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Distribution of Cadmium and Its Effect on Growth of *Zinnia elegans*

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ABSTRACT: Roots were the major site of Cd accumulation. Cd accumulation in stems was three folds higher than that of leaves, and each internode contained more Cd than that in each pair of leaves. The accumulation of Cd in plants was affected not only by external Cd concentration but also by the plants' age and organ development stages. The Cd accumulation ability of young leaves was much higher than that of older ones. Kinetic study on the time course of Cd absorption showed that the saturation time for different plant parts was slightly different, ranging from 8 to 14 days. Cd-treated plants could release the absorbed or adsorbed Cd from their plant body, and they also showed conspicuous redistribution of Cd in different plant parts after transferring to regular Hoagland's solution for 6 days. The redistribution patterns of Cd in plants were also affected by the concentrations of Cd in pretreatment. The lower the pretreatment Cd concentration, the lower the Cd redistribution. The loss of Cd from roots was tremendous. The time course study of Cd release showed that primary extrication of Cd occurred in the first two days, and lessened afterwards. A reduction in the dry weights of whole plants, roots and shoots was observed in Cd-treated plants. Dose response curves for different organs were quite different.

KEY WORDS: Accumulation, Cadmium, Growth retardation, Redistribution, Release, *Zinnia elegans*.

INTRODUCTION

Indicator plants have been widely used for monitoring environmental pollution. They were simple, and handy, and provided a certain degree of sensitivity for assaying pollutants from different sources (Bawazir and Idle, 1989). The success of monitoring systems depended on choosing the right plant species, developmental stage of plant organs or tissues, and range of assaying (Martyin and Coughtrey, 1982). Meanwhile, plants have also played an important role in the phytoremediation of heavy metals from polluted soil through the accumulation of minerals in different parts of plants (Woolhouse, 1983). There are two ways for phytoremediation of heavy metals in the field: 1) screening of tolerant plants; 2) nursing of fast growing ornamental plants.

The concentration of Cd on the earth's surface was originally tiny and negligible. However, soils contaminated with Cd came from zinc smelter and industrial sources, including battery manufacture, dye intensifier and polyvinyl chloride (PVC) processing (Woolhouse, 1983). Part of the Cd in soil was absorbed by plants and delivered to animals and humans through the food chain (Martin and Coughtrey, 1982).

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The Cd absorption through plant roots is dependent on pH, the presence of mineral and organic matters, and concentrations of Cd in soil (Culter and Rains, 1974). Almost all plants absorbed Cd from soil through active transport (Rauser, 1990; Oritz *et al.*, 1992). The occurrence of Cd in roots is much larger than that in stems and leaves. The fact that accumulation of Cd in roots related to plant resistance against Cd^{2+} toxicity has been proved (Coughtrey and Matin, 1978).

Most studies on Cd were related to its accumulation in different tissues or organs (Greger and Lindberg, 1986; Wong *et al.*, 1984). Symptoms of Cd toxicity in susceptible plants are stunt plant bodies, root growth retardation, and small leaves with necrosis. Physiological effects of Cd on plants are as follows: affects the absorption and transport of zinc (Root *et al.*, 1975); inhibits the activities of aminolaevulinic acid dehydratase, protochlorophyllide reductase and heme-related enzymes (Stobart *et al.*, 1985); stimulates lipoxygenase activity (Padmaja *et al.*, 1990) and reduces the rates of photosynthesis and respiration (Woolhouse, 1983).

The absorption and distribution of Cd^{2+} in *Zinnia elegans* plant were examined in this present study. The effects of Cd^{2+} on growth of plant were also reported.

MATERIALS AND METHODS

Plant materials

Seeds of *Zinnia elegans* were surfacely sterilized in 2 % sodium hypochloride solution with few drops of Tween-20 for 30 min. Seeds were sowed on the vermiculite and germinated in the dark after thoroughly washed with water. Young seedlings were hydroponically cultured in Hoagland's solution for 3 to 6 weeks with a light/ dark period of 13 h/11 h and a day/night temperature of 26℃/24℃. Three- to six-week-old plants were chosen for further experiments.

Treatment and detection of cadmium in different plant parts

Test plants were grown in nutrient solutions with addition of 10, 30, 60, 120 and 240 μM $Cd(NO_3)_2$ for 14 days. Coded numbers of leaves, internodes, cotyledons hypocotyls and roots were collected for Cd^{2+} quantification (Fig. 1). All measurements were triplicated.

The detection of Cd^{2+} in plants followed the methods of Ma and Chen (1999) with some modifications. Different parts of the roots, stems and leaves collected were oven dried at 70 ℃ for two days and then cut into pieces. Measured 0.2 g of dried plant material in quartz test tubes were mixed with 10 ml of 14 N nitric acid for one day. Then, the mixture was digested in Prolabo MX-350 Microdigesty (CEM, Mathews, NC, U.S.A) at steps of 1, 2, 3, 4, 5 and 6 until cleaning. They were properly diluted, filtered and finally detected by a Hitachi Z6100 atomic absorpton spectrophotometer (Hitachi, Tokyo, Japan).

RESULTS

The Cd accumulation in plant tissues varied with different plant organs, and their Cd contents were as follows: roots> stems> leaves (Fig. 2). The Cd content in plants was also affected by plant age (Fig. 2), the developmental stages of organs (Table 1) and treatment of

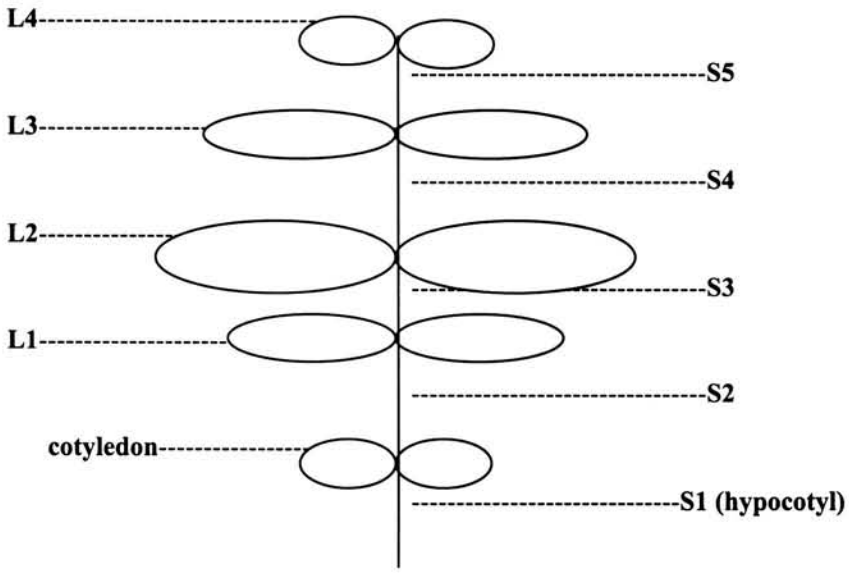

Fig. 1. Schematic illustration of plant shoots showing the relative positions of hypocotyl, cotyledons, internodes and leaves. S1, S2, S3, and S4 are the abbreviations for the first internode, second, third and fourth internode, respectively. L1, L2, L3, and L4 are the first, second, third and fourth pairs of leaves, respectively.

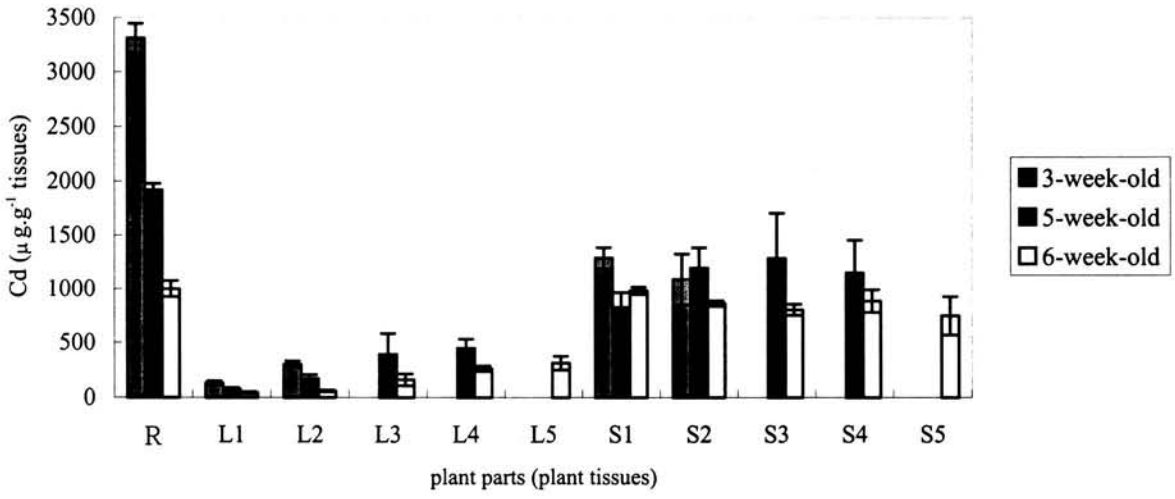

Fig. 2. Accumulation of cadmium in different parts of 3-, 5-, 6-week-old plants treated with 60 μM Cd nutrient solution for eight days. L1, L2, L3, and L4 are the first, second, third and fourth pairs of leaves, respectively.

Cd concentrations. As shown in Fig. 3, similar curves of accumulation in roots, stems and leaves were observed in plants treated with in different concentrations. Meanwhile, the 3-week-old plants had a higher capacity of Cd accumulation per plant organs than that of the 6-week-old ones.

In 60 μM Cd-treated plants, roots were the major site for Cd accumulation (Table 2). Meanwhile, Cd accumulation in stems was much higher than that in leaves, and each internode contained more Cd than that in each pair of leaves. It indicated that Cd was a mobile heavy metal, easily transported from root system to shoot systems. Moreover, Cd

Table 1. Cadmium distribution of the different parts of five-week-old *Z. elegans* plants grown in Hoagland's solution containing various concentrations of cadmium for eight days.

Cd (μM)	Cadmium of accumulation (μg·g^{-1} tissues)											
	R[a]	L1	L2	L3	L4	S1	S2	S2'[b]	S3	S3'	S4	S4'
10	720±128	4±2	31±13	104±10	101±14	337±4	218±13	222±13	233±4	245±29	274±58	228±55
30	1013±38	22±5	76±12	207±16	242±17	593±45	549±12	520±90	567±92	545±61	565±141	556±12
60	1781±64	49±3	155±39	387±50	420±25	887±104	1134±225	914±129	953±69	801±152	813±13	ND[c]
120	1902±265	83±39	191±26	434±19	642±28	1481±359	1379±111	1254±128	1287±131	1229±259	1138±61	ND
240	2592±497	79±24	104±31	322±191	550±153	1841±516	1400±54	1484±412	1308±428	1171±173	1229±235	ND

a. denotion of different parts of plant shoot as shown in Figure 1.

b. S2', S3'and S4' denoted the detrichomed internodes of S2, S3, and S4, respectively.

c. N. D. denoted non determined.

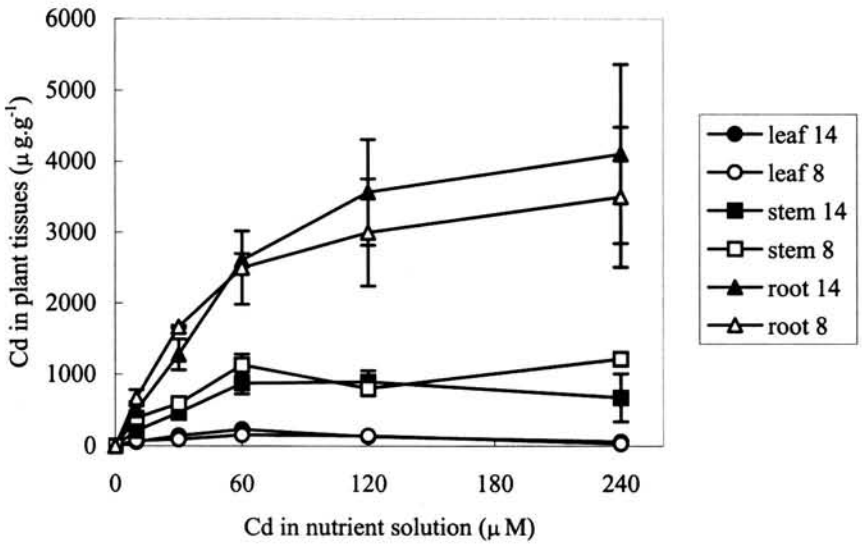

Fig. 3. Cadmium accumulation in roots, stems and leaves of 5-week-old plants treated with different concentrations of cadmium for eight and fourteen days.

Table 2. Effect of plant age on the distributions of cadmium in different organs of plants grown at 60 μM cadmium for eight days.

Plant age	Cadmium accumulation ($\mu g \cdot g^{-1}$ tissues)		
	Roots	Leaves	Stems
No. 1[a]	3311±136	432±26	2383±281
No. 2	1919±60	1091±176	4476±200
No. 3	1001±75	838±138	4301±159

a: No. 1, No. 2 and No. 3 denoted the plants in age of 3-, 5-, and 6-week-old, respectively.

accumulation in leaves was related to leaf age, showing that the younger leaf, the more Cd accumulation. It meant that the faster the leaf growth, the more Cd accumulation. A similar result was also observed in roots, but was not the same in different internodes.

Studying the time course of Cd accumulation in plants showed that the duration to reach the plateau of Cd accumulation in different plant parts ranged from 8 to 14 days (Table. 3). Plant roots absorbed external Cd in solution efficiently from the beginning (0 μg/g tissues) and reached the plateau (620 μg/g tissues) within 8 days. The kinetic studies of Cd accumulation in different internodes and leaves were different in 6 μM Cd-treated plants. In stems, days for reaching the accumulation plateau for S1, S2, S3, S4, S5 were around 6, 10, 14, 14 and 14 days, respectively. In leaves, the duration required for the accumulation plateau of L1, L2, L3, L4 and L5 were around 10, 8, 8, 8 and 12 days, respectively. Meanwhile, Cd accumulation in leaves of seedlings corresponded to the initial measurement. L1 (cotyledons) particularly showed its nutrient source characteristic in the experiment period: the detectible Cd in cotyledon tissues were only found in plants treated with external Cd longer than 4 days; the Cd accumulation in cotyledons was much less than that in ordinary leaves. These preliminary data provided good information for late developmental studies.

Table 3. Changes in Cd content in various plant parts after treatment with 6 μM Cd.

Days	Cadmium accumulation (μg·g^{-1} tissues)										
	R[a]	S1	S2	S3	S4	S5	L1	L2	L3	L4	L5
0	0	0	0	0	0	0	0	0	0	0	0
2	334±28	65±4	47±11	50±2	ND[b]	ND	0	8±5	21±13	ND	ND
4	318±28	118±3	75±3	96±11	ND	ND	0.5±0.9	11±4	44±15	ND	ND
6	470±52	184±2	116±26	134±15	ND	ND	0.8±1.4	9±1	52±7	ND	ND
8	620±47	195±22	135±28	161±26	168±40	ND	4±7	28±6	61±7	100±16	ND
10	555±25	210±17	173±16	180±0.2	194±17	ND	9±4	33±9	77±5	107±7	ND
12	568±59	183±8	179±10	196±33	224±4	186±40	8±6	20±3	57±12	97±21	100±17
14	476±38	198±48	198±54	243±18	229±34	246±37	12±3	42±16	57±17	103±11	101±10

a. denotion of plant parts was shown in Figure 1.

b. N. D.; non-determined.

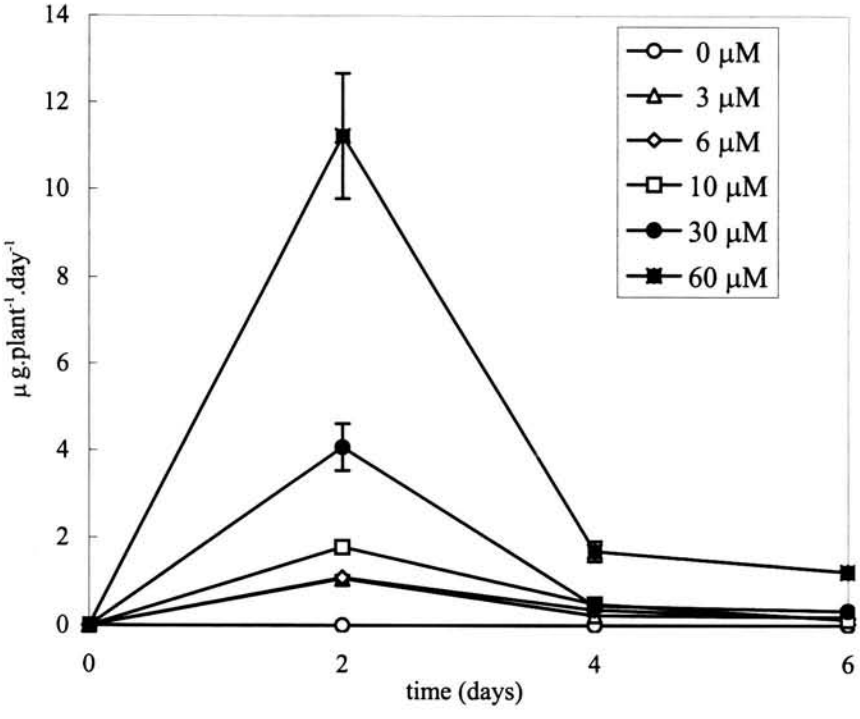

Fig. 4. Time course of released cadmium from plants pretreated in different concentrations of cadmium for eight days, and then grown in plain nutrient solutions for different periods.

The Cd released from Cadmium-treated plants transferred to plain Hoagland's solution increased in the first 2 days, and then declined (Fig. 4). The Cd-treated plants showed conspicuous redistribution of Cd in different plant parts after transferring to Hoagland's solution for 6 days (Table 4). The redistribution of Cd in plant parts was affected by the pretreatment Cd concentrations. The lower the pretreatment Cd concentration, the lower the amount redistributed. The Cd loss from roots was large and only two-fifths of Cd remained at 60 μM Cd-pretreated plants. However, nearly half of Cd was lost in second internodes, but not much Cd loss in third leaves. The higher amount of Cd loss in roots is related with the fraction of adsorbed Cd present in free spaces of roots. The Cd release from roots into the culture medium was also related to the initial Cd accumulation in plants pretreated with different Cd concentrations in culture medium for 8 days (Table 4). Plants pretreated at 60 μM Cd released more Cd to solutions than other lower Cd pretreatment. Studying the time course for Cd release showed that primary extrication of Cd occurred in the first two days and, then, lessened with time (Table 5). The above observations indicated that Cd was easily mobile and redistributed in plant bodies, and between plants and their environments.

The effect of Cd concentrations on plant growth was shown by the reduction in dry weights of whole plants, roots and shoots. However, their Cd-dose response curves for roots and shoots were quite different (Fig. 5). Root growth was retarded tremendously from 0 to 30 μM, mildly from 30 to 60 μM, and almost constantly from 120 to 240 μM. The dry weight of shoots in plants treated with Cd below 60 μM shapely and continuously decreased, and then decreased slowly down. It indicated that shoots were more severely susceptible than roots at

Table 4. Distribution of cadmium before and after transferring to Cd-gree Hoagland's solution in the root (R), second internode (S2), and third pairs of leaves (L3) of plants pretreated with different concentration of cadmium for eight days.

Cd (μM)	Cd concentrations ($\mu g \cdot g^{-1}$ tissues) in different parts of plant					
	Before transferring			6 d after transferring		
	R	S2	L3	R	S2	L3
0	0	0	0	0	0	0
3	379±73	57±19	37±7	243±41	46±14	34±7
6	575±109	111±27	69±16	260±76	81±7	66±11
10	695±136	260±34	142±31	366±43	115±13	100±13
30	1194±400	664±99	282±14	457±112	336±34	179±46
60	2271±312	1136±206	376±123	919±197	789±82	398±150

Table 5. Release of cadmium into Cd-free Hoagland's solution from plants pretreated with different concentrations of cadmium for eight days.

Treated Cd (μM)	Cd released ($\mu g \cdot$ plant^{-1} d^{-1})		
	2 d	4 d	6 d
0	0	0	0
3	1.064±0.020	0.225±0.003	0.190±0.026
6	1.088±0.054	0.356±0.014	0.159±0.011
10	1.781±0.059	0.486±0.028	0.125±0.043
30	4.072±0.537	0.447±0.091	0.324±0.072
60	11.226±1.445	1.686±0.228	1.214±0.076

0d , 2d, 4d denote the days of incubation in Hoagland's solution.

low Cd concentrations. Morphological abnormalities caused by cadmium toxicity were well described elsewhere (Chen *et al.*, 1999). Cadmiums' effect on growth of newly extending leaves and stretching internodes was shown in Fig. 6. Plants grown in 60 μM Cd showed that the effect of Cd on the S4 internode was not as distinct as that on the leaf length of L3. Meanwhile, the growth rates of internode and leaf width were less affected by Cd treatment. However, the growth rate of leaf length was reduced by 50% after Cd treatment (Fig. 7).

DISCUSSION

Zinnia elegans was highly sensitive to Cd toxicity: visible retardation of shoots and roots was observed at Cd as low as 3 μM (Tsay, 1993). Time for symptom expression of Cd

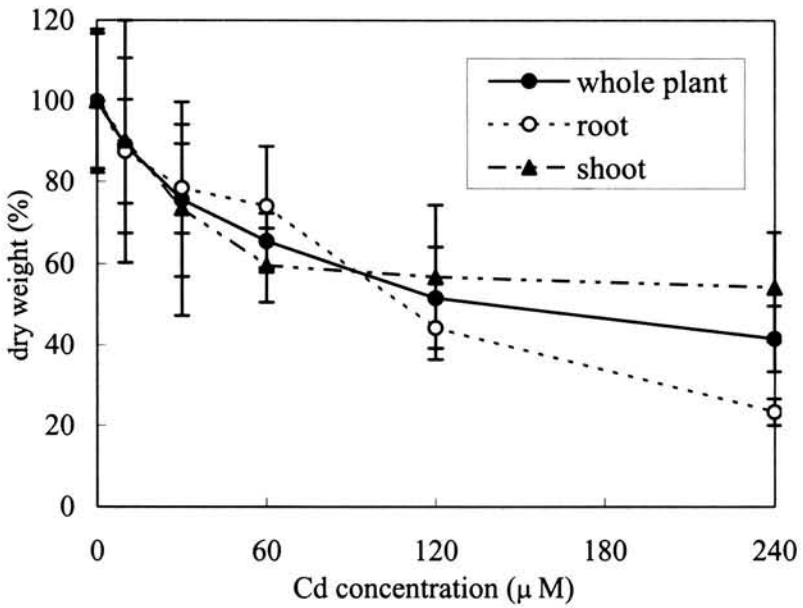

Fig. 5. Lowering in dry weight of roots, shoots and whole 5-week-old plants treated with different concentrations of cadmium for eight days.

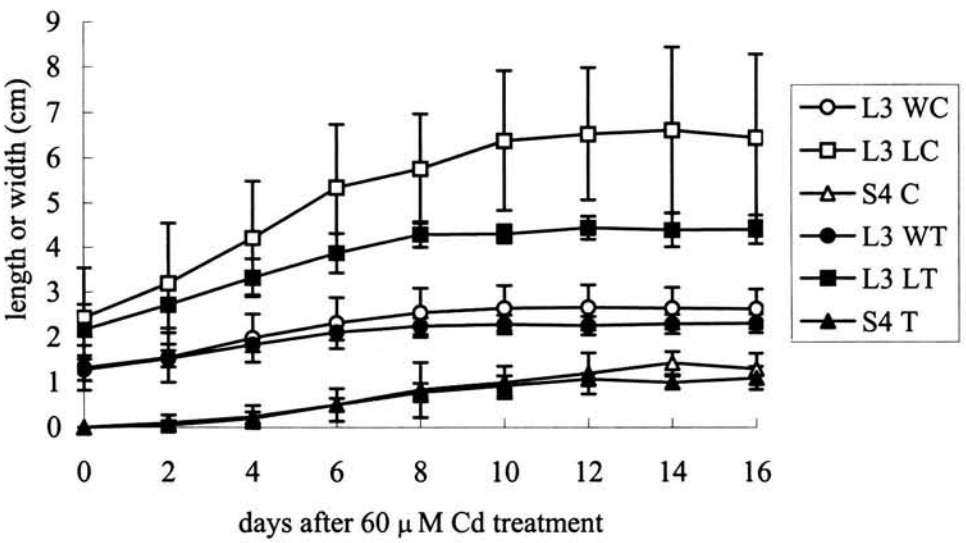

Fig. 6. Effects of cadmium on the growth of the youngest internode (S4) and extending leaves (L3). C and T denote control and treated plants, W and L denote width and length of examined plant organs, respectively.

toxicity was short. Plants treated with higher than 120 μM Cd resulted in browning of root hairs, and inhibition of root growth. A symptom of rotten roots was observed within a few hours. Studies on the internal structure of Cd affected stems showed a modification of vessels in number and size. In addition, the pattern of xylem transfer cells in the vascular bundle was changed in this ornamental plant (Chen *et al.*, 1999).

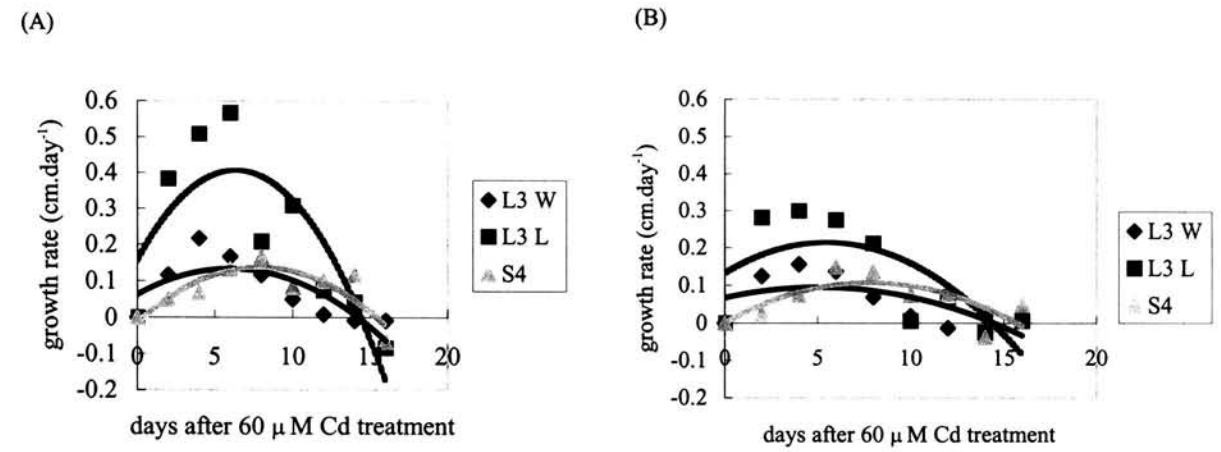

Fig. 7. Effect of cadmium on the growth rate of youngest internode (S4) and extending leaves (L4) of control (A) and Cadmium-treated (B) plants. W and L denote width and length of examined plant organs.

The elongation of stem cells of *Impatiens balsamina* was inhibited by the treatment of Cd higher than 1 μM (Aidid and Okamato, 1992). In this present study, different internodes and leaf pairs contained various amounts of Cd, and their response to Cd was quite different. The Cd content per gram dry weight of stems was three-folds higher than that of leaves. It has been suggested that trichomes on stem surfaces of *Brassica juncea* L. were the major sites of Cd accumulation in shoots (Salt *et al,* 1995). Sela *et al.* (1988) found that Cd deposition in dead *Azolla filiculoides* were 3-7 folds higher than that of living organisms, and cell walls played an important role in Cd adsorption. Apparently, further study on Cd localization and quantitation at cellular and subcellular levels is required. For this purpose, cryotechniques of energy dispersed x-ray (EDX) under transmission electron microscope might be a possible approach method.

The effects of Cd on leaves were related to the age of *Z. elagans* leaves. Cadmium toxicity was found to cause chlorosis of young leaves and enhancing senescence of old leaves (Chen *et al.*, 1999). Cd accumulation in young leaves was much higher than that of old leaves. The results were contrast to the observations of Cd-treated *Nicotiana tabacum,* in which old leaves were the major sites of Cd accumulation and Cd accumulation in midrib cells was slightly lower than that of mesophylls (Wagner, 1986). Possibly, the patterns of Cd transportation in plants varied from species to species.

Redistribution of Cd in plant body occurred when Cd-treated plants were transferred to Cd-free culture mediums, suggesting that Cd was readily transported in plants. The phenomenon of more Cd loss from roots than from shoots might be related to the adsorbed Cd present in free space of cell walls. In hydroponically cultured *Eichhornia crassipes*, the adsorbed Cd was related to the pH of the medium (Fett *et al.*, 1994). These phenomena were especially distinct when plants were treated with higher Cd concentrations. Apparently, further studies on Cd absorption and translocation are necessary.

ACKNOWLEDGEMENTS

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鎘在百日草植株中的分布及其對植株生長的影響

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摘　　要

植物吸收鎘離子主要存在於根部。將地上部區分節間與對葉一一對應時檢測各區的鎘分布情形，莖部的總鎘累積量約為葉部的三倍，同時各節間的鎘累積量也比各對葉多。鎘在植物體內的累積量與處理外界鎘濃度和植物生長發育時期有關，幼葉的鎘累積量比老葉高。鎘吸收的動力學研究顯示不同的區位其飽和時間約在 8 至 14 天之間。鎘處理的植株移置無鎘的營養液後六天，植物體內的鎘有重分布的現象，其中以根部的鎘流失嚴重；鎘的流失量與植物的先鎘處理處理有相關；植株內鎘的重分布以移置後兩天內最多，而後急速減少。植物各部對鎘毒害的反應曲線不一致，有關鎘在莖部的橫向運輸與其意義須待進一部的探討。

關鍵詞：累積量、鎘、生長抑制、重分布、釋出、百日草。

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