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Cadmium Sensitivity Inducing Structural Responses in *Salvinia molesta* Mitchell

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Salvinia molesta Mitchell a free floating freshwater fern is a major noxious weed, threatening aquatic ecosystems in tropical countries (Thomas and Room 1986). The ability to absorb high levels of mercury and copper by Salvinia natans has been demonstrated by Sen and Mondal (1987, 1990). Enhanced nutrient uptake ability of Salvinia has also been reported by Reddy and DeBusk (1985). Accumulation of heavy metals, especially lead, resulting in the reduction in the size and number of fronds was observed in Salvinia natans by Puckett and Burton (1981).In the case of cumulative toxins like Cd, visible signs of injury appear late in the development of a plant. Ultrastructural changes, however, could appear earlier and at low doses, and, thus, could serve as indicators of cadmium toxicity. Therefore, the effect of Cd on the morphology and growth characteristics of Salvinia molesta was studied.

MATERIALS AND METHODS

Vegetative clones of Salvinia molesta were cultured in 3% Hoagland's solution (EPA 1975) at 25±0.5 ° C, 16 hrs light/8 hrs dark photoperiod, 1600 ftc, fluorescent light (Chawla et al. 1989). Using AnalaR $CdCl_2.H_2O$ (Loba-Chemie Indoaustranal Co.) 0.025, 0.05, 0.075, and 0.1 ppm concentrations of Cd were prepared in 3% Hoagland's solution. Control experiments, using 3% Hoagland's solution only, were performed simultaneously. Young plant of Salvinia with 6-7 pairs of leaves was floated in triplicate for each group. To assess biomass, protein and Cd levels harvesting of 3 plants was done at periods specified. The plants were washed with deionised water, weighed, dried at 70 C for 48hrs and digested with 10N H_2SO_4, HNO_3 mixture. Cd was estimated using a Perkin Elmer 2380 Atomic Absorption Spectrophotometer (Detection limit for Cd =

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0.0005 ppm). Protein content was estimated by the method of Lowry et al. (1951), chlorophyll a and b according to Arnon (1949) and carotenoids by the method of Duxbury and Yentsch (1956). Student's 't' test described by Fisher (1950) was employed to calculate the statistical significance between the control and experimental values.

To determine effects of Cd on S. molesta using electron microscopy, materials were prefixed in 2.5% glutaraldehyde, followed by 1% osmium tetroxide. For SEM, specimens were dried in a critical point drier, coated with gold in an ion sputter coater and examined by stereo scanning electron microscopy at 30 tilt and 10 KV. For TEM the material was embedded in epon araldite, sections cut on a LKB ultramicrotome using glass knives, stained with aqueous uranyl acetate and lead citrate and examined under a Philips CM-10 transmission electron microscope.

RESULTS AND DISCUSSION

The growth pattern as followed from the number ofleaves per plant was unaffected by Cd at 0.025 ppm (Fig. 1). Cadmium at 0.05 and 0.075 ppm showed a small increase in leaves from 24 to 72 hrs and then a decrease. Higher concentrations not only prevented further leaf production, but led to decay of existing leaves especially after 72 hrs of exposure. At 0.1 ppm there was 35% death of the shoot by the 5th day, as evidenced from the decrease in leaves. In control plants there was a marginal increase in biomass upto 48 hrs, but at 72 and 120 hrs the weight gain was 12 and 15% as compared to zero time. There was an initial increase in the biomass of 0.025 ppm Cd-treated plants (Table 1), but at 120 hrs it was 13.53% less as compared to the control (100%). In all other concentrations the decrease was pronounced with the period of exposure. There was an increase at both 24 and 48 hrs at 0.05 ppm Cd.

There was a dose and time-dependent uptake of Cd (Fig.2). At 0.025 ppm and 48, 72 and 120 hrs the values were about 1.8 ($P<0.05$), 2.4 ($P<0.02$), and 2.6 ($P<0.02$) fold higher than those at 24 hrs. With other concentrations the Cd content also increased with time of exposure. The uptake levels at 24 hrs at 0.05, 0.075, and 0.1 ppm were about 1.3 ($P<0.1$), 1.3 ($P<0.1$) and 2.9 ($P<0.01$) fold higher than that at 0.025 ppm. A similar pattern was observed at other time periods as well. The maximum uptake of 1290 µg/g dry wt ($P<0.02$) occurred at 0.1 ppm at 120 hrs of exposure. Total chlorophyll content decreased due to Cd exposure in a dose and time-dependent manner (Table 2).

Table 3. Effect of different concentrations of Cd on carotenoid content in S. molesta at different time intervals.

Conc. of Cd (ppm)	Carotenoid content (mg/g fresh wt) 48 hrs	96 hrs	168 hrs
0.0	0.276±0.012	0.287±0.009	0.293±0.007
0.025	0.267±0.007*	0.251±0.002*	0.245±0.006*
0.05	0.256±0.003*	0.235±0.004**	0.221±0.013**
0.075	0.238±0.006*	0.195±0.033***	0.185±0.005***
0.1	0.216±0.006**	0.195±0.011***	0.182±0.005***

Each value is the arithmetic mean ± S.D. of triplicate

* Not significant; ** P<0.05; *** P<0.001

Table 4. Effect of different concentrations of Cd on protein content in S. molesta at different time intervals.

Conc. of Cd (ppm)	Protein content (mg/g fresh wt.) 24 hrs	48 hrs	72 hrs	120 hrs
0.0	10.19±1.6	15.08±0.65	14.79±1.26	13.57±1.02
0.025	9.48±0.96*	13.52±5.74	11.95±1.83*	10.26±2.01*
0.05	11.08±1.63*	12.13±3.77	9.73±2.71**	8.68±3.13**
0.075	10.94±3.06*	6.40±0.27**	6.92±2.24**	5.18±0.42**
0.1	9.95±1.40*	4.33±0.042***	5.72±0.86***	5.50±1.10***

Each value is the arithmetic mean ± S.D. of triplicate.

* Not significant; ** P<0.05; *** P<0.001

same concentration of Cd using younger plants twenty days old, the plants in 0.025 ppm also showed cell collapse and shrinkage of basal cells of the trichome on the abaxial surface (Fig. 6). They also showed tears in the guard cells on the adaxial surface as compared to the control where the basal cells of the trichome were swollen and stomata normal (Fig. 5). The epidermal cells appeared turgid and highly stretched. Browning of the roots was observed in 0.05 to 0.1 ppm Cd.

Sections of control leaf under TEM showed an intact plasma membrane, mitochondria, chloroplasts and some stages of vacuole formation (Fig. 7). Below 0.1 ppm there was no change in the ultrastructure. In leaves

Table 2. Effect of different concentrations of Cd on total chlorophyll content in S. molesta at different time intervals.

Conc. of Cd (ppm)	Total chlorophyll content (mg/g fresh wt.)		
	48 hrs	96 hrs	168 hrs
0.0	0.891 ± 0.127	0.997 ± 0.045	1.016 ± 0.051
0.025	0.816 ± 0.023 *	0.684 ± 0.050 **	0.631 ± 0.019 **
0.05	0.806 ± 0.002 *	0.724 ± 0.015 **	0.638 ± 0.024 **
0.075	0.753 ± 0.013 **	0.721 ± 0.043 **	0.640 ± 0.039 **
0.1	0.703 ± 0.018 **	0.597 ± 0.024 **	0.452 ± 0.029 ***

Each value is the arithmetic mean ± S.D. of triplicate

* Not significant; ** $P<0.05$; *** $P<0.01$

A 56% decrease was observed at 168 hrs and 0.1 ppm ($P<0.01$). However, the Chl a / Chl b ratio did not show any statistically significant change. Carotenoid content also decreased on Cd exposure and the effect was of a higher magnitude at later periods and higher concentrations, indicating a dose-time effect relationship. The largest decrease of 38% ($P<0.01$) was observed at 0.1 ppm and 168 hrs (Table 3).

Protein content was unaffected at 24 hrs (Table 4) but by 48 hrs, 0.075 and 0.1 ppm caused toxicity as evident from 58 ($P<0.05$) and 72% ($P<0.02$) decrease in values as compared to the control. A decrease in protein was manifested from 48 hrs onwards in the case of 0.05 ppm. The lowest dose of 0.025 ppm showed no significant change in protein.

The adaxial surface of leaves in controls showed the presence of egg-beater shaped unwettable, multicellular hairs (Fig. 3), a regular wax coating and sunken stomata. In plants treated with 0.05 ppm Cd the upper surface of cells had acquired a slightly circular shape with upturned walls as against hexagonal cells of the control. Treated plants showed detachment of the tip of the egg-beater shaped hairs, the extent of detachment increasing with the higher concentration of Cd (Fig.4). When the experiment was repeated with the

Table 3. Effect of different concentrations of Cd on carotenoid content in *S. molesta* at different time intervals.

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0.025	0.267±0.007*	0.251±0.002*	0.245±0.006*
0.05	0.256±0.003*	0.235±0.004**	0.221±0.013**
0.075	0.238±0.006*	0.195±0.033***	0.185±0.005***
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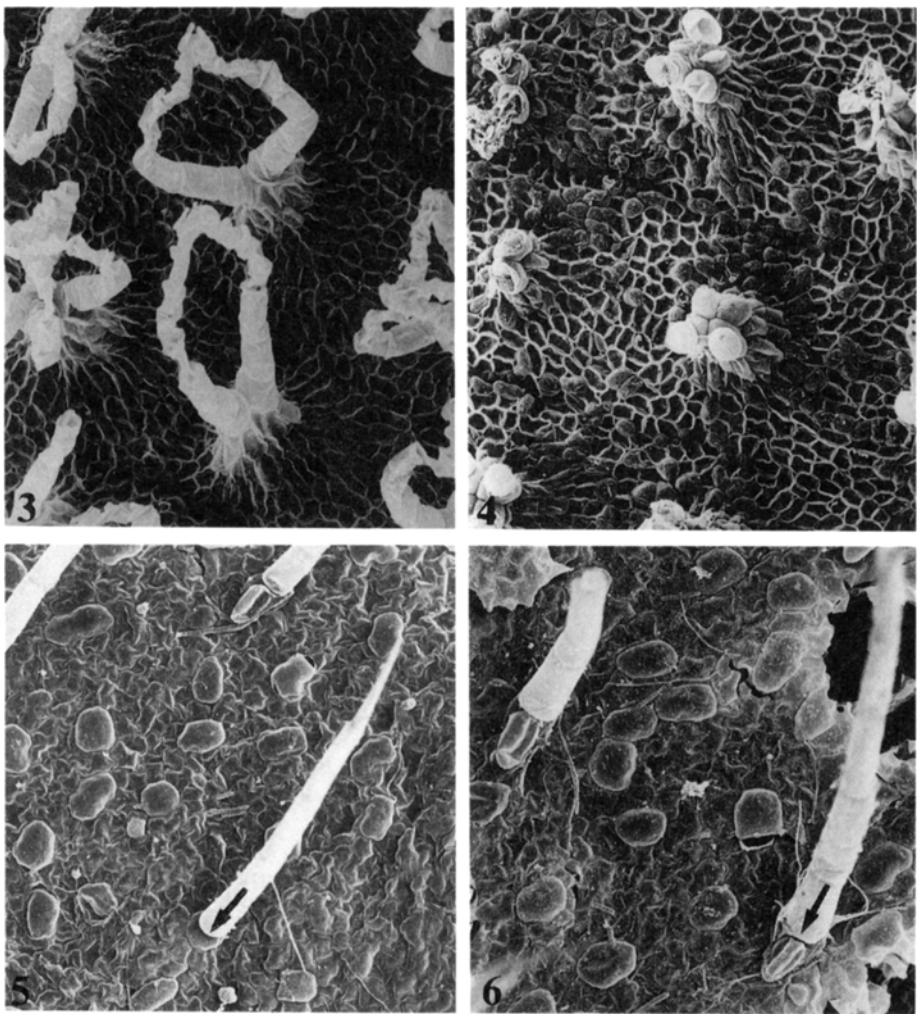
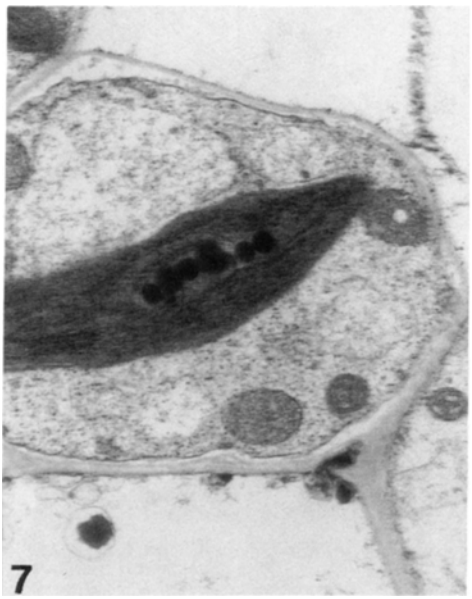
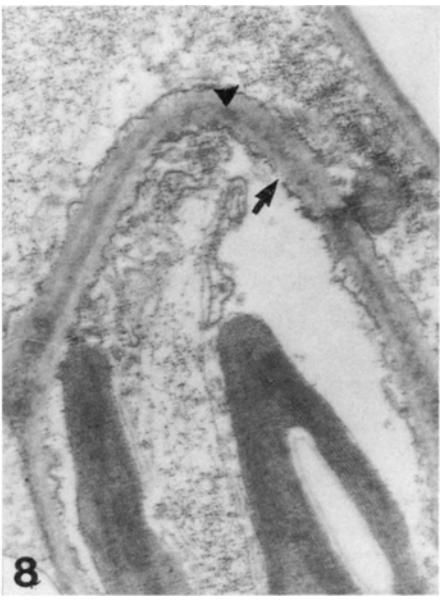


Figures 3-6. SEM, 3, 4 x 100 5, 6 x 200

3. Control adaxial surface of a leaf showing egg-beater shaped hairs.
4. Adaxial surface of leaf treated with 0.1 ppm Cd. Only the basal cells of the trichome are present; the upper cells are totally detached.
5. Abaxial surface of control leaf showing swollen hair bases (arrow) and epidermal cells.
6. Abaxial surface of leaf treated with 0.025 ppm Cd showing injury of the epidermal cells and shrunken base of the hairs (arrow).

treated with 0.1 ppm Cd a simiar cell showed shifting of the cytoplasm to one corner of a cell,disruption of the plasma membrane and deposition of electron opaque material along the middle lamella (Fig. 8) and discontinuity of the chloroplast envelope.





Figures 7, 8 x 38000. 7. TEM of bundle parenchyma of control leaf showing intact plasma membrane, mitochondria, chloroplast and stages in vacuole formation. 8. Similar stage of leaf treated with 0.1 ppm Cd showing shifting of cytoplasm, disruption of plasma membrane (arrow), and deposition of electron-opaque material along with the middle lamella (arrow head).

Salvinia molesta was found to be very sensitive to Cd even at 0.1 ppm, causing growth retardation and lethality (Hutchinson and Czyrska 1972). The present results show that sublethal concentrations of Cd produced significant morphological changes in Salvinia. Chawla et al. (1989) reported injury to the epidermal cells and severe damage of cuticular hairs of the upper surface in S. molesta treated with a detergent. Although epidermal damage was observed in this study, the hairs on the upper surface were not affected. An overall effect on the elasticity of the epidermis was noted. The reason for epidermal injury and the disruption of guard cells can be attributed to this loss of elasticity. At the ultrastructural level the disruption of the plasma membrane is an important factor. The plasma membrane, which acts as a regulatory barrier for the transport of vital molecules, and, in turn, influences the overall function of the cell constituents, appears to be the earliest target of phytotoxicity of Cd.

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