Bull. Environ. Contam. Toxicol. (1993) 51:914–919

Environmental Contamination and Toxicology

Uptake and Translocation of Cd^{109} by Two Aquatic Ferns in Relation to Relative Toxic Response

Jaswant Singh,[1] P. N. Viswanathan,[1] Manisha Gupta,[2] and Santha Devi[2]

[1]Industrial Toxicology Research Centre, Post Box 80, Lucknow-226001, India and [2]National Botanical Research Institute, Lucknow, India

Considerable variations exist in the phytotoxic response of different plants to cadmium exposure and uptake as observed in experimental and field studies (Sela et al. 1990 and Nir et al. 1990). Quantitative and qualitative variations in comparative anatomy, physiology and biochemistry could be responsible for selective toxicity (Albert 1979 and Barcelo et al. 1988). Variations in uptake (Taylor and Foy 1985), translocation (Van de Geijn and Petit 1978), sequestration by cell wall (Khan et al. 1984), phytochelation (Grill et al. 1985) or formation of inclusion bodies (Rauser and Ackerley 1987) have been reported in phytotoxic response to cadmium. Earlier studies by Singh et al. (1991) with the aquatic fern *Marsilea minuta* Linn showed Cd^{2+} induced both ultrastructural lesions and metallothioneins at concentrations above 0.5 ppm. However, another aquatic fern, *Ceratopteris thalictroides* (L.) Brongn was even more sensitive to cadmium (Gupta et al. 1992). In order to understand the basis of this variation, the comparative uptake and translocation of radioactive Cd^{109} by these plants was studied.

MATERIALS AND METHODS

Specimens of *Ceratopteris thalictroides* with four adult leaves were selected from original aseptic stock cultures maintained in 3% Hoagland's medium (EPA 1975) under laboratory conditions. Similarly, mature plants of *Marsilea minuta* were selected from aseptic stock cultures maintained in the laboratory (Singh et al. 1991). Throughout the experiment plants were maintained in a growth chamber under standard physiological conditions at 25±0.5 °C with 16 hr fluorescent light (1600 Ft.C.) and 8hr dark cycle.

Send reprint requests to J.Singh at the above address.

The Hoagland's medium was supplemented with cadmium chloride to provide Cd concentrations of 0.0, 0.05, 0.1, 1.0, 2.5, and 5.0 mg/l. These concentrations were selected on the basis of earlier data (Singh et al. 1991). Twelve 250-ml flasks containing 150 ml of amended media were established. Six plants of either C. thalictroides or M. minuta were put into each of six flasks. After 48 hr the plants were harvested, washed with cadmium free glass-distilled water, blotted dry and weighed. The samples were dried at 110 °C, digested in 10 N H_2SO_4 and HNO_3 mixture and cadmium content determined using a Perkin-Elmer, Model 5000 Atomic Absorption Spectrophotometer. Recovery of cadmium estimations was assessed by the analysis of wheat flour sample (National Institute of Standards and Technology, SRM 1567). The detection limit for cadmium was 0.02 µg/ml.

In another set of experiments the uptake of Cd^{109} was compared in the above two aquatic plants. Radioactive cadmium as $Cd^{109}Cl_2$ (specific activity 1mCi/µg Cd) was procurred from Radiochemical Centre Amersham, U.K. 0.1 µCI Cd^{109} was mixed in one liter of Hoagland's medium containing 0.05 ppm cadmium as $CdCl_2$. Seven plants of either C. thalictriodes or M. minuta were put into each of seven flasks, and maintained as above. After 2,4,8,14,24,32 and 48 hr one plant from each of the flasks was harvested, washed thoroughly with cadmium free glass-distilled water and blotted on paper. Each plant was devided into roots and remaining portion (designated as shoots), separately weighed and Cd^{109} incorporation was recorded on LKB ultrogamma counter. Student 't' test as described by Fisher (1950) was used to calculate the statistical significance between control and experimental values.

RESULTS AND DISCUSSION

The uptake of radioactive Cd^{109} showed a proportional increase with time of exposure in Marsilea minuta and Ceratopteris thalictroides (Table 1). The content of radioactivity in C. thalictroides in terms of tissue mass was considerably higher ($p<0.001$) than that in M. minuta at all the stages, even though the magnitude of increase was low. In M. minuta the root retained most of the radioactivity, since very few counts were detected in the shoot. There was generally an increasing trend in the Cd^{109} level in shoots, but it was always very low (less than 2%) in comparison to the levels in the roots. In C. thalictroides, the levels in shoots progressively increased along with uptake in the roots. Even in 2 hr, the concentration in shoots was more than half

Table 1. Mean uptake concentrations (± S.E.) of Cd^{109} by *Marsilea minuta* and *Ceratopteris thalictroides* plants in culture at different time intervals (counts per minute/g), N=7 (one plant from each of seven flasks).

Time (hr)	*M.minuta* Root	Shoot	*C.thalictroides* Root	Shoot
2	2506±444	292±47 (30.2)	3394±791	1865±74 (56.3)
4	3070±608	330±79 (11.2)	3845±258	2376±274 (53.8)
8	3477±246	381±69 (9.5)	4390±360	3031±602 (54.4)
14	4350±520	627±64 (14.0)	4959±382	4251±132 (36.9)
24	4686±258	701±75 (13.7)	5174±383	4830±88 (58.2)
32	4686±412	514±60 (13.6)	8936±1162	7670±113 (54.9)
48	6998±731	817±91 (13.8)	7934±453	7462±297 (54.0)

In paranthesis Cd^{109} content of shoot tissue expressed as percentage of uptake by whole plants (shoots and roots).

Table 2. Mean cadmium concentrations (±S.E.) in whole plants of *Ceratopteris thalictroides* and *Marsilea minuta* at 24 and 48 hr. Values are in μg/g dry weight, N = 6.

Cadmium (ppm)	*C.thalictroides* 24hr	48hr	*M.minuta* 24hr	48hr
0.0	4.1±0.98	3.8±0.82	2.05±0.40	4.56±1.3
0.05	135.5±8.1	154.8±29.4	8.2±0.4	9.7±1.3
0.1	176.8±18.9	169.3±20.6	10.2±1.9	15.7±3.5
1.0	1004±133.3	898.0±121.4	21.1±2.8	29.5±5.5
2.5	2428.8±265.7 ($p<0.02$)	1894±280.5 ($p<0.05$)	43.8±4.6 ($p<0.02$)	48.6±7.4 ($p<0.05$)

that in roots and this proportion increased regularly till the end of the study. At 48hr, the ratio of Cd 109 concentration in the shoot was about 95% that in the roots. The corresponding figure for *M.minuta* was less than 14%. The translocation in *M. minuta* was much less and slower than in *C.thalictroides*. This was indicated when the radioactivity in each whole plant was compared with that of the corresponding whole shoot tissue. Radioactivity levels in the shoot tissue of *M.minuta* after 2, 24 and 48hr were 30.3, 13.7 and 13.9% of the whole plant, whereas the corresponding figures for *C.thalictroides* were 56.3, 58.1 and 54.0%, respectively.

After homogenization in water and centrifugation at 12000g, the radioactivity levels in residue and suspended fractions in roots and shoots of both plants exposed for 48hr were recorded. Radioactivity in the residue fraction in both shoots and roots (36.9 and 31.5%) was much higher in *C .thalictroides* than in *M .minuta* (13.2 and 14.7%). This could be indicative of a higher level of Cd^{2+} sequestration by the cell wall or intracellular organelles leading to greater toxicity in *C. thalictroides* . Uptake of cadmium by *M. minuta* and *C. thalictroides* at 24 and 48hr is shown in Table 2. In both plants the uptake of Cd^{2+} from the medium showed a dose-dependent relationship. Cadmium concentrations were always higher at 48 hr, although statistical significance was low ($p<0.05$). However, cadmium concentrations in *M.minuta* were significantly lower than in *C.thalictroides* at all the test concentrations. Growth retardation, as calculated from biomass, was more in *C.thalictroides*, with 1.0 ppm showing the earliest effect at 48hr as compared to 2.5 ppm for *M.minuta*.

The uptake and translocation of Cd^{2+} has been very well studied in terrestrial plants (Koeppe 1977; Peterson 1977; Petit and Van de Geijn 1978; Van de Geijn and Petit 1978), but the uptake of trace metals by aquatic macrophytes has not. The relative uptake through roots and leaves is not yet clear for these plants. The uptake of Cd^{2+} by the aquatic fern *Azolla filiculoides* showed an initial rapid increase within the first hour, followed by a continuous gradual accumulation phase throughout the 77hr of the experiment. The Cd^{2+} content increased in the inner epidermis, cortex and bundle cell walls of the roots (Sela *et al*. 1990). The present data indicate that the rate of uptake and translocation of Cd^{2+} is much faster in *C.thalictroides* than in *M.minuta*. Dose-dependent effects on biomass, cadmium concentrations and ultrastructural changes were also more prominent in this plant (Gupta *et al.* 1992). The present data suggest that the higher sensitivity of *C.thalictroides*, as compared to *M.minuta*, could be due to faster uptake, translocation and higher interaction with membraneous structures.

Acknowledgments. The authors thank Dr. S.V. Chandra, Director, ITRC and Dr. P.V. Sane, Director, NBRI for their interest in this work. Word processing of the manuscript by Mr. Ram Vimal is also greatly acknowledged.

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Received January 22, 1993; accepted June 3, 1993.