

TOXIC EFFECTS OF CADMIUM CHLORIDE ON GROWTH AND OOGONIUM FORMATION IN *OEDOGONIUM HATEI*

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The effect of CdCl₂ on the germination and growth of swimmers and subsequent induction of oogonia was studied in *Oedogonium hatei* Kam (Oedogoniales, Chlorophyceae). CdCl₂ within the concentration range from 0.05 to 3.0 mg/l showed progressive reduction in the growth of alga. The percentage of oogonia formed and mature oogonia developed linearly decreased with increase in the concentrations of CdCl₂ employed. Three mg/l CdCl₂ proved to be highly toxic to the growth/multiplication of zoospores and further development of sexual structures in this alga. The germination of zoospores and growth of germlings were so markedly affected that induction of oogonia remained completely inhibited at 3mg/l CdCl₂. Five mg/l concentration of CdCl₂ proved to be completely lethal to the asexual swimmers.

Keywords: Cadmium chloride; Growth; *Oedogonium hatei*; Oogonium formation.

Cadmium is toxic heavy metal pollutants and widely distributed in aquatic environments which originate from geological weathering, industrial processing of ores and metals, leaching of metals from garbage and solid waste dumps and domestic sewage. Heavy metals cannot be eliminated from water bodies easily because they persist in sediments and pose serious hazards to aquatic organisms including algae. In spite of importance of algae in aquatic food chains relatively little attention has been focused upon them.

Wong, Burnison and Chau¹ reported acute toxicity of cadmium to green algae and has been shown to inhibit photosynthetic ¹⁴CO₂ uptake². Loss of motility and rounding of cells in cadmium treated *Euglena gracilis* has also been shown³. Chaudhary and Singh⁴ studied the toxic effects of mercuric chloride on akinete germination and akinete differentiation in *Pithophora oedogonia*. Singh and Chaudhary^{5,6} further investigated the effect of ZnSO₄ and CuSO₄ on the growth and induction of oogonia in *O. hatei*. Recently Singh⁷ studied the toxic effects of mercuric chloride on the growth and oogonium formation in the same alga. In the present study, the author has tried to assess the toxic effects of CdCl₂ on germination of zoospores, and the growth and subsequent differentiation of oogonia in macrocrouous heterothallic *O. hatei*, an integral component of aquatic ecosystem.

The macrocrouous heterothallic *Oedogonium hatei* Kam. was collected from fresh water ponds. A clonal culture was raised from a single female filament, which was later purified to auxenic state in godward medium, in thermostatically controlled culture chamber at 22 ± 1°C with an illumination of 2kl for 16h/day. Zoospores were obtained in nearly two month old cultures following the method of Howard and Horsley⁹ and Sarma and Singh¹⁰.

Equal amounts of Zoospore suspension were pipetted and spread on Chu¹¹ inorganic nutrient plates containing different levels of CdCl₂ viz. 0.05, 0.1, 0.5, 1, 2, 3 and 5mg/l. The plates were incubated in thermostatically controlled culture chamber in triplicate including control. The germination of zoospores and the initiation time of oogonial structures in germlings/filaments was analysed. To assess the growth, the average number of cells per filament was calculated from random sampling of different microscopic fields. For comparative assessment the number of vegetative cells and generative organs (oogonia) formed were ascertained on 9th and 15th day, respectively.

The dark green pear shaped motile swimmers settled on the agar surface within a few hours of inoculation. The free ends then elongated and divided through cell-plate formation to give rise to germlings which later grew into filaments through successive cell divisions. The divisions of zoospores was observed on second day of inoculation in controls and up to 1 mg/l of CdCl₂. The 5 mg/l concentration was lethal to all zoospores. The maximum growth, viz 22.2 cells/filament, on an average, was recorded in control on 9th day of inoculation of single celled zoospores, and then linearly decreased with increase in concentration of CdCl₂ (Table 1). In addition to decrease in number of cells per filament, a number of feeble, curved and short filaments appeared at 2.0 mg/l and above indicating its toxic and inhibitory effect on the growth of alga.

The initiation of differentiation of oogonia started on 10th day of inoculation of zoospores in the controls, and up to 0.5 mg/l concentration of CdCl₂. However higher concentration i.e. 1.0 mg/l and 2.0 mg/l of CdCl₂ delayed the process by two and three days respectively. Increasing concentration of CdCl₂ reduced the percentage of oogonia

Table 1. Effect of CdCl₂ on the growth of *Oedogonium hatei* *.

Concentrations mg/l	Days							
	2	3	4	5	6	7	8	9
0	1.8	3.5	5.8	8.4	10.0	14.6	16.3	22.2
0.05	1.8	3.3	5.5	8.0	9.2	14.0	15.2	21.1
0.1	1.7	3.1	5.0	7.5	8.7	12.3	13.6	19.4
0.5	1.5	2.8	4.5	7.0	7.5	10.2	11.5	15.5
1.0	1.3	2.5	4.0	5.4	6.0	7.9	9.5	11.2
2.0	1.0	2.0	3.5	4.8	5.5	6.1	7.5	8.6
3.0	1.0	1.3	1.8	2.0	2.6	3.1	3.5	3.8
5.0	-	-	-	-	-	-	-	-

* Average number of cells per germling and / or filament developed from single - celled zoospores.

Table 2. Effect of CdCl₂ on oogonium formation in *Oedogonium hatei*.

Concentrations (mg/l)	Initiation of oogonia (days)	percentage of oogonia on 15 th day of inoculation	Percentage of mature oogonia on 15 th day of inoculation
0	10	18.6	30.3
0.05	10	17.7	15.5
0.1	10	15.0	12.0
0.5	10	11.3	10.0
1.0	12	8.7	-
2.0	13	6.5	-
3.0	-	-	-
5.0	-	-	-

formed with concomitant decrease in mature oogonia as recorded on 15th day of inoculation of the zoospores. The maximum percentage of oogonia (18.6%) was recorded in control and the minimum (6.5%) in 2.0 mg/l concentration of CdCl₂ (Table 2). No oogonia formation occurred at and above 3.0 mg/l, either due to unhealthy growth of germlings or the lethal effect on the survival of zoospores.

The results (Table 1 and 2) show that cadmium chloride within the concentration range of 0.05 mg/l to 3 mg/l severely affected the initiation of germination, growth and initiation time and extent of oogonium formation. Doses between 0.05 mg/l and 2 mg/l of CdCl₂ contributed linearly to the severity of damage inflicted. Concentration above 3 mg/l were lethal to asexual zoospores of *O. hatei* substantiating the long held view that cadmium is a toxic heavy metal. Similar observations were made earlier by same authors in case of mercury (Chaudhary and Singh⁴ and Singh⁷) while working on akinete germination and akinete differentiation in *Pithophora oedogonia* and growth and induction of oogonium in *Oedogonium hatei* respectively. Singh and Chaudhary⁵, however observed that lower concentration (0.05-0.5 mg/l) of ZnSO₄ enhanced the growth rate as well as percentage of oogonia formed in

O. hatei under the same set of cultural conditions. Singh and Chaudhary⁶ further observed that 0.05 mg/l concentration of CuSO₄ promoted the growth but not the percentage of oogonia formed in comparison with controls in the same alga.

In the present work, increasing concentration of CdCl₂ linearly affected both growth and oogonium differentiation in alga which was more prominent at and above 2 mg/l CdCl₂ (Table 1,2). At 3 mg/l concentration filaments remained considerably shorter (up to 3-4 cell only) with smaller vegetative cells of faded colour, devoid of oogonia even on 15th day after inoculation of zoospores. Finally 5.0 mg/l CdCl₂ was lethal to all zoospores since no germlings developed at this concentration.

Cadmium toxicity in various algal system was reported by several other workers. Stration and Corke¹¹ noted cell lysis, filament elongation increased heterocyst frequency and loss of cellular contents from apical cells of the filaments. Conway¹² found a significant lowering of pigment content in *Asterionella formosa* after addition of Cadmium. Silverberg¹³ studied the effect of cadmium on fine structures of several fresh water algae and found changes in mitochondrial structure as a most prominent

symptoms of Cadmium injury.

The above findings also indicated the differential response of algae to heavy metals. This differential ability to accumulate and withstand varying levels of metals appears to be genetically determined property of algae. Thus, from present study and those made earlier, it is difficult to explain the actual cause of cadmium toxicity on the photoautotrophs. However, enzymatic inactivation and metabolic inhibition seems to be logical reasons for zoospore lethality with respect to germination and further oogonium formation in growing germlings. Further experiments are necessary to understand the actual mechanism of cadmium toxicity on vegetative and sexual phase of algae.

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