Journal of Algal Biomass Utilization
J. Algal Biomass Utln. - Copyright © PHYCOSPECTRUM
ISSN: 2229 – 6905

Toxicity of arsenic on nitrogen-fixing cyanobacteria

PREETI SHARMA and DILIP KUMAR RATHORE[1]
Department of Botany, Eklavya College Bundi-323001 Rajasthan (India)
[1]Department of Botany, Government College, Bundi-323001 Rajasthan (India). Email- bhadialdkrathore@gmail.com

ABSTRACT

In the present study the effect of arsenic on three nitrogen-fixing cyanobacteria *Nostoc muscorum, Anabaena doliolum* and *Aulosira fertilissima* have been analyzed in terms of Total growth, Total carbohydrate, Proteins and Amino acids using 1 mg/L to 15 mg/L concentrations of arsenic. Heterocyst frequency calculated after growth period of 18 days. Values of growth parameters studied decreased significantly in presence of arsenic ion into the medium without any increase in concentration range studied. *Aulosira fertilissima* found to be the most sensitive species.

Key Words *Cyanobacteria, Toxicity, Heavy Metals, purifying alga.*

INTRODUCTION

Cyanobacteria (blue-green algae) are valuable tools for bioassays of metal toxicity (Fatma and Sultan 1999; Kapoor *et al*. 1998a; Angadi *et al.,* 1996; Dubey and Rai 1989).They are endowed with property to cope up stressful conditions such as waters polluted with heavy metals. Although a considerable amount of information is available on metal interaction effects on eukaryotic algae (Strarodub *et al.* 1987; Chu and Lin 1997) but comparable information on cyanobacteria is lacking.

Arsenic is a heavy metalloid and acts sometimes as a metal, sometimes not. Arsenate is structurally highly related to PO_4^{3-}, thus, its main toxicity is the interference with the metabolism of the major bioelement phosphor. Because of its toxicity, arsenic has no function as a trace element. The IARC recognizes arsenic and arsenic compounds as group I carcinogens and the EV lists arsenic trioxide, arsenic pentoxide and arsenate salts as category I carcinogens.

Many studies has been done on effects of arsenate on different organisms, but there remain substantial gaps in our understanding of effects of arsenic on different kinds of organisms on different trophic levels and on ecosystems function itself. With such consideration taken into account, a study was proposed to observe toxic effect of arsenic on *Nostoc muscorum, Anabaena doliolum* and *Aulosira fertilissima* with reference to total growth, total carbohydrate, total proteins, total amino acids content and heterocyst frequency.

MATERIAL AND METHODS

Study was done in two stages. At first stage, *Nostoc muscorum, Anabaena doliolum* and *Aulosira fertilissima* were grown and maintained as unialgal, clonal and axenic cultures in nitrogen-free Allen and Arnon's culture medium (1955) at 1800 lux and 28± 2˚C. At second stage, stock solution of sodium arsenate was prepared and it was then diluted with sterile distilled water to get concentrations ranging between 1 to 15 mg/L. Experiments were carried out in triplicate in 125 × 25mm test tubes with a total volume of 15ml (medium plus toxicant). Controls were maintained. The readings were recorded after a growth period of 18 days.

Growth was measured by taking optical density of chlorophyll pigments at 630, 645 and 665nm by UV-VIS spectrophotometer Systronics-117. Total carbohydrate content was estimated by acid hydrolysis Anthrone reaction method (Plummer, 1979). Total protein content was estimated by Lowry's method (Lowry et al. 1951). Total content of amino acid was estimated by Ninhydrin method (Mahadevan A. and Sridhar R. 1982). Heterocyst frequency of exponentially growing cultures was determined after the growth period of 18 days by calculating an average of 5 fields under microscope. Percentage heterocyst frequency as represented indicates number of heterocyst per 100 vegetative cells.

Further analysis of variance, ANOVA**,** of various growth parameters studied were performed at 5% and 1% level of significance of total growth, total carbohydrate, total proteins, total amino acids and heterocyst frequency which indicate highly significant values (Tables 1-5).

RESULTS AND DISCUSSION

A very interesting trend was seen in results on effects of sodium arsenate on growth response of all theses

cyanobacteria, i.e., *Nostoc muscorum, Anabaena doliolum* and *Aulosira fertilissima*. Results showed that they could not tolerate the presence of metal into the medium and were highly sensitive as exhibited. In *Nostoc muscorum, Anabaena doliolum* and *Aulosira fertilissima* values of growth parameters e.g., total growth, total carbohydrate, total proteins, total amino acids and heterocyst frequency were studied and were found to decrease significantly in presence of the metal ion in the concentration range, i.e., 1 to 15 mg/L (Fig 1-5). In contrast to above *A. fertilissima* showed a small increase at 1 mg/L which is greater than control values. Beyond this concentration, a gradual decrease in all the parameters was recorded. It shows that tested organisms were greatly affected by presence of the arsenic ion in the medium.

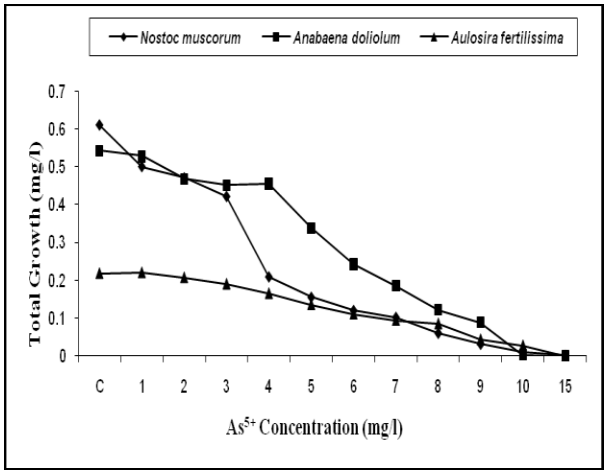

Fig.1 Effects of various concentrations of As^{5+} on Total growth (mg/l) of *N. muscorum, A. doliolum* and *A. fertilissima.*

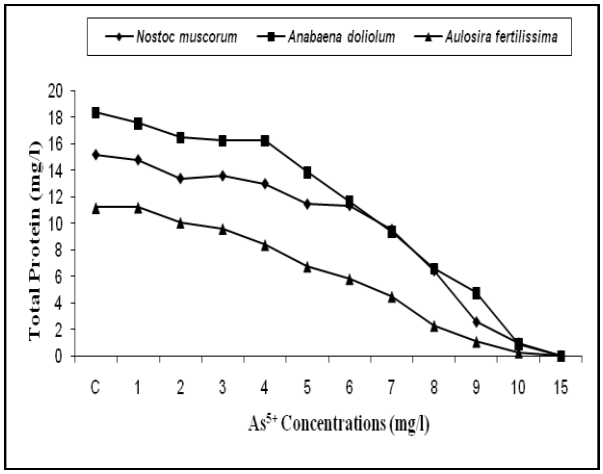

Fig.2 Effects of various concentrations of As^{5+} on Total carbohydrate (mg/l) of *N. muscorum, A. doliolum* and *A. fertilissima.*

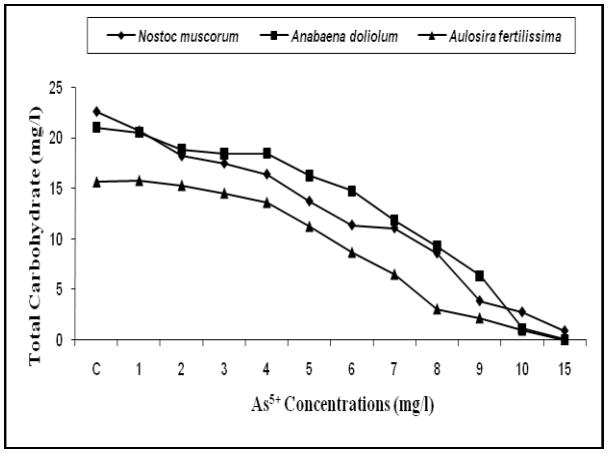

Fig.3 Effects of various concentrations of As^{5+} on Total protein (mg/l) of *N. muscorum, A. doliolum* and *A. fertilissima.*

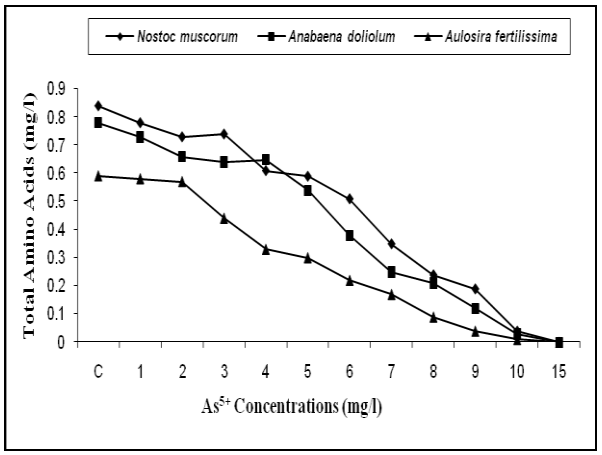

Fig.4 Effects of various concentrations of As^{5+}on Total amino acids (mg/l) of *N. muscorum, A. doliolum* and *A. fertilissima.*

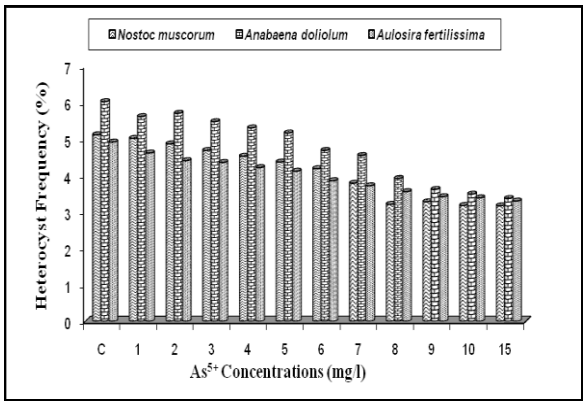

Fig. 5 Effects of various concentrations of As^{5+} on Heterocyst frequency (%) of *Nostoc muscorum, Anabaena doliolum* and *Aulosira fertilissima.*

Table 1 ANOVA for Total growth (mg/l) under the influence of As^{5+}:-

SOV	*Nostoc muscorum*			*Anabaena doliolum*			*Aulosira fertilissima*		
	DF	SS	MSS	DF	SS	MSS	DF	SS	MSS
Conc.	11	1.55	0.1411344*	11	1.37	0.1249499*	11	0.20	0.0177691*
Error	24	0.00	0.0000403	24	0.00	0.000062	24	0.00	0.000011
Total	35			35			35		

Table 2 ANOVA for Total carbohydrate (mg/l) under the influence of As^{5+}:-

SOV	*Nostoc muscorum*			*Anabaena doliolum*			*Aulosira fertilissima*		
	DF	SS	MSS	DF	SS	MSS	DF	SS	MSS
Conc.	11	1702.66	154.78722*	11	1793.86	163.07846*	11	1263.92	114.90174*
Error	24	2.71	0.1130015	24	2.96	0.1235011	24	1.64	0.0684349
Total	35			35			35		

Table 3 ANOVA for Total protein (mg/l) under the influence of As^{5+}:-

SOV	*Nostoc muscorum*			*Anabaena doliolum*			*Aulosira fertilissima*		
	DF	SS	MSS	DF	SS	MSS	DF	SS	MSS
Conc.	11	991.94	90.174249*	11	1433.31	130.30126*	11	599.82	54.52885*
Error	24	1.34	0.0559518	24	2.88	0.1200210	24	0.65	0.0271366
Total	35			35			35		

Table 4 ANOVA for Total amino acids (mg/l) under the influence of As^{5+}:-

SOV	*Nostoc muscorum*			*Anabaena doliolum*			*Aulosira fertilissima*		
	DF	SS	MSS	DF	SS	MSS	DF	SS	MSS
Conc.	11	2.89	0.262352*	11	2.68	0.243556*	11	1.68	0.152720*
Error	24	0.00	0.000153	24	0.00	0.000126	24	0.00	0.000066
Total	35			35			35		

Table 5 ANOVA for Heterocyst frequency (%) under the influence of As^{5+}:-

SOV	*Nostoc muscorum*			*Anabaena doliolum*			*Aulosira fertilissima*		
	DF	SS	MSS	DF	SS	MSS	DF	SS	MSS
Conc.	11	19.44	1.766928*	11	30.00	2.727316*	11	9.68	0.8795974*
Error	24	0.34	0.0142192	24	0.45	0.018800	24	0.42	0.0173283
Total	35			35			35		

A significant continuous decrease in heterocyst frequency observed in all the three tested cyanobcacteria on increasing the arsenic concentrations (Table 5). These observations show a reduction in growth as well as biochemical parameters confirming the arsenic toxicity in tested cyanobacteria.

Arsenic is used in arsenical pesticides and its main toxicity is the interference with the major biolelment phosphor. Arsenic is chemically similar to phosphorus, so it can substitute for phosphorus, however is doubtful. Three major modes of biotransformation of arsenic species have been found to occur in the environment: redox transformation between arsenite and arsenate, the reduction and methylation of arsenic, and the biosynthesis of organoarsenic compounds. Most of the arsenate is reduced, methylated and released to the surrounding media and there is great possibility of these cyanobacteria present in the waters to accumulate it in their cells.

A mechanism of toxicity of pentavalent inorganic arsenic, such as arsenate, is its reduction to a trivalent form, arsenite. Arsenite is more toxic than arsenate. Arsenate is known to uncouple phosphorylation. Thus, the coupled phosphorylation of ADP is abolished, the energy of ATP is not available, and the organisms slowly succumb as shown in our study.

The ability of arsenate to enter into the reactions in place of phosphate is probably the most important way in which arsenic acts as a toxicant.

Out of the three, *A. fertilissima* found to be most sensitive but this does not reduces the possibility of using other two cyanobacteria for purifying purposes.

REFERENCES

Allen, M. B. and Arnon, D. I. 1955 Studies on nitrogen-fixing blue-green algae I. Growth and nitrogen fixation by *Anabaena cylindrica* Lemm. *Plant Physiology* **30**: 366-372.

Angadi, S. B., Hiremath, S. and Pujari, S. 1996 Toxicity of copper, nickel, manganese and cadmium on cyanobacterium, *Hapalosiphon stuhlmannii*. *Journal of Environmental Biology* **17**: 107-113.

Dubey, S. K. and Rai, L. C. 1989 Toxicity of chromium and tin to *Anabaena doliolum*. Interaction with sulphur-containing amino acids and thiols. *Biology of Metals* **2**: 55-60.

Chu, S. Y. and Lin, L. P. 1997 Cellular growth and lead accumulation by *Anabaena cylindrica* and *Chlorella* sp. In cultures containing different lead concentrations. *J Chinese Agric Chem Soc*. **35**: 152-160.

Fatma, T. and Sultan, S. 1999 In: *Cyanobacterial and Algal Metabolism and Environmental Biotechnology.* Tasneem Fatma (ed.), Narosa Pub. House, New Delhi, pp 150-157.

Kapoor, K., Arora, L. and Bamniya, B. R. 1998a Cyanobacterial growth responses in presence of combinations of ions of heavy metals. *Res J Chem and Environ.,* **2(1)** : 59-62.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. 1951 Protein measurement with the folin-phenol reagent.. *J Biol Chem* **193**: 265-275.

Mahadevan, A. and Sridhar, R. 1982 *Methods in physiological plant pathology* (II edition) Sivkami Publications, Chennai.

Plummer, D. T. 1979 *An introduction to practical biochemistry*. Tata Mc Graw Hill Publishing Company Ltd., New Delhi.

Starodub, M. E., Wong, P. T. S., Mayfield, C. I. and Chau , Y. K. 1987 Influence of complexation and pH on individual and combined heavy metal toxicity to a freshwater green alga. *Canadian J Fish Aquat Sci* **44**: 1173-1180.