ORIGINAL ARTICLE

Cadmium accumulation by *Axonopus compressus* (Sw.) P. Beauv and *Cyperus rotundas* Linn growing in cadmium solution and cadmium-zinc contaminated soil

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Abstract

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Cadmium accumulation by *Axonopus compressus* (Sw.) P. Beauv and *Cyperus rotundas* Linn growing in cadmium solution and cadmium-zinc contaminated soil

Songklanakarin J. Sci. Technol., 2007, 29(3) : 881-892

This research investigated the phyto-remediation potentials of *Cyperus rotundas* Linn (Nutgrass) and *Axonopus compressus* (Sw.) P. Beauv (Carpetgrass) for cadmium removal from cadmium solution and cadmium-zinc contaminated soil. Plants growth in the solution showed that cadmium decreased the relative growth rate of both grasses. However, the amount of cadmium accumulated in shoot and root was increased with the increase in cadmium concentration and exposure time. Growth in fertile soil mixed with Cd-contaminated zinc silicate residue (65% Si, 19% Ca, 2% Zn, 1% Mg and 0.03% Cd) at the ratio of 50:50 (w/w) for 30 days showed that *C. rotundas* Linn accumulated cadmium in root and shoot to 2,178 and 1,144 mg

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Received, 22 May 2006 Accepted, 12 September 2006

kg^{-1} dry weight, respectively. *A. compressus* (Sw.) P. Beauv accumulated cadmium in root and shoot to 1,965 and 669 mg kg^{-1} dry weight, respectively. Scanning electron microscope connected to energy-dispersive X-ray spectroscopy suggested that the mechanism of cadmium accumulation by both grasses involved the cadmium precipitation in the stable form of cadmium silicate, which indicated that *C. rotundas* Linn and *A. compressus* (Sw.) P. Beauv could be grown to prevent soil erosion and to remediate cadmium-contaminated soil.

Key words : *Axonopus compressus* (Sw.) P. Beauv, *Cyperus rotundas* Linn, cadmium, phytoremediation

บทคัดย่อ

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การสะสมแคดเมียมโดย *Axonopus compressus* (Sw.) P. Beauv และ *Cyperus rotundus* Linn ภายใต้สภาวะการเจริญในสารละลายแคดเมียมและในดินเจือปนโลหะแคดเมียมและสังกะสี

ว. สงขลานครินทร์ วทท. 2550 29(3) : 881-892

งานวิจัยนี้ได้ศึกษาความเป็นไปได้ของการใช้หญ้าแห้วหมู (*Cyperus rotundas* Linn) และหญ้ามาเลเซีย (*Axonopus compressus* (Sw.) P. Beauv) ในการบำบัดโลหะแคดเมียมภายใต้สภาวะการเจริญในสารละลายแคดเมียมและดินผสมกากแร่สังกะสีซิลิเกต ผลการเจริญในสารละลายแคดเมียมพบว่าแคดเมียมมีผลทำให้อัตราการเจริญเติบโตของพืชลดลง โดยพบว่าการสะสมแคดเมียมในรากและยอดเพิ่มขึ้นเมื่อความเข้มข้นของแคดเมียมในสาร ละลายและเวลาในการสัมผัสเพิ่มขึ้น การเจริญในดินปลูกพืชผสมกากแร่สังกะสีซิลิเกตที่มีแคดเมียมเจือปน (65% Si, 19% Ca, 2% Zn, 1% Mg และ 0.03% Cd) ในสัดส่วนผสม 50:50% โดยน้ำหนัก เป็นเวลา 30 วัน พบว่าหญ้าแห้วหมูสะสมแคดเมียมในส่วนราก (root) และส่วนต้น (shoot) ได้ 2,178 และ 1,144 มก./กก. น้ำหนักแห้ง หญ้ามาเลเซียสะสมแคดเมียมในส่วนรากและส่วนต้นได้ 1,965 และ 669 มก./กก. น้ำหนักแห้ง ผลการศึกษาโดยใช้กล้องจุลทรรศน์อิเลคตรอนแบบส่องกราด (Scanning Electron Microscope) ต่อพ่วงกับ Energy Dispersive X-ray spectroscopy แสดงในเห็นว่ากลไกการสะสมโลหะแคดเมียมของหญ้าทั้งสองชนิดเกี่ยวข้องกับการตกตะกอนของแคดเมียมในรูปของแคดเมียมซิลิเกตที่มีความเสถียร จึงมีความเป็นไปได้ในการนำหญ้าแห้วหมูและหญ้ามาเลเซียมาใช้เพาะปลูกเพื่อช่วยป้องกันการกัดเซาะหน้าดินและช่วยบำบัดโลหะแคดเมียมในพื้นที่ซึ่งมีโลหะแคดเมียมเจือปนในดิน

Cadmium is a heavy metal naturally present in soil at concentrations of slightly more than 1 mg kg^{-1} (Peterson and Alloway, 1979). It is highly toxic to most organisms, having toxicity 2-20 times higher than many other heavy metals (Vassilev *et al.*, 1998). Cadmium content in soil has been dramatically increased from anthropogenic sources including smelters and agricultural applications of fertilizer and sewage sludge. Cadmium in soil can be bioavailable for plant uptake and subsequent human uptake, thus cadmium in the environment poses a significant health risk (McLaughlin *et al.*, 1999).

Phytoremediation is the use of plants in environmental remediation. The general strategies for phytoremediation of soil metals is to either: (1) *phytoextract* the soil elements into the plant shoots for recycling or less expensive disposal; (2) *phyto-*

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volatilize the soil trace elements e.g. generation of Hg^o enter the vapour phase; (3) *phytostabilize* soil metals into persistently nonbioavailable forms in the soil (Terry and Banuelos, 2000). Since cadmium cannot be degraded as organic molecules, only phytoextraction and phytostabilization are applicable to remediation (Terry and Banuelos, 2000; Lasat, 2000).

A mine in Tak province, Thailand, has used the growth of *Tectona grandis* L.f. (Family: Labiatae), *Eucalyptus camaldulensis* Dehnh. (Family: Myrtaceae) and *Vetiveria zizanioides* Nash (Family: Gramineae) on the heap of mine tailings for long-term phytoremediation. Therefore, *Axonopus compressus* (Sw.) P. Beauv, a cover crop, should be grown to protect erosion of cadmium-contaminated soil from floods. Our exploration of metal hyper-accumulative plants in the mine area found that *C. rotundas* Linn had potential for phytoremediation. The cadmium accumulations in its root (tuber and rhizome), leaf, and flower were 74, 29 and 19 g $kg\text{-}ash^{-1}$, respectively. It can survive in mine areas all year; dry and rainy seasons. Therefore, the cadmium accumulation by *A. compressus* (Sw.) P. Beauv and *C. rotundas* Linn were investigated and compared in this study. The plants' characteristics are shown in Table 1 (Smith and Valenzuela, 2002).

This research aimed to study the effect of cadmium on *A. Compressus* (Sw.) P. Beauv and *C. rotundas* Linn growing in cadmium solution and Cd-Zn contaminated soil. The amount of cadmium accumulated in phytomass (root or shoot) was investigated and compared to obtain a hyper-accumulative ability. In addition, scanning electron microscope connected with energy dispersive X-ray spectroscopy (SEM/EDX) was used to study the possible form of cadmium accumulated in plant tissue.

Materials and Methods

1. Plant materials

Cyperus rotundas Linn (Nutgrass) and *Axonopus Compressus* (Sw.) P. Beauv (Carpet-grass) were cultivated in fertile soil mixed with sand (Panpinit, 1980) in a yard of King's Mongkut University of Technology Thonburi, Thailand. The pH of fertile soil mixed with sand was 5.5-6.5. Plant materials were the last joint stolon of carpet-grass holding roots and the separated nutgrass holding one tuber. They were carefully rinsed with tap water to remove any contaminated substances, and then were selected for using in experiments by the criteria of similar size, similar fresh weight, and the same numbers of leaves and roots.

2. Growth treatment

Plants were grown in a controlled environment room under temperature of 25-30°C, humidity in 70-75%, a 16-h white light/8-h dark photoperiod with a photon flux density of 130 $\mu E/m^2$ s and no aeration in hydroponic system.

2.1 Growth in cadmium solution

The plant materials were acclimatised by growing in distilled water (pH 5.5±0.5) for 2 days. After acclimatisation, the healthy plants were selected and grown for 9 days in a deep-flat polyethylene bottle containing 50 ml of 5.0 mg l^{-1} cadmium solution (pH 5.5±0.5) prepared from cadmium nitrate ($Cd(NO_3)_2$) standard solution (Merck, Germany). A control system was the plant growing in deionised water (pH 5.5±0.5). One healthy plant was grown in each bottle in triplicate. The volume of water evaporated was compensated by adding deionised water (pH 5.5±0.5) before taking 2.0 ml of the solution every day. The sample solution was analysed for cadmium remaining and pH changing by an Inductively Coupled Plasma Spectrophotometer (ICP) (Jobin Yvon JY 124, France) and a pH meter (Metler Toledo Delta 304, USA), respectively. Plant growth was evaluated by counting the root and shoot numbers and weighting the fresh weight and dry weight by oven method (70°C for 2 days).

2.2 Growth in Cd-Zn contaminated soil

A Cd-contaminated zinc silicate residue was obtained from Padang Industry Public Company Limited, Tak Province, Thailand. The residue originated from chemical extraction of a zinc silicate high grade ore. A commercial fertile soil obtained from Nongporn Co. Ltd., Thailand was

Table 1. Characteristics of *Cyperus rotundas* Linn (nutgrass) and *Axonopus compressus* (Sw.) P. Beauv (carpetgrass) (Smith and Valenzuela, 2002)

Characteristics	***Cyperus rotundas* Linn**	***Axonopus compressus* (Sw.) P. Beauv**
Common names	Purple nutgrass	Carpetgrass
Life cycle	Perennial	Perennial
Growth habit	Graminoid	Stoloniferous
Photosynthetic	C4 photosynthetic	C4 photosynthetic
Stem height	Up to 40 cm	maximum height of about 20-50 cm.
Uses of plants	Use as a soil binder in India, and landscaping in China. Medicinally in China and India; tuber extracts may reduce nausea and act as a muscle relaxant. Carminative and energy and hormone regulating herb in traditional Chinese medicine.	For controlling erosion, suppressing weeds. Improved soil structure, better water infiltration rates, and increased soil water-holding capacity. Some research indicated that it can fix atmospheric nitrogen and can add this nutrient to the soil.
Reproduces	Primarily by tubers. Rapid growth The principle means of spread and reproduction is by creeping Underground stems (rhizomes) and tubers.	The creeping stems of carpetgrass are compressed and root at each joint. Spreads by both stolons and seed. It is usually only propagated vegetatively by stolons.
Climate	Tropical to warm	Tropical and subtropical areas. Requires a minimum annual rainfall of about 30 inches (750-775 mm.)
Habitat	All types of soils, disturbed soils, moist fertile soil, full sunlight	All type of soil, sandy soils with a high water table, full sunlight.
Tolerates	May remain dormant in soil for up to 10 years, waiting for an opportunity to germinate. It grows in all types of soils and can survive the highest temperature.	Acidic (pH 4.0-7.0) and low fertility soils, Shade tolerant, requires little fertilizer

used as soil sample to increase organic matter. Cd-contaminated zinc silicate residue was determined by ICP and CHNS analyzer (Leco CHN 2000, and Leco S-144DR, USA). It contained 65% Si, 19% Ca, 2% Zn, 1% Mg, 0.03% Cd, 0.31% C, 0.88% H, 0.08% N and 8.34% S. The fertile soil contained 20.17% C, 2.32% H, 0.44% N, and 0.14% S. Cd-Zn contaminated soil, was prepared from 1:1 mixture of Cd-contaminated zinc silicate residue and commercial fertile soil. The pH of Cd-Zn contaminated soil was 5.5-6.5.

The experiment pot was the plant growing in a 500 g of Cd-Zn contaminated soil. The size of plastic pots was 8.5 cm diameter and 6 cm high and they were placed in individual trays. Two controls were the plant growing in fertile soil and the Cd-Zn contaminated soil without a plant. One healthy plant was grown in each pot in triplicate. Pots were daily watered with a little of deionised water (2 ml) throughout the experiment of 30 days for growth. A 1 g sample of Cd-Zn contaminated soil was taken from each pot every ten days for analysis of cadmium remaining.

3. Analytical methods

3.1 Plant and soil analysis

At the end of the experiment period, the plants were harvested, washed with deionised water before being divided into shoots and roots, and then dried at 70°C for 2 days. Dried plant samples were digested by 3 ml of H_2SO_4 conc. mixed with 3 ml of HCl conc. The digestion solution was filtered and the volume adjusted before being analysed for cadmium concentration by ICP.

The sample of Cd-Zn contaminated soil was digested by aqua regia (the mixture of 35% (w/v) HCl and 65% HNO_3 as 3:1, 98% (w/v) H2SO4 and 50% (w/v) HF). The digestion method was adapted from ASTM E841-04 (ASTM, 2004). The digestion solution was analysed for cadmium concentration by ICP. The pH of Cd-Zn contaminated soil was determined by Carter's method (1994) by stirring 10 g of contaminated soil in 20 ml of deionised water for 30 minutes, and allowing it to settle for 1 hour before mesurement by the pH meter.

3.2 Microscopic analysis

A plant sample was harvested after 9 days of growing in 5.0 mg l^{-1} cadmium solution (pH 5.5±0.5), and dried at 70°C for 2 days. A small piece of leaf or root was put on aluminium straps, and coated with carbon before it was analysed by a Scanning Electron Microscope connected with Energy Dispersive X-ray spectroscopy (SEM/EDX) (JSM-6400, Japan). The percentages of elements on the plant surfaces were calculated from at least three areas of scanning.

3.3 Calculation of indices and statistics

The percentage of cadmium accumulation and the cadmium absorption capacity by phytomass (shoot or root) were calculated following Eqs.(1) and (2), respectively (Vazquez *et al.*, 1994). Relative growth rates (RGR) of plants growing in control and treatment systems were calculated by Eq (3) (Arduini *et al.*, 2004).

$$R = \frac{C_i - C_r}{C_i} \times 100 \qquad (1)$$

$$\frac{x}{m} = \frac{C_i - C_r}{m_c} \qquad (2)$$

$$RGR = \frac{\ln W_2 - \ln W_1}{t_2 - t_1} \qquad (3)$$

Where

R	is the percentage of cadmium accumulation,
x/m	is the capacity of cadmium absorbed into phytomass (mg g^{-1}),
m_c	is the concentration of phytomass in the solution (g l^{-1}),
C_i	is the initial concentration of cadmium at the beginning of experiment (mg l^{-1}),
C_r	is the residual concentration of cadmium at the end of the experiment period (mg l^{-1}),
W_1	is the dry weight at the beginning of experiment (t_1),
W_2	is the dry weight at the end of the experimental period (t_2).

The RGR will facilitate the assessment of change in the above-ground biomass during the experimental period. Plant monitoring indicated the feasibility and efficiency of both species for employing in treatment under real conditions. The uptake of cadmium by the two plant species was calculated based upon the plant dry weight and cadmium concentration in the harvested portions. The appearance of plant and the number of leaf and root was observed and recorded every day.

The data were statistically analysed by two-way variance analysis (ANOVA), and Duncan's multiple range test at $P<0.05$ significance level, using Sigma Stat software (SPSS Inc.). Three replications per treatment per cultivar were set in the experiments.

Results

1. Plant growing in cadmium solution

The growth of *C. rotundas* Linn and *A. compressus* (Sw.) P. Beauv was affected when grown in 5 mg l^{-1} of cadmium solution, pH 5.5±0.5. The numbers of roots and leaves were determined daily for 9 days. The three replicas of the numbers of roots and leaves are shown in Table 2. The plants exposed to deionised water after 9 days seemed to retain more freshness in appearance as a whole and have many new roots, but the plants

Table 2. Number of roots and leaves of *Cyperus rotundas* Linn and Azonopus compressus (Sw.) P. Beauv during growth in 5 mg l^{-1} of cadmium solution and deionised water (control)

Treatment	**Number of roots**						**Number of shoots**					
	Day 0	**Day 1**	**Day 3**	**Day 5**	**Day 7**	**Day 9**	**Day 0**	**Day 1**	**Day 3**	**Day 5**	**Day 7**	**Day 9**
C. rotundas Linn growing in deionised water	11	13	17	20	26	30	11	11	12	13	14	19
C. rotundas Linn growing in cadmium solution	14	14	14	15	15	16	8	8	8	8	8	9
A. compressus (Sw.) P. Beauv growing in deionised water	8	8	13	18	25	31	8	8	8	9	10	11
A. compressus (Sw.) P. Beauv growing in cadmium solution	12	12	13	13	14	14	14	14	14	14	14	14

exposed to the cadmium concentration seemed to lose freshness starting from Day 3.

Wet weight was examined throughout the experiment and determined by using a scale. It was evident that the plants of both control and treatment showed linear increases in wet weight. The rates of wet weight for both plants after treatment with cadmium solution were lower than for both plants exposed to deionised water (control) (Figure. 1). For *C. rotundas* Linn, the rate of wet weight in the cadmium solution was low, in the order of 0.0515 grams per day, as compared to the plant-wet weight growing in deionised water, which was in the order of 0.1581 grams per day. For *A. compressus* (Sw.) P. Beauv, the rate of wet weight in the cadmium solution was in the order of 0.0394 grams per day as compared to the plant-wet weight growing in deionised water, which was in the order of 0.1731 grams per day (Figure 1). Relative growth rate was used to illustrate the response of the plant to cadmium stress. Both plants had similar RGR values. The RGR values of *C. rotundas* Linn and *A. compressus* (Sw.) P. Beauv species were 0.078 and 0.077 g dry wt. day^{-1}, respectively (Table 3).

The residual cadmium concentrations after bioaccumulation by *C. rotundas* Linn and *A. compressus* (Sw.) P. Beauv for 9 days are shown in Figure 2. Both plants showed significantly different in cadmium uptake from cadmium solution. The

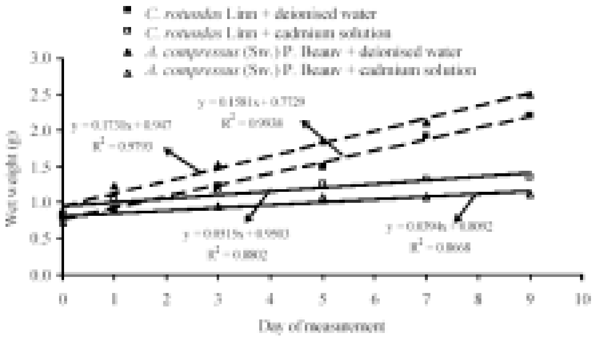

Figure 1. Wet weight of *Cyperus rotundas* Linn and *Axonopus compressus* (Sw.) P. Beauv during 9 days growth in 5.0 mg l^{-1} of cadmium solution and deionised water (control)

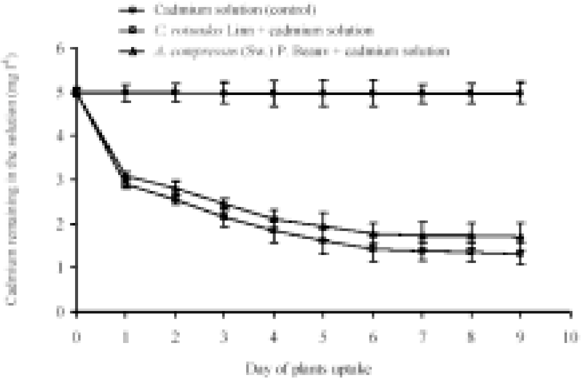

Figure 2. Cadmium remaining in the solution during the growth of *Cyperus rotundas* Linn and *Axonopus compressus* (Sw.) P. Beauv in 5.0 mg l^{-1} of cadmium solution for 9 days

Table 3. Cadmium accumulation in *Cyperus rotundas* Linn. and *Axonopus compressus* (Sw.) P. Beauv after growing in 5 mg l^{-1} of cadmium solution for 9 days

Plants' samples	Dry weight		Relative growth rate (g dry wt day^{-1})	Cadmium accumulation (mg kg^{-1} dry weight)	
	Day 0	Day 9		Shoots	Roots
C. rotundas Linn growing in deionised water	0.0351	0.0816	0.092	0.0	0.0
C. rotundas Linn growing in cadmium solution	0.0352	0.0715	0.078	1,193±0.04	1,800±0.04
A. compressus (Sw.) P. Beauv growing in deionised water	0.0392	0.0925	0.095	0.0	0.0
A. compressus (Sw.) P. Beauv growing in cadmium solution	0.0351	0.0705	0.077	1,032±0.03	1,675±0.04

Table 4. Cadmium accumulation in *Cyperus rotundas* Linn and *Axonopus compressus* (Sw.) P. Beauv after growing in Cd-Zn contaminated soil for 30 days

Plants' sample	Cadmium accumulation (mg kg^{-1} dry weight)	
	Shoots	Roots
C. rotundas Linn growing in fertile soil	0.00	0.00
C. rotundas Linn growing in Cd-Zn contaminated soil	1,144±0.03	2,178±0.04
A. compressus (Sw.) P. Beauv growing in fertile soil	0.00	0.00
A. compressus (Sw.) P. Beauv growing in Cd-Zn contaminated soil	669±0.02	1,965±0.04

cadmium concentration remaining at Day 9 in the solution of *C. rotundas* Linn (1.32 mg l^{-1}) was lower than the cadmium concentration remaining in the solution of *A. compressus* (Sw.) P. Beauv (1.71 mg l^{-1}). After 9 days of cultivation in 5 mg l^{-1} of cadmium solution, *C. rotundas* Linn accumulated cadmium to 1,800 mg kg^{-1} dry weight in the roots and 1,193 mgkg^{-1} dry weight in the shoots. While *A. compressus* (Sw.) P. Beauv accumulated cadmium to 1,675 mg kg^{-1} dry weight in the roots and 1,032 mg kg^{-1} dry weight in the shoots (Table 3).

2. Plant growing in Cd-Zn contaminated soil

Cadmium could be accumulated from Cd-Zn contaminated soil (252 mg Cd kg^{-1}) by *C. rotundas* Linn and *A. compressus* (Sw.) P. Beauv. The residual cadmium concentrations in Cd-Zn contaminated soil after bioaccumulation during Day 0 to Day 30 are shown in Figure. 3. Both plants showed significantly different in cadmium uptake from the Cd-Zn contaminated soil. The cadmium concentration in the Cd-Zn contaminated soil significantly decreased when the exposure time was increased. The cadmium remaining in the Cd-Zn contaminated soil after 30 days of treatment by *C. rotundas* Linn and *A. compressus* (Sw.) P. Beauv were 21.64 mg Cd kg^{-1} and 28.03 mg Cd kg^{-1}, respectively. Table 4 shows the amounts of cadmium accumulated in both plants after 30 days of treatment. *C. rotundas* Linn accumulated cadmium into the roots and shoots to 2,178 and 1,144 mg kg^{-1} dry weight, respectively. While *A. compressus* (Sw.) P. Beauv accumulated cadmium in roots and shoots to 1,946 and 669 mg kg^{-1} dry weight, respectively. Cadmium accumulation in *C. rotundas* Linn was higher than in *A. compressus* (Sw.) P. Beauv.

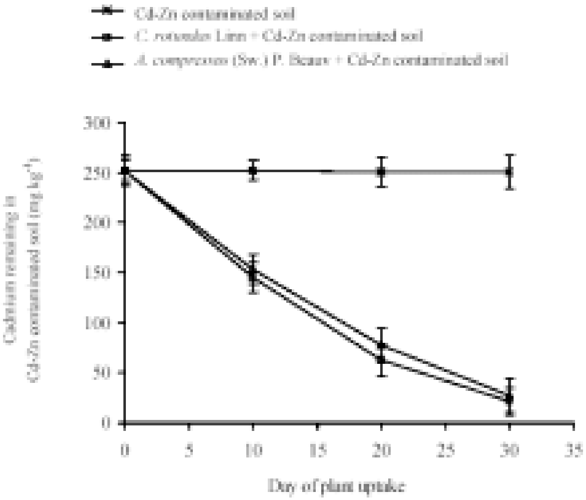

Figure 3. Cadmium remaining in Cd-Zn contaminated soil during the growth of *Cyperus rotundas* Linn and *Axonopus compressus* (Sw.) P. Beauv in the soil for 30 days

3. Microscopic analysis by SEM/EDX

A Scanning Electron Microscope (SEM) connected to an Energy Dispersive X-ray (EDX) was used to study the images of the plants' surface and the percentages of elements. The SEM images of the root and leaf of *C. rotundas* Linn after growing in 5.0 mg l^{-1} of cadmium solution for 9 days are shown in Figures. 4(b) and 4(d), respectively. Although the SEM images show unclear precipitation of metals, the percentages of elements in the root and leaf of *C. rotundas* Linn were analysed by EDX as listed in Table 5. Cadmium concentration of *C. rotundas* Linn's root and leaf were 2.6% and 1.0%, respectively, where the sum of all metals measured were 100%, whereas no cadmium was detected in the root and leaf of *C. rotundas* Linn growing in deionised water (control). The proportions of K and Ca in the root and leaf of *C. rotundas* Linn growing in cadmium solution were dramatically lower than the plant growing in deionised water, whereas the proportion of Si was noticeably higher (Table 5).

The SEM images of the root and leaf of *A. compressus* (Sw.) P. Beauv are shown in Figures. 4(f) and 4(h), respectively. Their SEM images also show unclear precipitation of metals. The percentages of elements in the root and leaf of *A. compressus* (Sw.) P. Beauv analysed by EDX are shown in Table 6. Cadmium concentrations in the

Table 5. The percentage of elements studied by SEM/EDX in the root and leaf of *Cyperus rotundas* Linn before and after growing in 5 mg l^{-1} of cadmium solution for 9 days

Element	% Element in root		% Element in leaf	
	Control	Treatment	Control	Treatment
Na	N/D	2.4	11.1	1.6
Al	26.9	29.4	2.2	27.7
Si	6.0	48.0	1.8	53.1
S	11.2	4.4	14.4	2.6
Cl	8.8	3.2	16.2	2.4
K	14.5	2.9	24.0	3.8
Ca	21.0	1.0	25.0	0.9
Fe	11.5	6.0	5.2	6.7
Cd	N/D	2.6	N/D	1.0
Total	100	100	100	100

N/D = not detected

Table 6. The percentage of elements studied by SEM/EDX in the root and leaf of *Axonopus compressus* (Sw.) P. Beauv before and after growing in 5 mg l^{-1} of cadmium solution for 9 days

Element	% Element in root		% Element in leaf	
	Control	Treatment	Control	Treatment
Na	N/D	1.2	6.5	2.0
Al	42.8	20.6	7.7	43.2
Si	49.4	54.4	39.6	43.2
P	N/D	1.8	N/D	N/D
S	2.4	2.1	20.4	1.7
Cl	1.0	3.5	3.2	1.7
K	2.1	3.6	12.2	1.3
Ca	N/D	1.7	3.7	0.4
Fe	1.3	7.1	6.4	5.6
Cu	1.0	2.2	N/D	N/D
Cd	N/D	1.9	N/D	0.4
Total	100	100	100	100

N/D = not detected

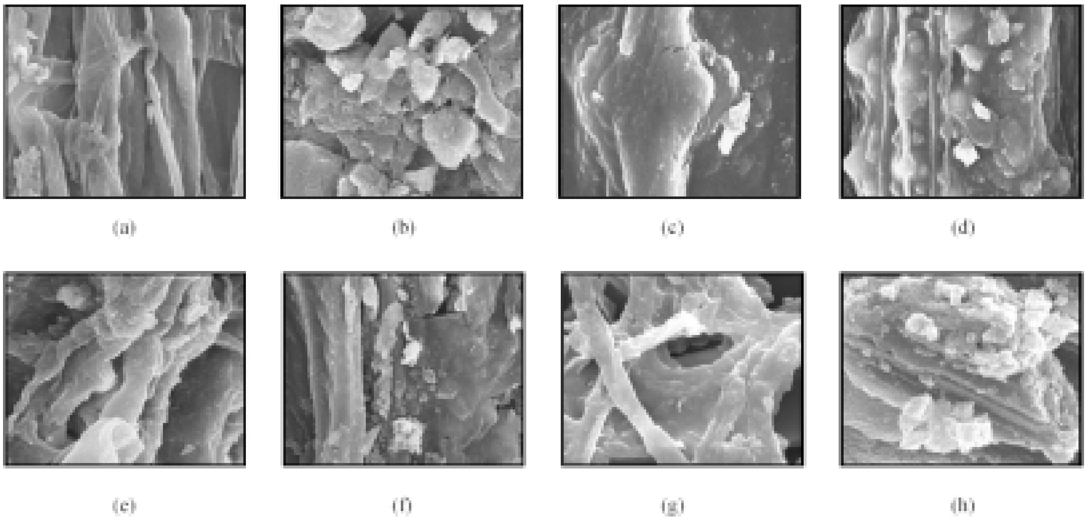

Figure 4. **SEM images of root and leaf of *Cyperus rotundas* Linn and *Axonopus compressus* (Sw.) P. Beauv growing in deionised water and 5.0 mg l^{-1} of cadmium solution for 9 days**

(a) Root of *C. rotundas* Linn growing in deionised water
(b) Root of *C. rotundas* Linn growing in 5.0 mg l^{-1} of cadmium solution
(c) Leaf *C. rotundas* Linn growing in deionised water
(d) Leaf *C. rotundas* Linn growing in 5.0 mg l^{-1} of cadmium solution
(e) Root of *A. compressus* (Sw.) P. Beauv growing in deionised water
(f) Root of *A. compressus* (Sw.) P. Beauv growing in 5.0 mg l^{-1} of cadmium solution
(g) Leaf of *A. compressus* (Sw.) P. Beauv growing in deionised water
(h) Leaf of *A. compressus* (Sw.) P. Beauv growing in 5.0 mg l^{-1} of cadmium solution

root and leaf of *A. compressus* (Sw.) P. Beauv growing in cadmium solution were 1.9% and 0.4%, respectively, whereas there was no cadmium in the root and leaf of the grass growing in deionised water (control). The proportions of Si in the root and leaf of *A. compressus* (Sw.) P. Beauv growing in cadmium solution were higher than the plant growing in deionised water, while only in the leaf the proportions of K and Ca were lower after cadmium treatment (Table 6).

Discussion

Cd-uptake and accumulation in *C. rotundas* Linn and *A. compressus* (Sw.) P. Beauv was studied in high-level Cd exposure for a short time to elicit responses. Results from hydroponics' culture experiments were crucial to highlight the physiological response of plants to toxic (cadmium 5 mg l^{-1}) and non-toxic (deionised water) condition. Cadmium decreased the relative growth rate of both grasses, and the amount of cadmium accumulated in the roots of plants to a higher level than in their shoots. Such levels of cadmium might immediately affect plant-water relationships through the alteration of the permeability of root membranes and the decrease of transpiration flow. Then both phenomena strongly reduced plant growth and the transport of cadmium to the shoot (Haag-Kerwer *et al.*, 1999). Root morphology of grasses growing in cadmium solution was short length and brown colour. These changes are consistent with the hypothesis that Cd induces an abnormal proliferation of root cells, as suggested by Beyersmann (2002), in consequence of the reduction of water flow and mineral uptake (Varga *et al.*, 1999).

Silicon not only is a cell wall incrustation, responsible for the rigidity of leaves in monocots, but also is involved in physiological process (Hodson and Evans, 1995; Epstein, 1999). The results of SEM/EDX study implied that *C. rotundas* Linn and *A. compressus* (Sw.) P. Beauv might alleviate the cadmium toxicity by precipitation in the form of cadmium silicate and insoluble inorganic compound. Silica also effectively alleviated the cadmium toxicity in maize (*Zea mays* L.) (Liang *et al.*, 2005). The formation of Zn-silicate is part of the heavy metal tolerance mechanism and might be responsible for the amelioration of the Zn toxicity in *Cardaminopsis halleri* (Neumann and Nieden, 2001). Concluding from the colocalisation of Si and Al in *Picea* needles, *Sorghum*, *Triticum* and *Pinus* roots it was suggested that the formation of extracellular insoluble Al/Si compounds is responsible for the amelioration effects (Hodson and Sangster, 1993; Turnau *et al.*, 1993).

The decrease in the percentage of K and Ca in *C. rotundas* Linn and *A. compressus* (Sw.) P. Beauv after exposure to cadmium solution (Tables 5-6) indicated that cadmium caused a disturbance of K and Ca nutrition. This result was also found in the study of Ghnaya *et al.* (2005), in which Cd in the medium culture decreased K concentrations in different organs of Sesuvium portulacastrum and *Mesembryanthemum crystallinum*. A dramatic decrease of K shoot content was observed in white lupin (Zornoza *et al.*, 2002) and *Pinus sylvestris* (Kim *et al.*, 2003) under cadmium treatment. Cadmium seems to complex with ATP and reduce the availability of energy for the functioning of membrane transport systems. Therefore, the roots of stressed plants decrease the uptake of K and cause a fall of K concentration in some organs (Asp *et al.*, 1994; Ouzounidou *et al.*, 1994). The effects of cadmium on calcium nutrition have shown conflicting results. Some studies showed that cadmium supply reduced Ca in leaves of *Fagus sylvatica* (Breckle and Kahle, 1992), *Pinus pinea*, *Pinus pinaster* and *Fraxinus angustifolia* (Arduini *et al.*, 1998) and in shoots of *Betula pendula* (Gussarsson, 1994).

C. rotundas Linn and *A. compressus* (Sw.) P. Beauv. was able to accumulate cadmium from Cd-Zn contaminated soil containing 252 mg Cd kg^{-1} and pH 5.5-6.5. The higher Cd in soil, low pH (within the range of >5) and coarser texture were also associated with higher Cd concentration and Cd uptake by *Thlaspi caerulescens* (Yanai *et al.*, 2006), and the uptake of Cd by willow found tended to increase with increasing soil Cd (Vandecasteele *et al.*, 2002).

C. rotundas Linn growing in cadmium solution and Cd-Zn contaminated soil accumulated cadmium in both shoots and roots to a higher level than *A. compressus* (Sw.) P. Beauv (Table 3-4). These results might be because (1) *C. rotundas* Linn has tubers that effectively accumulate nutrient and metals (Baghour *et al.*, 2002); and (2) *A. compressus* (Sw.) P. Beauv produced higher phytomass, therefore the value of cadmium accumulated per gram dry weight was lower than in *C. rotundas* Linn.

Conclusions

The ability of *C. rotundas* Linn and *A. compressus* (Sw.) P. Beauv to accumulate cadmium depended on the cadmium concentration and exposure time. Cadmium accumulation in *C.rotundas* Linn was higher than the cadmium accumulation in *A. compressus* (Sw.) P. Beauv. Growth in cadmium solution and Cd-Zn contaminated soil showed that the percentage of cadmium accumulated in the root was higher than that in the leaf. Microscopic analysis by SEM/EDX implied that *C. rotundas* Linn and *A. compressus* (Sw.) P. Beauv might alleviate the cadmium toxicity by precipitation in the form of cadmium silicate, which is not toxic to plants, and cadmium tended to affect a disturbance of K and Ca nutrition, especially *C. rotundas* Linn. Although the growth of both plants were significantly inhibited by cadmium, they could be grown to protect erosion and to remediate cadmium-contaminated soil due to high cadmium accumulation in the stable form of cadmium silicate.

Acknowledgement

The authors gratefully acknowledge the supporting grant from The Joint Graduate School

of Energy and Environment, King Mongkut's University of Technology Thonburi.

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