Nature Environment and Pollution Technology © Technoscience Publications	Vol. 6	No.1	pp. 159-162	2007

IMPACT OF CHROMIUM CHLORIDE ON HETEROCYST FREQUENCY OF *NOSTOC CARNEUM* AGARDH

Latika Sharan
Department of Botany, Ranchi Women's College, Ranchi-834 001, Jharkhand, India

ABSTRACT

The paper deals with the impact of different concentrations (0.001, 0.01, 0.1 µg/mL) of chromium chloride on heterocyst frequency of *Nostoc carneum* under laboratory culture condition (2200 lux light intensity, 7.5 pH and 28 ± 2°C temperature). The results showed a statistically significant decline in heterocyst frequency under treated condition with respect to control. In control, heterocyst frequency increased from 3.017 ± 0.273% (4^{th} day) to 6.026 ± 0.315% (24^{th} day) while it was 2.137 ± 0.255% and 5.622 ± 0.415% when treated with 0.001 µg/mL $CrCl_2$ on the similar days respectively. In case of 0.01µg/mL concentration, the percentage of heterocyst frequency on 4^{th} and 24^{th} days was 1.952 ± 0.115 and 3.536 ± 0.211 which declined to be 1.587 ± 0.094% and 1.117 ± 0.218% under the toxic influence of 0.1 µg/mL $CrCl_2$.

The two way ANOVA revealed a significant variation in heterocyst frequency as an impact of concentration of chromium chloride ($F = 30.161$, $df = 5, 3$; $p < 0.001$) and duration of treatment ($F = 4.961$, $df = 5, 3$; $p < 0.05$) reflecting the considerable influence of both concentration and duration of heavy metal on heterocyst frequency of *N. carneum*. The relation between heavy metal toxicity and reduction in heterocyst frequency has been discussed in the paper.

INTRODUCTION

Heterocysts are differentiated cells specialized for nitrogen fixation in anaerobic environment (Singh et al. 1999). Most of the cyanobacteria, growing diazotrophically produce these specialized cells. The vegetative cells provide heterocysts with photosynthetically generated carbohydrate which acts as a source of reductants and ATP is needed for nitrogen fixation. In turn, heterocyst fix and assimilate nitrogen as glutamine which is then released to the neighbouring vegetative cells (Wolk 1982). Differentiations of heterocysts and their functional significance have been taken as physiological division of labour, pattern formation, differentiation of cell, intracellular communication and developmentally regulated gene expression (Apte 1992, 1993).

Nitrogenase activity and heterocyst differentiation in nitrogen fixing cyanobacteria are usually interrelated (Geetha & Anand 2004). Nitrogen deficiency is one of the causative factors for heterocyst differentiation from vegetative cells but there are different factors to control the heterocyst differentiation leading to reduced frequency. Inhibitory substances produced by heterocysts if not metabolized by vegetative cells, where they reach by cell-to-cell diffusion; stops initiation of differentiation. The inactive form of an inhibitor of heterocyst development is produced by vegetative cells and is either activated by a co-inhibitor derived from developing or matured heterocyst or by high concentration of fixed nitrogen (Adams 1992). Xenobiotic toxicants like herbicides and heavy metals have been reported to have inhibitory impact and heterocyst differentiation and thereby decreasing its frequency (Pandey 1985, Pandey & Tiwari 1986, Mishra & Tiwary 1986). The heterocyst frequency assessment has now been considered as a tool of bioassay of environment but due to paucity of knowledge and lack of data pertaining to concentration of xenobiotics and heterocyst frequency, standard index has not been proposed.

The present communication records the heterocyst frequency with respect to different concentration of chromium chloride on *Nostoc carneum* (Ag.) in ideal culture conditions as an attempt to establish relation between concentration of xenobiotic toxicity and heterocyst frequency.

MATERIALS AND METHODS

Four sets of conical flasks (250 mL size), each with four replicates containing 50 mL of basal nutrient media (BM) with 2 mL of pure culture of *Nostoc carneum* were taken for experiment. One set of inoculated flasks (four flasks) was kept as control. To other three sets 0.001, 0.01 and 0.1 µg/mL $CrCl_2$ were introduced. All the flasks were kept covered in aseptic condition under 2200 lux light intensity while 28 ± 2°C temperature and 7.5 pH was maintained.

The heterocyst frequency was estimated by counting vegetative and heterocystic cells. A drop of the *Nostoc carneum* culture from control flask as well as from different flasks treated with 0.001, 0.01, 0.1 and 1.0 µg/mL $CrCl_2$ was taken out and put on clear glass slides. After covering it by cover slip the slide was examined under microscope. In each focus both number of vegetative cells and heterocysts were counted. Ten such random countings were made for each reading. The process was repeated on 4^{th}, 8^{th}, 12^{th}, 16^{th}, 20^{th} and 24^{th} day of culture, taking samples from both control and treated flasks. The heterocyst frequency was calculated as:

$$\text{Heterocyst frequency (\%)} = \frac{\text{Number of Heterocysts}}{\text{Total number of vegetative cell + heterocysts}} \times 100$$

OBSERVATIONS

The data generated on heterocyst frequency of *Nostoc carneum* have been set in Table 1 of 4^{th}, 8^{th}, 12^{th}, 16^{th}, 20^{th} and 24^{th} day of chromium chloride treatment of different concentrations with respect to the control. The treatment with different concentrations of chromium chloride has resulted into significant decline in heterocyst frequency from control. In case of control the heterocyst frequency gradually increased from 3.017% (4^{th} day) to 6.026% on 24^{th} day. A gradual increase was observed in 0.001 µg/mL $CrCl_2$ treated condition ranging from 2.137% to 5.622% from 4^{th} day to 24^{th} day. This increase was considerably lower than normal condition. Under treatment with 0.01 µg/mL of $CrCl_2$ a slow increase in heterocyst frequency was observed from 4^{th} day to 20^{th} day (1.952% to 4.017% respectively) but heterocyst frequency declined on 24^{th} day of treatment (3.536%). In case of 0.1 µg/mL $CrCl_2$ treatment the heterocyst frequency slightly increased (from 1.587 to 2.50%) up to 8^{th} day of treatment and thereafter a gradual decline was observed up to 24^{th} day. To analyse the significance of the data between the concentration of treatment and the duration of treatment (days) were subjected to two way analysis of variance (ANOVA, Table 2). The analysis revealed the variation of heterocyst frequency with respect to different concentrations of treatment and different durations of treatment which were statistically significant ($F = 30.161$; $df = 3, 5$; $p < 0.001$; $F = 4.961$; $df = 3,5$; $p < 0.05$ respectively).

DISCUSSION

Algal forms readily respond showing morphological changes as a consequence of cytological effects caused by higher concentrations of heavy metals (Venkataraman et al. 1992). However, no definite pattern of change can be postulated for any specific metal as it widely varies with algal form and growth and nutritional conditions.

Table 1: Variation in heterocyst frequency (%) in different concentrations of chromium chloride.

Days	C (BM)	Chromium chloride Concentration (µg/mL) with BM		
	0.00	0.001	0.01	0.1
4th	3.017 ± 0.273	2.137 ± 0.255	1.952 ± 0.115	1.587 ± 0.894
8th	4.982 ± 0.149	4.535 ± 0.275	3.355 ± 0.181	2.507 ± 0.118
12th	5.126 ± 0.008	4.817 ± 0.435	3.490 ± 0.091	2.110 ± 0.211
16th	5.872 ± 0.028	4.981 ± 0.210	4.042 ± 0.102	1.562 ± 0.317
20th	5.983 ± 0.210	5.017 ± 0.325	4.017 ± 0.117	1.312 ± 0.419
24th	6.026 ± 0.315	5.622 ± 0.415	3.536 ± 0.211	1.117 ± 0.218

BM = Basal medium

Table 2: Two-way analysis of variance of heterocyst frequency.

Source of Variation	Sum square	df	MS	F	Significance level
Between days	11.37941	5	2.275881	4.961552	$p < 0.05$
Between treatments	41.50507	3	13.83502	30.16114	$p < 0.001$
Error	6.86055	15			

The results during present investigation reveal a gradual decrease in heterocyst frequency with an increase in chromium chloride concentration and duration of treatment reflecting a relation between heterocyst frequency and dose as well as duration. Toxicity of heavy metal has been reported to be affected by environmental factors like light intensity, pH (Rai et al. 1990), chelating agents (Shanmukhappa & Neelakantan 1990) and amino acids (Rai & Raizada 1985, 1988).

From the data obtained (Table 1) it may be noted that heterocyst frequency decreased with increasing concentration of chromium chloride with the age of culture. Sarada (1989) reported 50% decrease in heterocyst frequency of *Anabaena oryzae* by treatment with 0.1 µg/mL mercuric chloride and 125 µg/mL zinc oxide. The heterocyst morphogenesis and frequency is a sensitive measure of the factors that affect the growth under any given set of experimental conditions (Fogg 1949, Tyagi 1973).

Geetha & Anand (2004) reported interrelationship of heterocyst differentiation with nitrogenase activity in nitrogen fixing cyanobacteria. They observed marked decrease in heterocyst frequency in *A. ambigua* when treated with various chemicals in BG II medium.

The inhibitory action of heavy metals on heterocyst frequency has been attributed to chlorophyll pigment which directly or indirectly affects the photosynthetic electron transport system. Chromium has been reported to be highly toxic to photosystem (PS) II (Husaini et al. 1991). The high sensitivity of PS II has been attributed to the alteration of energy transfer capacity of phycobilisomes, the main energy capturing antenna pigment for PS II. Heterocyst formation mainly depends upon interrelationship of metabolites which provide necessary energy during morphogenesis. Hence, chromium toxicity appears to produce inhibitory effect on photosystem causing inhibition of photosynthesis and the interrelated pathway leading to the formation of heterocyst.

Since heterocyst is the site of nitrogen fixation, the heavy metal treatment impairs the nitrogen fixation process in *Nostoc carneum* by not allowing differentiation of heterocysts. Such xenobiotic substances, may it be from any source natural or anthropogenic in a habitat, will adversely affect the

nitrogen fixation by capable cyanobacteria. The level of impact depends upon the level of concentration and duration of persistence of xenobiotic substances.

ACKNOWLEDGEMENT

The author is thankful to U.G.C., New Delhi for providing financial assistance for the project (PSJ-007/03-04-ERO) and also to Dr (Mrs.) Manju Sinha, Principal, and Dr. (Mrs.) Sandhya Sinha, Head of the Department, Botany, Ranchi Women's College, Ranchi for providing facilities.

REFERENCES

Adams, D.G. 1992. The effect of DL7-azatryptophan on heterocyst development in the cyanobacterium *Anabaena cylindrica.* J. Gen. Microbiol., 138: 355-362.

Apte, S.K. 1992. Molecular biology of cyanobacterial nitrogen fixation: Recent advances. Indian J. Microbiol., 32: 103-106.

Apte, S.K. 1993. Cyanobacterial nitrogen fixation: Molecular genetic aspects. Proc. Indian Natl. Sci. Acad., 59: 367-386.

Fogg, G.E. 1949. Growth and heterocyst production in *Anabaena cylindrica* Lemm. II. In relation to carbon and nitrogen metabolism. Ann. Bot. NS, 13: 241-259.

Geetha, S. and Anand, N. 2004. Physiological changes during akinete differentiation in *Anabaena ambigua* Rao. J. Ind. Bot. Soc., 83**:** 46-53.

Husaini, Y., Singh, A.K. and Rai, L.C. 1991. Cadmium toxicity of photosynthesis of associated electron transport system of *Nostoc linckia.* Bull. Environ. Contam. Toxicol., 46: 146-150.

Mishra, A.K. and Tiwari, D.N. 1986. Effect of tryptophan on 2,4-dichlorophenoxyacetic acid toxicity in the nitrogen fixing cyanobacterium *Nostoc linokia.* J. Basic Microbiol., 26: 49-53.

Pandey, A.K. 1985. Effect of Propanil on growth and cell constituents of *Nostoc calcicola*. Pesticide Biochemistry and Physiology, 23: 157-162.

Pandey, A.K. and Tiwari, D.N. 1986. Action of 2,4-Dichlorophenoxyacetic acid on *Nostoc linckla*: Impact of glucose and tryptophan. Folia Microbiol., 31: 50-55.

Rai, L.C. and Raizada, M. 1988. Impact of chromium and lead on *Nostoc muscorum.* Regulation of toxicity by ascorbic acid, glutathione and sulphur containing amino acids. Ecotoxicology Environ. Safety, 15: 195-205.

Rai, L.C. and Raizada, M. 1985. Effect of nickel and silver ions on survival, growth, carbon fixation and nitrogenase activity of *Nostoc muscorum*: Regulation of toxicity by EDTA and calcium. J. Gen. Appl. Microbiol., 31: 329-337.

Rai, L.C., Singh, A. K. and Mallick, N. 1990. Employment of CEPEX enclosures for monitoring toxicity of Hg and Zn on *in situ* structural and functional characteristics of algal communities of river Ganga in Varanasi. Ecotoxicol. Environ. Safety, 20: 211-221.

Sarda, K. 1989. Effect of heavy metals on *Anabaena oryzae.* Ad. Plant Sci., 2(2): 204-215.

Shanmukhappa and Neelkantan, K. 1990. The influence of humic acid on the toxicity of copper. Environ. Contam. Toxicol., 44: 840-843.

Singh, B., Chauhan, V.S., Gour, R.K., Singh, S. and Bisen, P.S. 1999. Heterocyst: Current status and future prospects. J. Indian Bot. Soc., 78: 25-32.

Tyagi, V.V.S. 1973. Effect of some metabolic inhibitors on heterocyst formation in the blue-green alga *Anabaena doliolum.* Ann. Bot., 37: 361-368.

Venkataraman, L.V., Suvarnalatha, G. and Manoj, G. 1992. Uptake, accumulation and toxicity of heavy metals in algae. Phykos, 31(1 & 2): 173-195.

Wolk, C.P. 1982. Heterocysts. In: Biology of Cyanobacteria (Eds. Carr N.G. & B.A. Whitton) Oxford Blackwell Scientific Publications, pp. 359-386.