that in stolons (10 μg/g dry weight) and leaves (77 μg/g dry weight). Ye et al. (1997) reported that the contents of cadmium, lead and zinc in the natural plant population of *Typha latifolia* maintained 0.2-0.8 μg Cd/g, 4.7-40 μg Pb/g and 22-122 μg Zn/g dry weight in the leaves, and 1.0~17 μg Cd/g, 25-3,628 μg Pb/g and 46-946 μg Zn/g dry weight in the roots. They stated that the bioaccumulation amount of heavy metals in *T. latifolia* increased in the order of leaf < rhizome < root. Our previous studies demonstrated that the underground parts (roots) of *Polygonum thunbergii* showed higher metal contents than in the aboveground parts (leaves and stems) (Kim et al., 2003). Therefore, comparing with the results of other macrophytes and wetland plants, we confirmed that the bioaccumulation patterns of cadmium in *M. korsakowi* (capsule < leaf < petiole < root) in this study are similar with those of other submerged-hydrophytes.

Total chlorophyll content (Chl a + Chl b) is a unifying parameter for indicating the effect of heavy metals. According to Vangronsveld and Clijsters (1994), the changes in photosynthetic pigments content in response to heavy metal stresses not only indicate damage to the photosynthetic apparatus and capacity, but also have consequences for reduced carbon assimilation, growth, survival, reproduction and the production of carbon-based products. In this study, the total chlorophyll content did not vary greatly with cadmium treatment, but chlorophyll a or chlorophyll b contents were decreased or increased distinctly by various cadmium concentrations. The biomass of *M. korsakowi* exposed to cadmium was decreased at the all plant parts (leaves, petioles, capsule, and roots). We assumed that chlorophyll a content was more affected by cadmium treatment than chlorophyll b, and biomass reduction could be interpreted as an effect of the accumulated cadmium in *M. korsakowi*. The variations of chlorophyll content

induced by cadmium were also observed by Greger and Ogren (1991) and Ewais (1997). According to Abdel-Basset et al. (1995) and Ewais (1997), changes in the contents of chlorophyll *a* or chlorophyll *b* and the ratio of Chl *a*/Chl *b* are an important parameter, which always have been taken under consideration when estimating the effect of an environmental stress in plants. In this study, we found that the higher cadmium doses severely attenuated the Chl *a*/Chl *b* ratio. A similar reduction in the Chl *a*/Chl *b* ratio induced by cadmium was observed in *Cyperus difformis*, *Chenopodium ambrosidides* and *Digitaria sanguinolis* (Ewais, 1997).

The responses of plants to cadmium stress are reported to a number of defense systems, such as immobilization in cell wall (Wagner, 1993), exclusion (Costa et al., 1997), the synthesis of phytochelatin (Grill et al., 1985), the modification of antioxidant enzyme (Kim et al., 2002; Romero-Puertas et al., 2002) and the increase of antioxidant (Iannelli et al., 2002). Among these defense systems, the antioxidant systems in plants play a role to perform an additive role in the detoxification mechanisms (Dixit et al., 2001). Our results on total antioxidative capacity demonstrated that the increase of cadmium content in the leaves influenced on the increase of total antioxidative capacity in the plants (Fig. 3).

Plants have the diverse ability in accumulating and removing heavy metals. A threshold of tolerance in each plant species to the heavy metal accumulation is various shown by a number of environmentally, physiologically and genetically determined reasons (Rai et al., 1995; Salt et al., 1995; Sharma and Gaur, 1995). Our study clearly indicated that *M. korsakowi* could accumulate an amount of cadmium without the occurrence of phytotoxicity. We considered these results as indicative of the ability of *M. korsakowi* plants to take up cadmium from wetlands.

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