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EFFECT OF CADMIUM TOXICITY AND NITROGEN AND PHOSPHORUS DEFICIENCY ON THE GROWTH AND MORPHOLOGY OF *STIGEOCLONIUM TENUE KUTZ.*

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The influence of different concentrations of cadmium (Cd) and deficiency of nitrogen (N) and phosphorus (P) on the growth and morphology of *Stigeoclonium tenue* Kütz. has been carried out. Hair-like out growths were formed when *Stigeoclonium* was grown under N and P deficiency, whereas these hairs were totally absent when grown on basal medium. Low concentration of Cd (10 µM and 50 µM) reduced the cell length in both prostrate and erect systems. Higher concentrations of Cd drastically reduced growth in general by reducing the cell length and width and severely reduced the erect system and branching. The toxic effect of Cd was more under N and P deficiency. The deficiency of N and P seem to effect the growth and enhance the toxicity of Cd in *Stigeoclonium*.

Keywords : Cadmium; Erect system; Hair formation; Morphology; N & P deficiency; Prostrate system; *Stigeoclonium tenue*.

Introduction

Industrialization and urbanization has resulted in the environmental pollution by herbicides, pesticides, heavy metals and other potential toxicants. The toxic levels of heavy metals results from anthropogenic acttivities[1]. At sub-acute levels the pollutants gradually accumulate in aquatic organisms and through food chain, reach a higher tophic level leading to ecological backlash and biomagnification[2,3]. Certain algal species can accumulate tremendous amounts of heavy metals despite a very low concentration in the ambient medium[4,5]. Several algal species have been used as indicators of heavy metal pollution in marine and fresh water systems[6-8]. The molecular biology of heavy metal toxicity and tolerance has recently been reviewed[9-10]. *Stigeoclonium tenue* has been shown to be resistant to metallic pollution[11].

The alga *Stigeoclonium tenue* Kütz. chosen for the present study occurs in diverse habitats including polluted waters. It has been used as an indicator of different types of pollution[12-14]. The alga isolated from Ramagundam thermal power plant effluents polluted with heavy metals was used in the present investigation.

Materials and Methods

Stigeoclonium Kütz. was collected and isolated from the industrial effluents of Ramagundam thermal station (AP). After isolation axenic cultures were obtained throgh a series of washing with sterile double distilled water and mercuric chloride treatment (0.1% $HgCl_2$ for 10 min.) and finally treated with Streptomycin. The pure axenic cultures of the alga were grown on modified Bold's medium[15] at 18± 1°C with cool flourescent light intensity of 6000 lux.

For the preparation of inoculum for various treatments, the alga was grown for 14 days on the basal medium and equal amount of the inoculum was added to all treatments. The toxicity test and tolerance index concentration (TIC) for cadmium on a semi-quantitative scale was determined as per Harding and Whitton[16].

The concentration range of cadmium chosen for the study was - 0 µM (control), 10 µM, 50 µM, 100 µM, 150

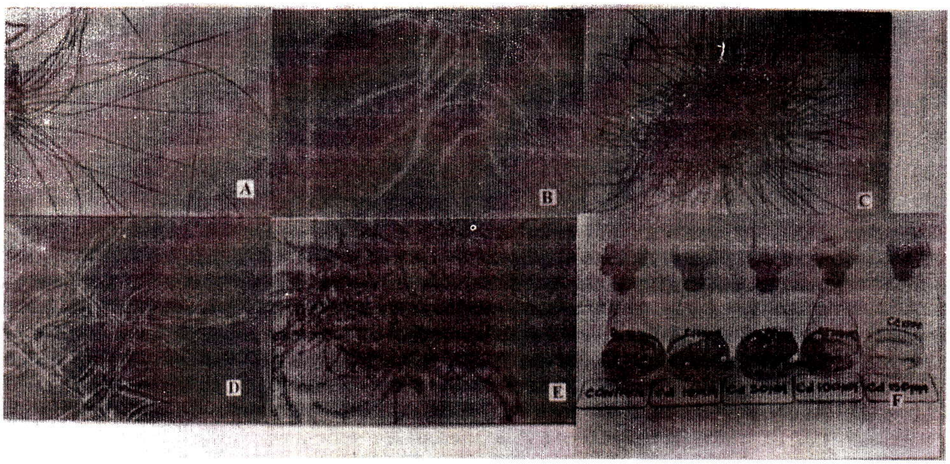

PLATE-I : Effect of different treatments on the morphology of *Stigeoclonium tenue* Kütz. A- Conrol; B- hair formation under nitrogen deficiency; C- hair formation under phosphorus deficiency; reduction in the erect system with D- Cd 100 µM; E- Cd 150 µM; F- growth of the alga at different Cd concentrations.

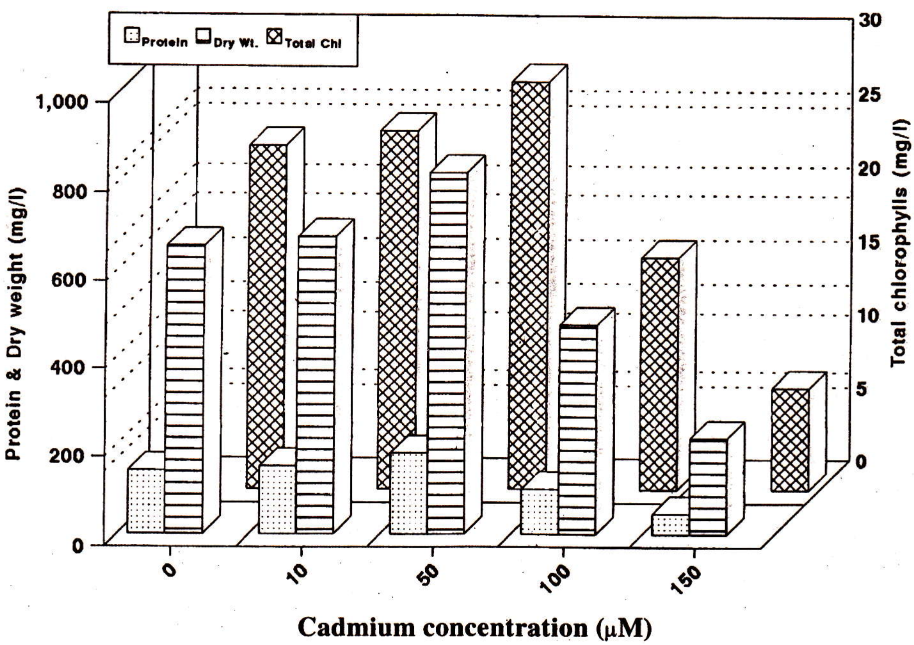

Fig.1 Effect of cadmium on the growth of *Stigeoclonium tenue*

Table 1. Effect of cadmium and deficiency of nitrogen and phosphorus on the morphology of *Stigeoclonium tenue*.

S.No.	Treatment	Prostrate system		Erect system		No. of branches/ 10 cells	No. of hairs/ 10 br.	Length of hairs/ (µM)
		Cell length (µM)	Cell width (µM)	Cell length (µM)	Cell width (µM)			
1.	Control	17.6	11.4	37.2	5.2	6.3	0	–
2.	Cd 10 (µM)	15.5	13.4	36.2	3.7	4.3	0	–
3.	Cd 50 (µM)	11.4	10.3	30.0	3.1	2.3	0	–
4.	Cd 100 (µM)	11.4	9.3	21.7	3.1	1.7	0	–
5.	Cd 150 (µM)	14.5	10.3	14.5	3.4	1.3	0	–
N-deficient								
6.	Cd 0 (µM)	12.4	10.3	24.8	3.6	5.3	8.7	258
7.	Cd 10 (µM)	12.4	10.3	24.5	3.8	6.0	7.7	249
8.	Cd 50 (µM)	11.4	10.3	22.7	3.1	4.3	4.3	67
9.	Cd 100 (µM)	10.3	10.3	20.7	3.1	2.3	0	–
10.	Cd 150 (µM)	10.3	9.3	18.6	3.1	1.7	0	–
P-deficient								
11.	Cd 0 (µM)	14.5	10.3	35.1	5.2	5.7	9.0	442.4
12.	Cd 10 (µM)	17.6	12.4	38.2	4.2	6.7	8.3	373.6
13.	Cd 50 (µM)	16.5	10.3	33.1	3.1	5.0	5.7	217.9
14.	Cd 100 (µM)	14.5	9.3	28.9	3.1	3.7	1.7	13.3
15.	Cd 150 (µM)	11.4	9.3	21.7	3.1	2.3	0	–
N&P-deficient								
16.	Cd 0 (µM)	14.5	8.8	31.0	3.3	4.0	8.3	467
17.	Cd 10 (µM)	17.6	9.3	28.9	4.7	5.2	7.0	411
18.	Cd 50 (µM)	15.5	9.3	20.7	3.6	4.1	6.7	236
19.	Cd 100 (µM)	14.5	12.4	22.7	3.1	3.7	0	–
20.	Cd 150 (µM)	16.5	11.4	36.2	3.1	2.3	0	–

µM. For nitrogen deficiency studies the nitrogen source in the basal medium $NaNO_3$ was replaced by NaCl and for phosphorus deficiency studies, KH_2PO_4 in the basal medium was replaced by KCl and the pH of the medium adjusted accordingly.

The growth of the alga was estimated in terms of (i) chlorophyll content (ii) protein content and (iii) dry weight. The chlorophyll content was estimated as per Strain *et al.*,[17] and protein as per Lowry *et al.*,[18]. For the estimation of dry weight cultures grown in 100 ml medium were harvested by filtering and washed thoroughly with double distilled water. The algalmat was carefully transferred to pre-weighed aluminum foil dish and dried over night at 80°C. The weight of the algalmat was calculated after deducting the foil dish weight. Morphological studies were carried out by microscopic examination. To assess the influence of Cd as well as deficiency of N and P on the morphology the following growth characters were observed.

i. Cell length and width of prostrate and erect filaments.

ii. Number of branches (erect filaments) arising from 10 celled length of prostrate filaments.

iii. Presence of hairs if any and the length and frequency of the hairs formed.

For the growth and morphology studies, *Stigeoclonium* was harvested and studied at the end of the experimental period of 14 days.

Results and Discussion

Under normal culture conditions when the alga was grown on basal medium (control), *Stigeoclonium tenue* never formed hair like structures (Plate-I A). Low concenrations of Cd (10 and 50 μM) reduced the cell length in both erect and prostrate systems (Table 1), whereas at higher concentrations (100 and 150 μM) the general growth, cell length and cell width were severely reduced. The erect system and branching pattern were restricted at higher concentrations of cadmium (Plate-I D&F). At higher concentrations of Cd (100 and 150 μM) the growth was decreased to 30% of the conrol (Fig.1 and Plate-I F). Dry weight increased by 25% at 50 μM Cd over control, whereas at higher concentrations it decreased by 37% at 100 μM and 67% at 150 μM Cd over control. The protein content, chlorophyll and dry weight also showed a similar trend like dry weight (Fig.1).

Stigeoclonium tenue belonging to chaetophorales shows heterotrichous habit with well developed prostrate and erect systems (Plate-I A). Upon exposure to Cd, the erect system showed more sensitivity than the prostrate system. The branching pattern also was affected due to the increasing concentrations of Cd (Plate-I D&E). At much higher concentration of 200 μM Cd (not shown in the present study) the prostrate and erect systems dissociated with the formation of spherical akinete like cells. Severe morphological changes in *Stigeoclonium* were observed at high chloride levels[19], palmella-like stages under unfavourable growth conditions[20-22] and high osmotic pressure[21]. Thus under toxic conditons the cells and the growth of erect and prostrate systems of *Stigeoclonium* may undergo morphological change as an adaptive defense mechanism.

The cell division in the erect filaments of *Stigeoclonium* is inter-calary. Therefore the dimension of the cells in the erect system were more effected than the cells in the prostrate system (Table-1). At higher concentrations, Cd completely eliminated the erect system. Similar inhibitory effects were observed for higher toxic levels of chlorine[19] and ammonium-N in *Stigeoclonium*[23].

The deficiency of N and P induced the most distinct and general effect on hair formation (compare plate-I B&C with A). Hair cell formation in Chaetophorales due to nutrient depletion has been reported[24,25], nitrate depletion[26]. Light and temperature have also been shown to induce hair formation[23,27]. The formation of hairs may provide more absorbing surface for the alga and under nutrient deficient and unfavourable conditions[23, 28-30]. The number and length of hairs produced under phosphorous deficiency was more than the nitrogen deficient conditon. Cadmium not only reduced the growth of the alga in general under N and P deficiency, but also reduced the hair frequency and length. This could be the toxic effect of Cd on growth of the cells in general.

Thus the present investigation has shown that cadmium toxicity causes reduction in growth and elimination of erect system. The nitrogen and phosphorus deficiency induces the formation of hair like out growths and the toxic effect of cadmium is more pronounced under nutrient deficient conditions. These studies help in evaluating the algal systems for using as indicators of pollution and also as biosystem for the treatment of industrial effuents to remove heavy metals.

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