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CADMIUM CHLORIDE INDUCED DOSE AND DURATION DEPENDENT BIOMASS VARIATION IN *NOSTOC PUNCTIFORME* Ag. IN CULTURE

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ABSTRACT
Bioaugmentation is one of the essential components of bioremediation, a biotechnological method of recent origin. Bioaugmentation is the process of introducing a known veriety of bioremedial agent into contaminated environment to initiate and sustain a specific bioremedial process. The paper deals with the biomass production of Nostoc punctiforme under the influence of different concentrations of cadmium chloride in laboratory culture. The success of bioremedial process by *N. punctiforme* depends upon its biomass production, which in due course removes the heavy metal.In control culture the biomass of *N. punctiforme* as mg /50ml varied from 13.79 (0 day) to 22.88(25th day). The corresponding values for 0.01 and 0.5 ppm cadmium chloride treatment after 25 days were 20.68, 14.98 and 10.01 mg/50ml respectively, indicating a decline in biomass of *N. punctiforme* with increase in concentration. A two way ANOVA revealed the variation of biomass with respect to concentration and duration of treatment statistically significant ($F = 10.494$, df5,3; $p < 0.001$ and $F = 20.004$, df 5,3; $p < 0.001$). The results indicate better perfomance of *N. punctiforme* at lower concentrations.

INTRODUCTION

The use of toxic chemicals in agriculture, industries and other sectors has grown phenomenally, as per estimations derived from correlation between hazardous waste and toxic chemicals (Sahu and Panigrahi, 2002). Every industry creates pollutants in the form of smoke, effluents, noise etc. A significant amount of these pollutants are released into the environment, affecting the flora and fauna. Many inorganic elements such as mercury (Hg), lead (Pb) Arsenic (As) and Cadmium (Cd) are biological poisons at concentrations even in parts per billion (ppb) range. Once these chemicals find their way into the environment, a major portion reaches the sand and sediments which in turn serve as sink (Haqne, 1975). Leaching of chemicals pose ground water contamination problems (Hamaker, 1972). Widespread contamination by these chemicals or compounds is a serious problem faced by the mankind. With increasing pollution and health hazard, a number of methods have been developed to mitigate the impact or remove the contaminants from the natural systems. Among the various technologies employed for the purpose, bioremediation is becoming an increasingly popular technology for remediation of the contamination. Bioremediation is a treatabilty technology that uses the biological activity to reduce the concentration or toxicity of a pollutant. It commonly uses the process by which microorganisms transform or degrade chemicals in the environment "(King *et al.*,1998). The process of bioremediation includes biostimulation of naturally occurring microbial communities or bioaugmentation with specific microbial strains known to biodegrade the oxygenate. (Smith *et al.*, 2005).

Bioaugmentation is a process of introducing a known variety of bio-remanant to initiate and sustain a specific bio-remedial process. The sucess of the bioremedial process depends upon biomass production of the strain used as bioremedial agent. The more biomass is produced the more contaminants are removed. With this idea the present project has been taken up to study the influence of Cadmium chloride on biomass production of *Nostoc punctiforme* at different durations and different concentrations.

MATERIALS AND METHODS

Pure strain of *Nostoc punctiforme* was cultured in BGII medium (Stanier *et al.*, 197). Before the inoculation, the culture medium was subjected to autoclaving for sterilization. The cyanobacterial culture was maintained under continuous white light (2200 lux) at the temperature

28 ±2° C. Besides the culture flasks were subjected to hand shaking intermittently.

Experimental setup

Conical flasks of size 250 ml were used for the experiment. Four sets of flasks each with four replicates containing 50 ml of basal nutritional media (BNM) were taken. To each flask 2ml of the *N. punctiforme* culture was introduced. One set of inoculated flask (four flasks) was kept as control. To the other three sets 0.01, 0.1 and 0.5 ppm of cadmium chloride ($CdCl_2$) was introduced. All the conical flasks were maintained at the temperature 28 ±2⁰ C under continuous white light (2200 lux).

Estimation of biomass

For the estimation of biomass the total content of the cultured flask was filtered through a pre-weighed Whatman No. 41 filter paper. After filtration, the filter papers containing the algal mass were dried keeping in an air oven at 80⁰C for 2 hrs and at 30⁰C for 12 hrs. The final biomass of dried cyanobacteria was calculated from the difference between the final wight of filter paper cantaining the dried cyanobacterial biomass and and the initial weight without cyanobacteria.

Estimation of biomass production

Biomass production was calculated from the summation of all positive increments in the biomass over a period of 25 days of culture.

Estimation of biomass turnover

Turnover of the biomass was calculated by using the formula.

$$\text{Turnover} = \frac{\text{sosisn osos}}{\text{vere sisos}}$$

Estimation of Instantaneous Growth Rate (IG)

Instantaneous growth rate was determined following Brafrild and Llewellyn (1982).

$$IG(\%) = \frac{\log_{10} YT - \log_{10} Yt}{T - t} \times 2.3026 \times 100$$

where t = time at the beginning of the observation,

T = time at the end of the observation,

YT = weight at time T,

Yt = weight at time t and

2.3026 is conversion factor.

Statistical Analysis

The data obtained were subjected to the analysis of variance (ANOVA) following Shedecor and Coachran (1967).

OBSERVATIONS

The biomass of *N. punctiforme* ranged from 1.79 mg / 50ml (on 0 day) to a maximum of 22.88 mg/50 ml / on 25th day) in the control condition. However the corresponding values for different concentrations of $CdCl_2$ i.e. 0.01, 0.1 and 0.5 ppm after 25 days (mg/50 ml) were found to be 20.68, 14.98 and 10.01 respectively (Table 1), which shows a decline in biomass of *N. punctiforme* with the increase of $CdCl_2$ concentration. The data were subjected to two way analysis of variance. The analysis revealed the variation of biomass statistically significant with respect to different concentrations of $CdCl_2$ treatments and different days (Table 2).

Table 1: Effect of different concentrations of Cadmium chloride on biomass (mg/5ml) of *Nostoc punctiforme* (Mean ± SEM of 4 data)

Days	Control	Concentration of cadmium chloride		
		0.01	0.1	0.5
0	1.79 ±0.254	1.79±0.254	1.79±0.254	1.79±0.254
5	6.19 ± 0.108	6.07±0.182	5.59±0.135	3.69±0.158
10	15.11 ± 0.540	12.14±0.187	10.12±0.297	6.19±0.108
15	18.8 ± 0.393	17.49±0.248	11.66±0.302	7.85±0.248
20	20.21 ± 0.284	19.01±0.212	13.01±0.225	8.82±0.129
25	22.88 ± 0.336	20.68±0.197	14.98±0.301	10.01±0.248

Table 2: Analysis of variance (Two way)

Variation	SS	df	MS	F	Significance
Between days	794.028	5	158.8057	20.0046	P<0.001
Between conc.	249.931	3	83.3106	10.4946	P<0.001
Error	119.076	15	7.9384		

The biomass production varied from 4.40 mg /50ml in control whereas in case of 0.01, 0.1 and 0.5 concentration it ranged from 4.28 to 18.89, 3.80 to 13.19 and 1.90 to 8.22 mg/50ml respectively. The variation implies the gradual decline in biomass production with increasing concentration of $CdCl_2$.

he biomass turnover value was 11.78 in control while in 0.01, 0.1 and 0.5 concentrations of $CdCl_2$ treatment, the values were found to be 10.55, 11.40 and 4.59 respectively (Table 3). Out of the 3 treatment conditions, maximum value was found with 0.01 ppm concentration and least with 0.5 ppm which shows that 0.5 ppm adversely affect the biomass.

The IG (% per day) of *N. punctiforme* in the control condition ranged from 24.8 (on 5th day) to 1.44 (on 20th day) while in dose of 0.01, 0.1 and 0.5 concentrations of $CdCl_2$ treatment showed the variation from 23.98 (on 5th day) to 3.2 (on 25th day), 22.76 (on 5th day) to 2.18 (on 20th days) and from 14.46 (on 5th day) to 2.32 (on 20th day) Table4.

DISCUSSION

In the present study different concentrations of $CdCl_2$ (0.01, 0.1 and 0.5 ppm) showed a significant ($F = 10.4946$, $df = 3$, $P < 0.001$) decline in the biomass of *N.*

Table 3: Range of biomass production of*Nostoc punctiforme* and biomass turnover value in different concentrations of Cadmium chloride

S. No.	Biomass production/ turnover	Control	Concentration of cadmium chloride (ppm) 0.01	0.1	0.5
1	Biomass production	4.4 mg/ 50 ml to 21.09 mg/ 50 ml	4.28 mg/ 50 ml to 18.89 mg/ 50 ml	3.80 mg/ 50ml to 13.19 mg/ 50 ml	1.90 mg/ 50ml to 2.22 mg/ 50 ml
2	Biomass turnover	11.78	10.55	11.40	4.59

punctiforme. The biomass production was also found to be adversely affected with increase in concentration of Cadmium chloride. A similar trend was observed with 0.1, 0.5 and 1.0 ppm of Butachlor treatment by Pandey and Tiwari (1986)Roychoudhury and Kaushik (1986), Goyal, Roychoudhury and Kaushik (1991) and Patel (1997) reported a marked inhibition on the cyanobacterial biomass as a result of Butachlor treatment. As per their observation, Butachlor concentration higher than 1.0 ppm significantly inhibited the biomass production in *Tolypothrix ceyloneica* and *Scytonema cincinnatum*. However in the present study a significant ($F = 20.004$, $df = 5$, $p < 0.001$) decline in the biomass production of *N. punctiforme* over a period of 25 days (with an interval of 5 days) at even 0.5 ppm of $CdCl_2$ concentration was observed.

Table 4: Instantaneous growth of biomass of *Nostoc punctiforme* in control and Cadmium chloride treated cultures (% per day)

Days	Control	Conc. of Cadmium chloride in ppm. 0.01	0.1	0.5
0	0	0	0	0
5	24.8	23.98	22.76	14.46
10	17.84	13.86	11.86	10.34
15	4.36	7.3	2.82	4.74
20	1.44	3.66	2.18	2.32
25	2.48	3.2	2.8	2.52

The biomass turnover generally reflects the rate of replacement of individual organism and is considered, as an important ecological index, which can provide an insight into the functioning of the individual under certain conditions. In the present study, the turnover rate of *N. punctiforme* in the $CdCl_2$ treated condition was found maximum with 0.01 ppm and minimum with 0.5 ppm which reveals that 0.5 ppm of $CdCl_2$ treatment adversely affected the rate of replacement of *N. punctiforme*. A similar trend was observed with 0.1, 0.5 and 0.01 ppm concentrations of Butachlor treatment Patel (1997).The study revealed that lower concentration of Cadmium chloride provides better opportunity for biomass growth of the species.

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