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Effect of Cadmium on Spore Germination and Gametophyte Development in Some Ferns

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Fern spores have received the attention of environmental scientists as a model for in situ mutagenicity screening (Klekowski and Davis 1977), for phytotoxicity evaluation of pollutants (Francis and Petersen 1983a, b; Petersen et al. 1980) and more recently as aeroallergens (Yasmeen et al. 1988, Singh et al., 1989). Earlier, the effect of linear alkylbenzene sulphonate, a detergent component of relevance to water pollution, on the spores of Diplazium esculentum and Ceratopteris thalictroides was reported from this laboratory (Devi and Devi, 1986; Singh and Devi 1989). A roadside environment contaminated by cadmium, copper and lead is reported by Ho and Tai (1985) and Largerwerff and Specht (1970). Several terrestrial ferns grow on the roadside or by sewage drains (Singh and Devi 1987). There have also been reports regarding Cd contamination in rice growing soil and sewage (Bingham et al. 1975; Dabin et al. 1978). Ceratopteris is a water fern known to grow on rice fields and contaminated waters in closed bodies. Since Cd is a major toxic pollutant of soil and water, its effect on the spore germination and early development of the gametophytes of some terrestrial, epiphytic and aquatic ferns was compared in the present study.

MATERIALS AND METHODS

Spores of aquatic (Ceratopteris thalictroides (L.) Brogn), epiphytic (Drynaria quercifolia (L.) J. Sm.) and terrestrial (Christella parasitica (L.) Tardieu, Pteris ensiformis Burm., P. vittata L., Thelypteris augescence (Link.) Munz. and Johnston, Ampelopteris prolifera (Retz.) Copel., and Adiantum lunulatum Burm.) were collected from fertile fronds from the greenhouse of the National Botanical Research Institute, Lucknow, and stored in a dessicator under sterile conditions. Solid Knop's medium (Miller and Greany 1979) with agar was used for spore cultures. The culture medium in petriplates was dosed with cadmium chloride to attain the following concentrations of Cd: 0.0, 0.1, 1.0, 2.5, 5.0 and 10.0 ppm. One mg of spores was then sprinkled with a piece of glazed paper into the culture medium under asceptic conditions and the petriplates were maintained

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Table 1. Percent spore germination as affected by cadmium in different taxa.

Taxa	Cd concentrations					
	Control	.1 ppm	1 ppm	2.5 ppm	5 ppm	10 ppm
P. vittata	38.81 ±2.45	35.74 ±4.02	29.63 ±1.52	23.27 ±2.49	13.87 ±.57	4.63 ±.94
P. ensiformis	81.83 ±2.92	67.93 ±6.09	61.8 5.06	63.71 ±12.8	61.12 ±4.74	66.17 ±7.85
A. lunulatum	91.4 ±5.07	79.64 ±7.68	66.13 ±6.75	43.55 ±3.54	11.65 ±3.74	6.27 ±2.22
C. thalictroides	7.73 ±1.83	7.73 ±.84	7.07 ±1.15	6.81 ±.59	4.97 ±.80	4.41 ±.51
T. augescence	55.04 ±3.61	46.74 ±4.64	43.64 ±4.02	43.49 ±1.51	43.60 ±6.02	41.2 ±7.62
D. quercifolia	58.84 ±1.18	58.96 ±22.11	52.89 ±8.13	18.10 ±3.05	17.27 ±8.59	12.83 ±4.26
A prolifera	52.66 ±8.70	55.87 ±9.98	38.03 ±6.73	32.94 ±8.7	20.25 ±4.11	6.75 ±.28
C. parasitica	98.25 ±1.51	92.95 ±4.20	92.23 ±3.71	90.77 ±5.34	82.34 ±4.36	65.53 ±4.26

Each value is the arithmetic mean ± SD. The experiment was done in triplicate. n= 1500.

under standard physiological conditions of 25±0.5°C, 16 hrs light/8 hrs dark photoperiod, 1600 ft c, fluorescent light. The cultures were run in triplicate and maintained until germination. After determining the completion of the germination period which varied with the species, only 500 spores were scored from each plate. Percent germination was calculated for each replicate. Statistical analyses were done with the three sets for each concentration by the Students 't' test, with the total number of spores in each case being 1,500.

For calculating the onset of sex organs, the date at which the earliest manifestation was observed was taken as the index for each species. Since the number of gametophytes formed from the original 1500 spores varied from plate to plate, statistical evaluation in terms of the production of the sex organs was not justifiable; thus, comparisons were made on the basis of the date of initiation.

RESULTS AND DISCUSSION

Germination of spores started after 2 days in C. parasitica, 3 days in D. quercifolia and A. prolifera, 5 days in T. augescence and C. thalictroides, and 7 days in the others. The data

Table 2. Onset of the development of sex organs in different taxa. Values represented in days.

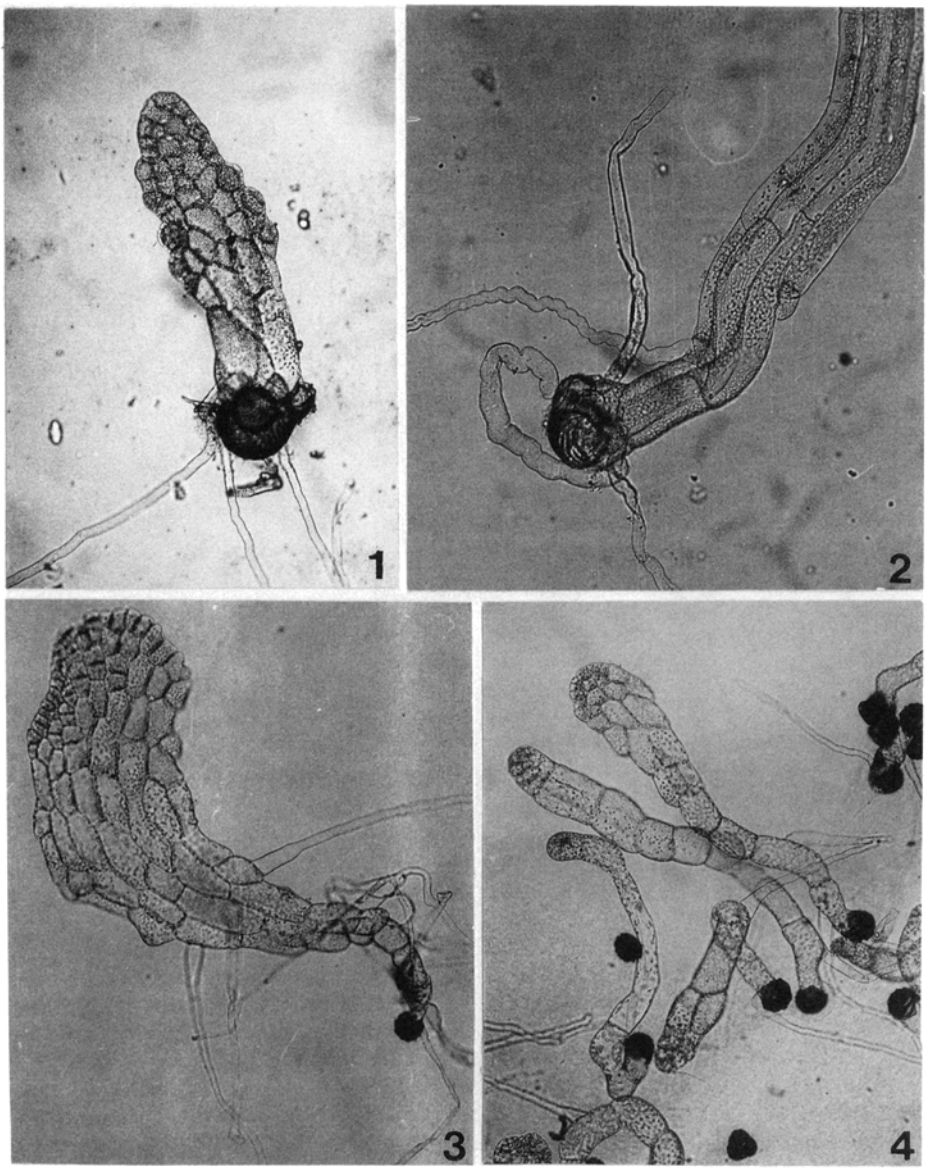
Taxa	Cd concentrations					
	Control ♂/♀	.1 ppm ♂/♀	1 ppm ♂/♀	2.5 ppm ♂/♀	5 ppm ♂/♀	10 ppm ♂/♀
P. vittata	39 53	39 60	39 60	39 60	39 60	60 85
P. ensiformis	45 76	53 82	59 82	66 82	76 104	82 104
A. lunulatum	45 92	59 92	66 92	98 98	98 98	104 104
C. thalictroides	18 38	20 55	x x	x x	x x	x x
T. augescence	67 55	67 55	67 58	67 58	67 58	67 58
D. quercifolia	65 103	85 103	85 x	85 x	103 x	103 x
A. prolifera	50 90	50 90	70 x	x x	x x	x x
C. parasitica	44 34	44 41	44 41	44 41	49 48	50 50

x=nil
Values are expressed as the first day of initiation of sex organs which was the same in all the triplicates in the same group.

on the percent spore germination are given in Table 1. The pattern of percent spore germination and viability in different concentrations of Cd was different in individual species. C. parasitica was the most viable (98%) and C. thalictroides (8%) the least. In all speices there was a gradual reduction in the germination rate from 2.5 ppm onwards except in P. ensiformis where the reduction was from control to 1.0 ppm after which the pattern was steady. T. augescence showed no significant inhibition in any concentration. Stimulation of growth was observed in 0.1 ppm Cd in D. quercifolia and A. prolifera.

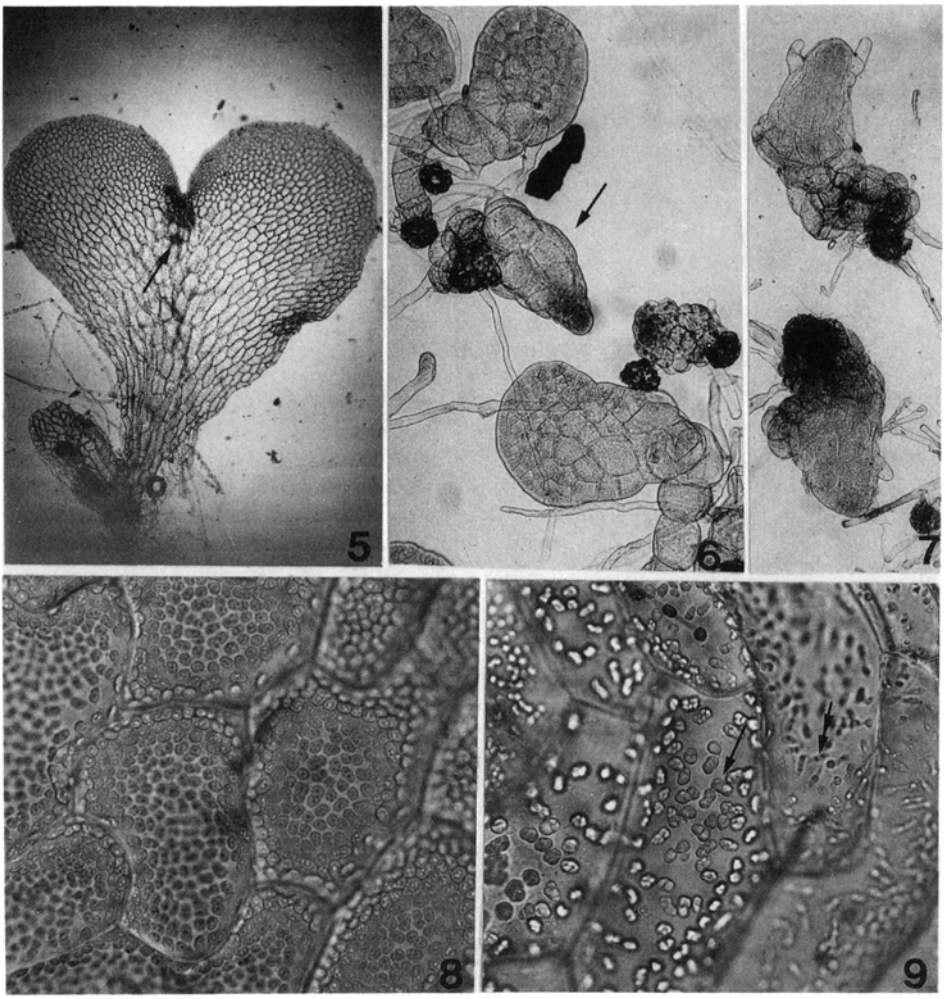
Taking the germination index of the control as 100%, the EC 50 (50% decrease in germination) was calculated by the Trimmed Sperman Karber computer program (Hamilton et al. 1978). The values are 2.7 ppm for P. vittata, 1.7 for A. lunulatum, .4 for C. thalictroides, 2.1 for D. quercifolia and 2.8 for A. prolifera. In the other species the EC 50 values could not be calculated because the mortality even at the highest concentrations was below 50%.

The morphological charcters of the rhizoids were affected in



All Figures x 100.

1. C. thalictroides control showing straight rhizoids. After 15 days.
2. Portion of C. thalictroides gametophytes. .1 ppm Cd showing swollen, curled rhizoids. Thallus highly elongated. After 15 days.
3. P. ensiformis control thallus showing meristematic tissue. After 30 days.
4. P. ensiformis thalli showing delayed development at 10 ppm Cd. Meristem not formed. After 30 days.



Figs. 5-7 x 100, 8,9 x 400

5. P. vittata control cordate formation and initiation of sex organs (arrow). After 53 days.

6. P. vittata, 5 ppm Cd, showing bunch of bulbous cells and another one with the apogamous sporophyte formation (arrow). After 50 days.

7. P. vittata 5 ppm Cd, fully developed apogamous sporophyte from bunch of bulbous cells showing two palaeate hairs and a meristematic tip. After 60 days.

8. C. thalictroides. Control. Surface view of the mature gametophyte showing normal chloroplasts. After 40 days.

9. C. thalictroides, 2.5 ppm Cd showing reduction in the density of the chloroplasts. Note the cleavage (arrow) and elongation (double arrow) of the chloroplast.

many taxa. In C. thalictroides while the rhizoids were thin and straight in the control (Fig. 1) the treated ones were swollen and curly (Fig. 2) and often discharged the contents on pressure. C. parasitica, P. vittata and A. lunulatum also produced swollen, curly rhizoids. In gereral, the thalloid form of the prothalli was obtained comparatively later than the control (Figs.3 & 4) in all species. Development of the sex organs was also delayed in those samples treated with cadmium (Table 2). There was no archegonia production after 1 ppm onwards in any concentration in C. thalictroides. Antheridia formation, however, continued. In P. vittata the gametophytes were almost normal in 1 ppm as compared to the control (Fig. 5), while in higher concentrations, after 1-2 cells, a bunch of bulbous cells was differentiated out of which one cell would become meristematic and produce a young sporophyte directly from the gametophyte (Fig. 6). In none of the concentrations, however, did sporophyte formation continue beyond the cylindrical stage with few palaeate hairs and conical tips (Fig 7). After attaining this stage, the apogamous sporophyte died off. This type of abnormal gametophyte occured in 50% of the germinated spores in 0.1 ppm, 60% in 1.0 ppm, 68% in 5.0 ppm and 80% in 10.0 ppm Cd. The rest of the gametophytes either developed into normal thalloid structures bearing sex organs or into filamented, branched sterile or male thalli.

In control gametophytes the chloroplasts were oval or circular and evenly distributed (Fig. 8), while elongation and cleavages were observed in sparsely distributed chloroplasts in all treated materials (Fig. 9).

Above, 5 ppm Cd there was an evident inhibition of development in germination and gametophyte formation in all the taxa studied. Changes in the chloroplasts structure and bulbous form of cells in P. vittata clearly indicates inhibitory effects. The role of physiological factors responsible for the low degree of germination need further investigation.

This study reveals biologically observed effects of Cd on the gametophytic generation of ferns. A comparative evaluation of EC-50 values of the 8 taxa studied shows that the toxic effect of Cd is variable at different concentrations in different taxa. It is concluded that morphological characters can be used as a significant parameter in toxicity studies, and the utility of fern spore germination bioassay in toxicity testing can be stressed as pointed out earlier by Francis and Petersen (1983), Petersen et al. (1980) and Singh and Devi (1989).

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